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Isolated Distal Vein Deep Vein Thrombosis: Diagnosis and Management Strategies

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TABLE OF CONTENTS

Abstract	5
Declaration	7
Acknowledgements	8
Abbreviations	9
Chapter 1: Introduction and Literature Review	12
1.1 Introduction	12
1.2 Pathophysiology of Venous Thromboembolism (VTE).....	13
1.3 Anatomy of DVT	15
1.3.1 Venous Anatomy of the Lower Limb	15
1.3.2 Anatomy of Distal DVT	16
1.4 Natural History of IDDTV	18
1.5 Epidemiology of Venous Thromboembolism	18
1.5.1 Epidemiology of IDDTV	21
1.6 Diagnosis of Venous Thromboembolism	21
1.6.1 Clinical Presentation	21
1.6.2 Clinical Prediction Rules	22
1.6.3 D-dimers	23
1.6.4 Imaging	23
1.6.5 Diagnosis of Recurrent DVT	25
1.7 Thromboembolic Risk Relating to IDDTV	27
1.7.1 Thromboembolic Risk Relating To Distal DVT: Studies Comparing 2-Point C-CUS vs C-CUS	28
1.7.2 Thromboembolic Risk Relating To Distal DVT: A Study Comparing 2-Point CUS vs Venography.....	29
1.7.3 Thromboembolic Risk Related to Isolated Muscular Calf Vein Thrombosis	30
1.8 Treatment of Venous Thromboembolism.....	31
1.8.1 Anticoagulation.....	31
1.8.2: Duration of Treatment	37
1.9 Post-Thrombotic Syndrome (PTS)	41
1.10 Conclusion	43
1.11 Research Proposal.....	44
Chapter 2: Materials and Methods.....	45
2.1 The TWISTER Study	45
2.2 Eligibility	45
2.2.1 Inclusion Criteria	45
2.2.2 Exclusion criteria	46
2.3 Treatment.....	46
2.4 Endpoints	46
2.5 Definitions	47
2.6 Study Flow (figure 7)	47
2.7 Recruiting Centres.....	50
2.8 Statistical Analysis	50
2.9 Adjudication of VTE recurrence/extensions	52
Chapter 3: Interim Analysis	53
3.1 Pre-adjudication.....	53
3.2 Post-adjudication	53
3.3 Post-Thrombotic Syndrome (PTS)	55
3.4 Bleeding events.....	55
3.5 All-cause mortality	56

3.6	Conclusion	56
3.7	Data safety monitoring board	56
3.8	Post-interim analysis.....	56
Chapter 4: Results	58	
4.1	Baseline Demographics	58
4.2	Distribution/Characteristics of IDDTV	59
4.3	Compression Stockings	60
4.4	Two-Week Visit and Treatment Allocation	62
4.5	Adjudication (Second analysis).....	65
4.6	Primary Outcome.....	69
	4.6.1 Symptomatic Distal DVT Recurrences in Experimental Group (C)	69
	4.6.2 Asymptomatic Proximal DVT Recurrence.....	69
4.7	Secondary Outcomes	70
	4.7.1 Asymptomatic Proximal Thrombus Extension at Two Weeks.....	70
	4.7.2 Time Course to Symptom Resolution.....	71
	4.7.3 Post-Thrombotic Syndrome (PTS)	72
	4.7.4 All-Cause Mortality	74
	4.7.5 Predictors of Recurrent or Progressive DVT/PE	74
4.8	Treatment Group Characteristics.....	75
4.9	Six-Week Visit (Symptomatic Arm) - Group B.....	76
4.10	D-dimer Sub-Study.....	76
4.11	Bleeding.....	79
4.12	Muscular Vein Sub-analysis.....	80
Chapter 5: Discussion.....	82	
5.1	Choice of Study Design.....	82
5.2	Baseline Demographics	83
5.3	Primary Outcome.....	83
5.4	Asymptomatic Proximal DVT Recurrence.....	86
5.5	Symptom Resolution	87
5.6	Post-Thrombotic Syndrome.....	87
5.7	D-dimer Sub-study	89
5.8	Bleeding.....	89
5.9	Predictors of Recurrent or Progressive DVT/PE	91
5.10	Treatment Group Allocation.....	92
5.11	Muscular Vein Sub-analysis.....	93
5.12	Compression Stockings	94
5.13	Rivaroxaban Sub-Analysis	95
5.14	Challenges in Conducting Clinical Trials.....	95
	5.14.1 Recruitment.....	96
	5.14.2 Loss To Follow-Up.....	97
	5.14.3 Adjudication.....	99
Conclusion	100	
References.....	101	
Appendices	110	
Appendix 1: TWISTER Protocol	110	
Appendix 2: D-dimer Sub-Study.....	131	
Appendix 3: Ethics Approval	137	
3.1 New Zealand Ethics Approval.....	137	
3.2 Australian Ethics Approval.....	139	
Appendix 4: Publications and abstract presentations during PhD candidature	140	
Appendix 5: Funding Sources	141	

Abstract

Approximately 50% of symptomatic deep vein thrombosis (DVT) are confined to the calf veins (distal DVT). The proportion of distal DVT that propagate to proximal veins, increasing the risk of pulmonary embolism (PE), is unknown but likely low (10%). The management of isolated distal deep vein thrombi (IDDVT) is therefore controversial. Treatment strategies range from withholding anticoagulation and repeating the ultrasound in 7-10 days (and anticoagulating if thrombi that have propagated into proximal veins), through to three months of full dose anticoagulation.

This multicentre prospective cohort study examined that hypothesis that a two-week duration of anticoagulation would lead to resolution of symptoms, with low rates of recurrent venous thromboembolism (VTE) during 3 month follow-up. Secondary outcomes included three and six-month post-thrombotic syndrome (PTS) rates, bleeding, and clinical predictors of extension/recurrence in IDDVT patients, including D-dimers (D-dimer sub-study).

Patients were enrolled within 72 hours of diagnosis of a first IDDVT, and treated with full dose enoxaparin or rivaroxaban for two weeks. At two weeks, patients had repeat complete compression ultrasound (CUS) and clinical review. If patients were asymptomatic with no extension on CUS, anticoagulation was stopped. If patients had ongoing symptoms and/or extension within distal veins on CUS, anticoagulation was continued for another four weeks. Symptomatic patients were reviewed at six weeks with repeat CUS; treatment was stopped if patients were asymptomatic with no extension on CUS. Patients in both groups were reviewed at 3 and 6 months. If the two-week CUS showed proximal extension, patients were taken off study and treated at the investigator's discretion. Patients recorded symptoms daily while on anticoagulation.

200 patients were enrolled at eleven sites in Australia and New Zealand; one was withdrawn on day 5 due to a clinically relevant non-major bleed (CRNMB). Of the remaining 199 patients, 140 (70%) were assigned to group C (asymptomatic, two weeks anticoagulation); 56 (28%) to group B (ongoing symptoms; 6 weeks anticoagulation); and three patients (2%) had proximal extension on 2-week CUS (group A).

Two patients in the two-week anticoagulation arm (group C) had symptomatic VTE recurrence, both distal DVT; VTE recurrence rate 2/131, or 1.5% (95% CI 0.19-5.41%).

There were no symptomatic proximal DVT/PE recurrences. Eighty percent of patients had complete resolution of symptoms within 2 weeks.

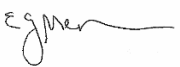
Of 158 patients with six-month follow-up data, seven patients (4.3%, 95% CI 1.7-8.6%) had mild PTS (using Villalta scale), with no cases of severe PTS.

Twenty-five patients (12.5%) developed bleeding, with five CRNMB (2.5% of patients) but no major bleeds. No predictors for VTE extension or recurrence were identified, including D-dimers.

Conclusion: For patients with IDDTV, two weeks of treatment is sufficient to achieve symptomatic relief in 80%. In these patients, if a repeat CUS does not show extension of the thrombus into the proximal veins, then stopping anticoagulation is associated with a low risk of VTE recurrence (1.5%) and no proximal DVT recurrence or PE. Based on this data, the previous standard of care, 6-12 weeks of anticoagulation, is not required for the majority of IDDTV patients.

Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

Signature: ... 

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Abbreviations

ACCP	American College of Chest Physicians
APLS	Antiphospholipid antibody syndrome
AT	Antithrombin
BMI	Body mass index
CACTUS	Randomised Controlled Trial of Anticoagulation versus Placebo for a First Symptomatic Isolated DVT
C-CUS	Complete compression ultrasound
CI	Confidence interval
CRNMB	Clinically relevant non-major bleed
CTPA	CT pulmonary angiography
CUS	Compression ultrasound
DOAC	Direct oral anticoagulant
DOTVAK	Dure'e Optimale Traitement AntiVitamines K study
DSMB	Data safety monitoring board
DVT	Deep vein thrombosis
ECS	Elastic compression stockings
FV	Factor V
FX	Factor X
HIT	Heparin induced thrombocytopenia
HMB	Heavy menstrual bleeding
HRT	Hormone replacement therapy

IDDVT	Isolated distal deep vein thrombosis
IGSVT	Isolated gastrocnemius and soleal vein thromboses
INR	International normalised ratio
IPG	Impedance plethysmography
ISI	International Sensitivity Index
ISTH	International Society of Thrombosis and Haemostasis
LMWH	Low molecular weight heparin
MRDTI	Magnetic resonance direct thrombus imaging
MRI	Magnetic resonance imaging
OAC	Oral anticoagulant
OR	Odds ratio
PCCs	Prothrombin complex concentrates
PE	Pulmonary emboli
PT	Prothrombin time
PTS	Post-thrombotic syndrome
RCT	Randomised controlled trial
RR	Risk ratio
TICT	Treatment of isolated calf thrombosis study
TF	Tissue factor
TWISTER	Two Weeks of Low Molecular Weight Heparin for Isolated Distal Vein DVT
UFH	Unfractionated heparin
USS	Ultrasound
VAS	Visual analogue scale

VKA	Vitamin K antagonist
V-Q	Ventilation perfusion
VTE	Venous thromboembolism
VWF	Von Willebrand Factor
WHO	World Health Organisation

Chapter 1: Introduction and Literature Review

1.1 Introduction

It is estimated that approximately 10% of pulmonary emboli (PE) are rapidly fatal, with an additional 5% causing death at a later time point, despite diagnosis and treatment (2, 3).

Most PE originate from a thrombus in the proximal veins of the leg; therefore treatment of lower limb deep vein thrombosis (DVT) with anticoagulant therapy can prevent the development of PE.

Full dose anticoagulant therapy is standard of care for treatment of proximal vein DVT (those involving the popliteal veins and above). Most patients with proximal vein DVT will receive a minimum of 3-6 months of treatment, as anticoagulant treatment for less than 3 months has been associated with substantially higher rates of VTE recurrence(4). Anticoagulant therapy is associated with a risk of major bleeding of 1-3% per year(5), with an estimated case fatality rate for major bleeding of 13.4%(6); there is a greater incidence of bleeding during the first 3 months of treatment (7, 8). Treatment of DVT is also costly (average cost \$10,000 Australian per case of VTE in 2008 (Access Economics Report 2008) and inconvenient. For all of these reasons, it is important to establish the diagnosis of DVT accurately and treat appropriately.

Conversely, the treatment and management of isolated calf vein thrombi or isolated distal vein DVT (IDDVT) is controversial. Treatment strategies range from withholding anticoagulation and repeating the ultrasound in 7-10 days (with anticoagulation of thrombi that have propagated into proximal veins), through to three months of full dose anticoagulation. In this literature review, I will summarise the pathophysiology, epidemiology, diagnosis and management of VTE, with an emphasis on IDDVT, and highlight areas where further research is needed.

1.2 Pathophysiology of Venous Thromboembolism (VTE)

In 1856, Rudolf Virchow described a triad of factors thought to contribute to thrombosis (Virchow's triad): stasis, changes in the vessel wall, and changes in the blood (hypercoagulability)(9).

Stasis Causes of stasis include venous stasis, varicose veins, prolonged immobility, and lengthy surgical operations. Venous valves are areas where stasis may occur, with blood contrast shown to linger for up to 27 minutes post-venography (10). Valvular venous stasis has been associated with an increased haemocrit and hypoxia, the latter potentially leading to down-regulation of the regionally expressed antithrombotic proteins, thrombomodulin and endothelial protein C receptor, and an upregulation of procoagulant activity including tissue factor (TF) on the endothelium (10).

Hypoxia also upregulates the expression of P-selectin on the endothelium(10). P-selectin is expressed by both activated platelets and endothelial cells, and mediates leucocyte adhesion (11). Both P-selectin and tissue factor have been demonstrated in monocyte-derived microparticles(11). Animal models show that both tissue factor and P-selectin are necessary for thrombus formation, and conversely, that blockage of P-selectin inhibits thrombus formation(12).

In recent years, neutrophil extracellular traps (NETS) have been demonstrated to play an important role in thrombosis. NETS are important in trapping and killing bacteria, fungi and even viruses, by creating fibrous nets with antimicrobial properties(13, 14). It is likely that hypoxia drives the release of von Willebrand factor (VWF) from Weibel-Palade bodies in endothelial cells. Hypoxia also induces the release of hypoxia-inducible factor 1 α (HIF-1 α), which is implicated in the formation of NETS (termed NETosis)(13). NETS are present in close association with VWF in baboon and mouse models of thrombosis (Blood 2014). Inflammation is a known risk factor for thrombosis, and many of the risk factors for DVT, such as trauma, surgery, infection, immobilisation and hypoxia, are associated with NET formation (Blood 2014). In accordance with this, levels of extracellular DNA and myeloperoxidase (MPO) have been shown to be significantly elevated in patients with VTE compared with patients without VTE(15).

Venous stasis may also occur due to extravascular venous obstruction from bulky tumours or enlarged lymph nodes, or intravascular obstruction by residual thrombosis (16).

Changes in the Vessel Wall In comparison to animal models, this is a far less common cause of venous thrombosis, except in cases of surgery-associated thrombosis (10). Therefore the rest of this section will focus on hypercoagulability.

Hypercoagulability Increased levels of coagulation factors, especially Factor VIII and von Willebrand factor (VWF), factor VII and prothrombin are associated with an increased risk of thrombosis (10). Both estrogens (e.g. in oral contraceptives and hormone replacement therapy, HRT) and cancer can lead to activation of thrombosis (16). Levels of VWF increase markedly during pregnancy and only return to non-pregnant levels during the late puerperium (17). Levels of the clotting factors I, II, VII, VIII, IX and XII also increase, while protein S levels decrease, and there is also an inhibition of fibrinolysis (18). Similarly, the estrogen-containing oral contraceptive pill (OCP) increases the levels of fibrinogen, prothrombin, factors VII, VIII and X. At the same time, the OCP reduces the levels of proteins which contribute to inactivation of anticoagulation i.e. antithrombin (AT), protein S, and tissue factor pathway inhibitor (TFPI), as well as causing resistance to activated protein C(19).

Cancer patients have a 4-7 fold increased relative risk of VTE when compared with the non-cancer population, and 20-30% of all first VTE are associated with cancer(20). The type and stage of malignancy are directly related to the degree of VTE risk, with the highest VTE risk associated with pancreas, brain, lung and ovarian cancer(21). Overexpression of tissue factor (TF), procoagulant tumour-derived microparticles and carcinoma mucins may all contribute to systemic hypercoagulability in a range of cancers (10, 20). Suppression of tumour suppressor genes, and activation of oncogenes, can lead to TF overexpression(21). TF-bearing microparticles are shed from platelets, erythrocytes, tumour cells, endothelial cells, lymphocytes and monocytes(22). Elevated TF levels stimulate angiogenesis by increasing platelet activation, increasing thrombin levels, and cleaving fibrinogen; thereby enabling tumour growth and metastasis(22). Chemotherapy also contributes to VTE risk through a number of mechanisms, including endothelial damage, suppression of the natural anticoagulants, stimulation of platelet aggregation and cytokine release(22). Recent surgery, hormonal therapy, antiangiogenic drugs, immunomodulatory agents, erythropoiesis-

stimulating agents, blood transfusions, and central venous catheters also increase VTE risk(21).

Major trauma has also been shown to induce a hypercoagulable state by leading to significantly increased, persistent, thrombin generation (23).

Hereditary reductions in proteins C, S and antithrombin, termed the major thrombophilias, are recognised as risk factors for VTE. The risk of a first VTE in those with major thrombophilias ranges from 1.7-18% in cohorts with a family history (24). The weaker thrombophilias, such as Factor V (FV) Leiden heterozygosity (leading to resistance to activated protein C), and the heterozygous prothrombin gene mutation, are associated with a risk of first-time VTE of 1.5-3.9% in those with a family history; in the homozygous or combined homozygous forms, the first-time VTE risk is similar to the major thrombophilias (24).

Not all thrombophilias are hereditary. Antiphospholipid antibody syndrome (APLS) is an important example of an acquired thrombophilia. Antiphospholipid antibodies, comprised of the lupus anticoagulant, anticardiolipin antibodies, and beta-2-glycoprotein-1, lead to an increased susceptibility to thrombosis through incompletely understood mechanisms. However, the pathogenesis of thrombosis in these patients is likely to be multifactorial, involving oxidative stress, reduced plasma nitrate, and upregulation of proadhesive and procoagulant molecules such as tissue factor (25). It is likely that the antiphospholipid antibodies disrupt the endothelium but need a second ‘hit’ to potentiate thrombus formation, such as infection or inflammation (26). Patients with nephrotic syndrome are also prone to thrombosis, due to loss of antithrombin in the urine (27).

1.3 Anatomy of DVT

1.3.1 Venous Anatomy of the Lower Limb

The calf has three groups of paired deep veins – the anterior tibial veins, the peroneal veins and the posterior tibial veins (**figure 1**). The calf muscles are drained by the muscular veins, the soleal and gastrocnemius veins. As the paired deep calf veins become more proximal, they unify to form a confluent segment, which is referred to as the ‘trifurcation’ area. After a few centimetres the confluent segments unify to become the popliteal vein, which is also

occasionally paired (1). This area is usually found at the level of the knee joint but may also be above or below the knee joint.

The popliteal vein then courses through the adductor canal, at which point it becomes the femoral vein. The femoral vein was previously referred to as the superficial femoral vein, but this is incorrect, as it is a deep vein. In the thigh, the femoral vein and the deep femoral vein (also called the profunda femoris) unite to form the common femoral vein. Once this passes above the groin crease, it becomes the external iliac vein. At the level of the sacroiliac joint, it unites with the hypogastric vein to form the common iliac vein. The two common iliac veins then unite at the level of the fifth lumbar vertebra to form the inferior vena cava.

1.3.2 Anatomy of Distal DVT

Distal or calf vein DVT (**figure 1**) refers to thrombosis involving the veins below the level of the popliteal vein (the **infra**-popliteal veins), as detailed above. Together these can be encompassed by the term '**Isolated Distal Deep Vein DVT**' (IDDVT). Some clinicians refer to IDDVT as 'below knee' DVT, however this term is misleading and should not be used, as in some cases the popliteal vein terminates below the knee.

The trifurcation area is variably referred to as proximal or distal; anatomically it is more correct to call it distal as it lies below the level of the knee joint. However in more recent times, with the advent of ultrasonography, the trifurcation area is more commonly classified as proximal (1). This may account for some of the differing outcomes in studies on IDDVT, as one would expect the more distal thrombi to be smaller, with a lesser chance of embolisation to the pulmonary arteries (28).

Distal DVT occur with an equal distribution between the left and right legs, whereas proximal DVT are more frequently left-sided (1.32:1 overall; but 2.4:1 for iliac DVT) (29). A congenital stenosis of the left common iliac vein by pressure from the overlying right common iliac artery (May-Thurner syndrome), is likely the reason why more proximal vein DVTs, particularly during pregnancy, occur in the left leg than in the right.

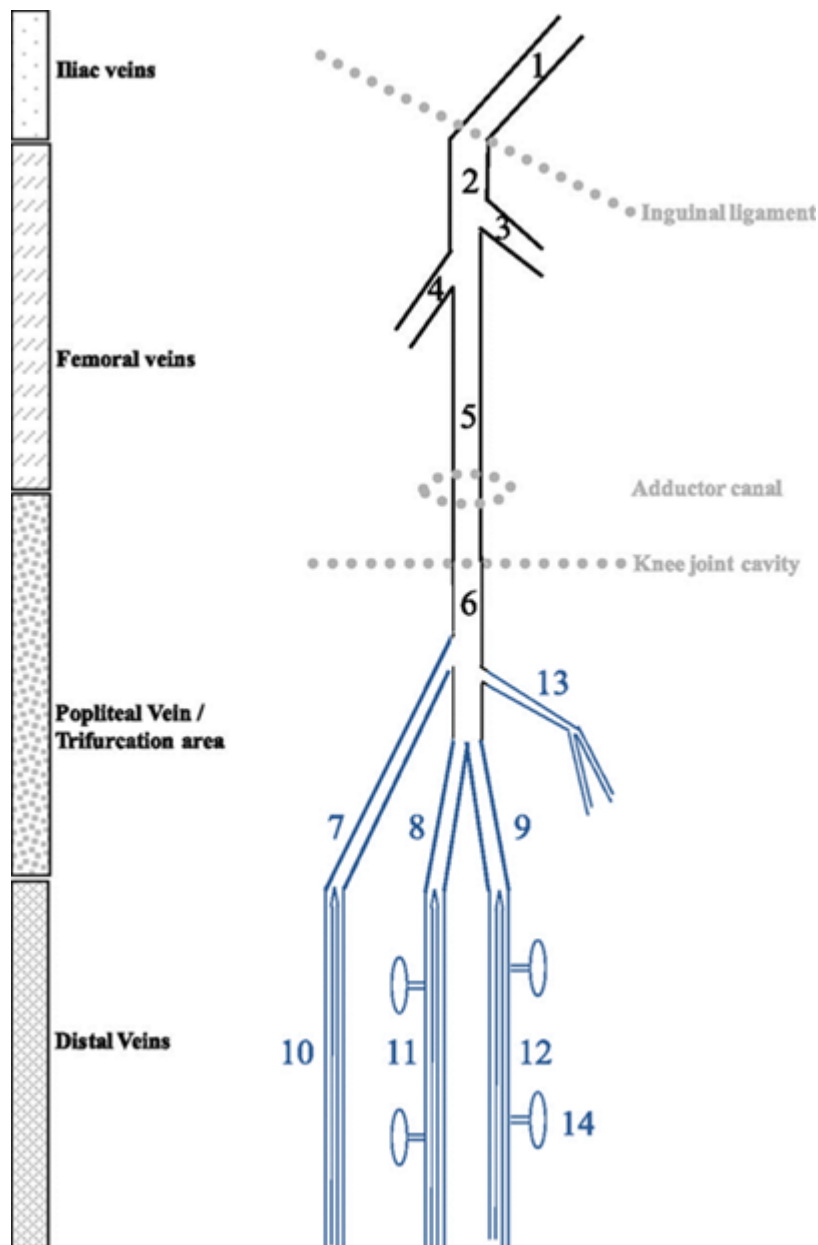


Figure 1. Anatomy of distal veins of lower limb; reproduced/adapted with permission from Palareti & Schellong, JTH 2012(1).

Blue = distal veins; black = proximal veins. 1, external iliac vein; 2, common femoral vein; 3, greater saphenous vein; 4, profound femoral vein; 5, (superficial) femoral vein; 6, popliteal vein; 7, anterior tibial confluent segment; 8, posterior tibial confluent segment; 9, peroneal confluent segment; 10, anterior tibial veins; 11, posterior tibial veins; 12, peroneal veins; 13, gastrocnemius muscle veins (medial head); 14, soleal muscle veins.

1.4 Natural History of IDVT

Most DVT start in the veins of the calf (2), with IDVT comprising 31-56% of all diagnosed leg DVTs (1). It is estimated that 10-20% enlarge and propagate to the proximal leg veins (2, 30) in the absence of anticoagulation. Propagation primarily occurs within the first week after detection of a distal DVT (2). Conversely, 80-90% (30) of small calf vein thrombi are thought to lyse spontaneously and therefore may be of minimal clinical consequence.

Estimates of the proportion of distal vein DVT propagating to proximal veins vary widely in the literature, from 0-44% (31). A recent systematic review of IDVT showed a propagation rate to the popliteal vein from 0-35%, with a mean of 8.9%(32); this included studies using both observation (no anticoagulation) and varying regimens of anticoagulant treatment. This correlates well with a systematic review from 2006, showing an estimated rate of proximal extension of 10% (95% CI 7-12%) in untreated patients with distal DVT, compared with 4% (95% CI 3-6%) in anticoagulated patients(33).

1.5 Epidemiology of Venous Thromboembolism

The annual incidence of venous thromboembolism (VTE), which includes DVT and PE, is approximately 70 per 100,000 Australians (Access Economics Report 2008). Of these, 56% were PE and 44% were DVT. New Zealand data shows a similar rate of 82 per 100,000 population (34). Around the rest of the world, VTE rates of 104 to 183 per 100,000 person-years are reported in those of European ancestry(35, 36), with VTE rates varying from 79 per 100,000 in Hong Kong to 269 per 100,000 in Denmark. This reflects the different ethnic composition of populations in these countries, and is discussed further below.

In the majority of VTE, a provoking factor is present. Conversely, idiopathic VTE (with no readily identifiable risk factor) comprise 25 to 40% of VTE events occurring in European and African populations, but only 19% of VTE events occurring in those of Asian and Pacific Island ethnicity(35, 37).

Established risk factors for VTE can be categorised using Virchow's triad and include hypercoagulability (estrogen-containing preparations, active malignancy, pregnancy and the post-partum period, major trauma, family history of VTE, presence of a hereditary or acquired thrombophilic defect, increasing age, surgery), stasis (immobility, varicose veins, neurological disease with paresis), and changes in the vessel wall (surgery, trauma), including

that due to foreign bodies (central vein catheterisation or transvenous pacemakers), and previous superficial vein thrombosis (38). More recent studies have shown that that inflammatory bowel disease, chronic kidney disease (especially nephrotic syndrome), congestive heart failure, smoking, obesity, and hypertension also increase the risk of VTE (39, 40), mostly through inducing a hypercoagulable state.

The incidence of VTE increases with age (**figure 2**). VTE is rare prior to adolescence(35), and rises from an annual rate of less than 5 per 100,000 in children <15 years of age, to 450-600 per 100,000 (approximately 0.5%) in adults over the age of 80 years (37). There are no overall differences in VTE incidence between men and women, despite the use of combined oral contraceptives and hormone replacement therapy in women (37).

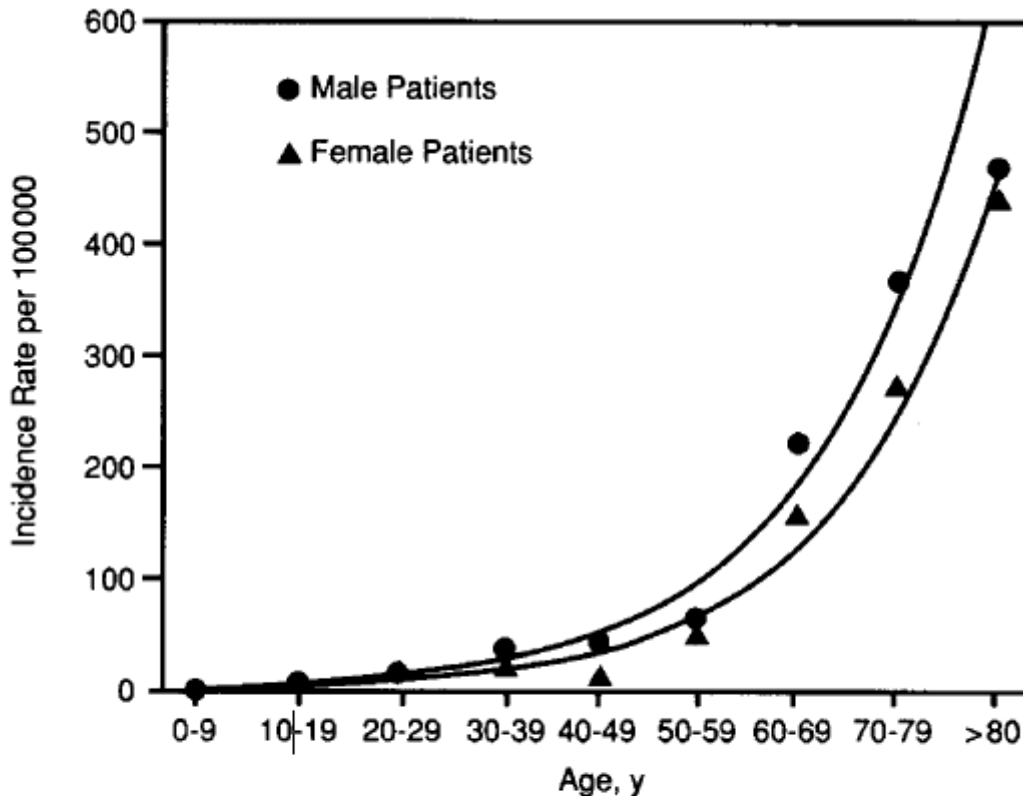


Fig 1.—Incidence rate of clinically recognized deep vein thrombosis and/or pulmonary embolism per 100 000 population. The increase in rates for both male and female patients is well approximated by an exponential function of age. The modeled rate for male patients (upper curve) is significantly higher ($P<.05$) than that for female patients (lower curve).

Figure 2: Incidence of VTE According to Age and Sex (Figure reproduced with permission; Anderson FA, et al. Arch Internal Med. 1991)(41)

With regards to ethnicity, the incidence has been reported as similar or higher among African-Americans and lower among Asian and native Americans (38). A New Zealand study showed that the VTE incidence rates were lower in Maori, Pacific Island, Chinese and Indian subjects than in European subjects (34). However, recent epidemiological data from Asian populations show that the incidence may not be as low as previously thought, and may be similar to European VTE rates(42). The differences in VTE rates in different ethnic groups are likely due to both genetic and environmental factors (34, 37).

Air travel and the risk of VTE have received much media attention. However, the risk is very small, with a systematic review showing a rate of 27 PE per million flights and 0.05% DVT

diagnosed through screening ultrasounds (43). The risk was significantly increased for flights of ≥ 6 -8 hours as opposed to less than 6 hours duration (OR 0.011). One study including 964 passengers returning from flights of duration ≥ 8 hours diagnosed VTE in 2.8% passengers compared with 1.0% of controls, RR 2.83(44). Most of the VTE events (2.1%) were comprised of muscular vein thromboses, compared with 0.8% controls (RR 2.52). The most commonly cited risk factor is “prolonged sitting in cramped quarters”(45), however most patients with air travel related VTE have pre-existing risk factors, such as thrombophilia. Other cabin-related factors thought to contribute to the increased risk of VTE include hypoxia, low humidity and smoking(45).

1.5.1 Epidemiology of IDVT

IDVT are usually associated with transient risk factors, such as hospitalisation, recent surgery or trauma, and recent travel. Lower limb varicosities are also associated with an increased risk of IDVT(46). Whilst IDVT overall comprise 50% of all diagnosed DVT(30), IDVT is less prevalent in the elderly (≥ 75 years), in pregnancy and the puerperium (6% of all pregnancy-associated DVT in one series)(47), in those with malignancy (approximately one-quarter of all diagnosed DVT,)(48), and in those with a previous history of VTE (1, 46). In patients presenting with bilateral distal DVT, a systemic aetiology, such as malignancy or a prothrombotic condition, should be considered.

1.6 Diagnosis of Venous Thromboembolism

1.6.1 Clinical Presentation

Lower limb DVT may be suspected when patients present with swelling or pitting oedema (usually unilateral), redness, dilated superficial veins and tenderness. However clinical manifestations alone cannot be used to diagnose DVT due to the poor specificity of these signs and symptoms. Only 20% of patients with clinically suspected DVT or PE subsequently have this diagnosis confirmed on imaging(49). Diagnostic algorithms using a combination of pre-test clinical probability and D-dimers are therefore useful to reduce the need for imaging in patients deemed to be at low risk.

1.6.2 Clinical Prediction Rules

The most commonly utilised score for DVT is the Wells score. The Wells' DVT score is comprised of ten items. The traditional score assigned patients to low probability (prevalence of DVT less than 5%), intermediate or high probability (prevalence DVT 15 and 70%, respectively) (50); however the binary Wells score is now used instead (51) (**figure 3**). This score is easy to use and well validated. However, clinical decision rules cannot safely exclude the presence of DVT alone, and should be used in combination with a D-dimer. It should be noted that these models are not validated for the diagnosis of calf vein DVT.

Table 1. Clinical Model for Predicting the Pretest Probability of Deep-Vein Thrombosis.*	
Clinical Characteristic	Score
Active cancer (patient receiving treatment for cancer within the previous 6 mo or currently receiving palliative treatment)	1
Paralysis, paresis, or recent plaster immobilization of the lower extremities	1
Recently bedridden for 3 days or more, or major surgery within the previous 12 wk requiring general or regional anesthesia	1
Localized tenderness along the distribution of the deep venous system	1
Entire leg swollen	1
Calf swelling at least 3 cm larger than that on the asymptomatic side (measured 10 cm below tibial tuberosity)	1
Pitting edema confined to the symptomatic leg	1
Collateral superficial veins (nonvaricose)	1
Previously documented deep-vein thrombosis	1
Alternative diagnosis at least as likely as deep-vein thrombosis	−2

* A score of two or higher indicates that the probability of deep-vein thrombosis is likely; a score of less than two indicates that the probability of deep-vein thrombosis is unlikely. In patients with symptoms in both legs, the more symptomatic leg is used.

Figure 3: Wells Score for DVT (Reproduced with permission from Wells PS et al, NEJM 2003; Copyright Massachusetts Medical Society) (51)

1.6.3 D-dimers

D-dimer units are composed of a pair of identical subunits derived from two fibrin molecules, and generated by plasmin-mediated degradation of cross-linked fibrin(52). Small amounts of D-dimers are found in the blood of all healthy individuals, but levels are increased in conditions associated with fibrin formation, such as venous thromboembolism. D-dimer levels are also increased in malignancy, inflammation, infection, trauma, pregnancy, after surgery, extensive burns or bruises, stroke, ischaemic heart disease and peripheral vascular disease (52). Monoclonal antibodies are used to measure the D-dimer level, with commercially available tests including ELISA, latex tests and whole blood agglutination tests.

D-dimers have been demonstrated to have a high sensitivity but limited specificity for the presence of VTE, with a high negative predictive value(51). The D-dimer test is therefore a useful 'rule-out' test, particularly when combined with a low clinical probability, thus averting the need for imaging in many cases. D-dimers appear to have a lower sensitivity and lower negative predictive value in patients with IDVT(53), with one meta-analysis showing all D-dimer assays to have a lower sensitivity for distal compared with proximal DVT: 86% (95% CI 84-88) vs 98% (95% CI 97-99) for ELISA, 79% (95% CI 75-83) vs 94% (95% CI 92-95) for latex, and 64% (95% CI 55-73) vs 84% (95% CI 80-88) for whole-blood agglutination(54). Despite this, management studies have demonstrated that the three-month risk of VTE is very low (<1%) when using a strategy combining a negative D-dimer and low clinical risk score(55). In addition to this, due to the poor performance of CUS in the distal veins, it is possible that some of the detected thrombi were 'false positive' results, which would correlate with some of the negative D-dimer results (56).

1.6.4 Imaging

Venography is traditionally the gold standard test for diagnosis of DVT, both distal and proximal, but is now rarely used as it is costly, invasive, and time consuming. However it is not ideal for diagnosis of IDVT due to issues with underfilling of vessels, and vessels overlying one another, leading to high inter-observer variability (57).

In previous years, fibrinogen leg scanning and impedance plethysmography (IPG) were widely used non-invasive investigations for diagnosis of DVT. The limitations of I¹²⁵-

fibrinogen leg scanning include delay in diagnosis (it can take up to 72 hours after injection of isotope to show a positive finding in some patients with DVT), and lack of sensitivity in certain patient groups.

IPG is based on blood volume changes in the calf produced by inflation and deflation of a pneumatic thigh cuff. This leads to changes in electrical resistance, or impedance, in areas of thrombosis (58). The sensitivity for diagnosis of symptomatic DVT is 72%, but only 13% for asymptomatic DVT (59). IPG may not detect non-occlusive proximal DVT or occlusive proximal DVT present in parallel venous systems, such as paired femoral veins. Additionally, IPG cannot detect DVT confined to calf veins (60). Ginsberg et al showed that it was safe to withhold anticoagulation in patients with a combination of a negative IPG and normal D-dimer (61), with a negative predictive value of 98.5% for DVT during 3-month follow-up. It should be noted that the sensitivity for calf vein DVT was still only 70% using this method (62).

Venous ultrasonography is now the most widely used diagnostic modality for diagnosis and exclusion of DVT. There are several types of venous ultrasonography, including compression ultrasound (B-mode imaging only), duplex ultrasound (B-mode imaging and Doppler waveform analysis), and colour Doppler imaging alone (60). Compression ultrasound is primarily used for imaging proximal veins, whereas a combination of compression ultrasound and colour Doppler imaging is often used to image the distal and iliac veins.

Historically, compression was applied to the common femoral vein at the groin and the popliteal vein at the popliteal fossa (2-point ultrasonography). This had the advantages of being rapid, broadly available and reproducible, with sensitivity and specificity of 97% and 94% respectively for proximal DVT (63). The main disadvantage is the need to repeat the ultrasound after one week to detect distal DVT extending to proximal veins (serial 2-point ultrasonography).

In recent years, colour-coded Doppler USS scans have been employed to evaluate the whole leg (entire deep venous system, including the calf veins), termed complete compression ultrasound (C-CUS). The presence of non-compressibility is the most important aspect of this evaluation, as the use of colour tends to be unreliable and does not add much to the diagnosis. This requires an experienced operator but has the advantage of requiring only a single test, as the incidence of subsequent confirmed DVT during the 3-month period after an

initial negative C-CUS has been shown to be low at 1.2% (64). However, this technique also enables detection of isolated distal DVT (thrombus involving the infra-popliteal vessels only), with such events making up approximately 50% of all diagnosed DVT (30).

The reported performance of CUS in the distal veins is much lower than for proximal vein CUS. A meta-analysis of diagnostic accuracy of USS for clinically suspected DVT (65) reported an overall sensitivity for USS of 63.5% (95% CI 59.8-67.0) for distal DVT, compared with an overall sensitivity of 94.2% (95% CI 93.2-95.0) for proximal DVT, and specificity for both of 93.8% (95% CI 93.1-94.4). False positive results occur in patients with obesity, oedema, casts, bandages and immobilisation devices (66).

Magnetic resonance direct thrombus imaging (MRDTI) has been used in recent years, and has been shown to be highly sensitive and specific for diagnosis of DVT, including isolated calf DVT (57). This type of imaging offers the advantage of visualising the thrombus directly, and thrombus more than six months old is not visualised. However MRI is costly and access is limited in many centres, therefore the routine use of MRI for diagnosis of DVT cannot be advocated at present.

It should be noted that it is still debated whether a whole leg USS is necessary in all patients, and in many countries around the world (e.g. North America), it is not routine to scan the deep calf veins. The ACCP guidelines (2016) recommend against routinely examining the calf veins in patients in whom proximal DVT has been excluded(67). Their rationale for this is that (1) other assessments/clinical risk scores may have already deduced that distal DVT is unlikely to be present, or if present, is unlikely to cause complications; (2) in patients deemed at higher risk, serial USS scans can be performed to detect possible DVT extension and treatment given if extension is present; and (3) false-positive findings are more likely with USS scans of the distal venous system as opposed to the proximal venous system. However, for the reasons stated above, in many countries a whole-leg compression USS as a single, definitive examination is preferred.

1.6.5 Diagnosis of Recurrent DVT

Diagnosing recurrent ipsilateral DVT is a diagnostic challenge, as it can be difficult to differentiate between new and residual thrombosis. Residual thrombus is present in

approximately 40% of patients three months after a DVT, falling to 30% by two years(68). Most of the reduction in thrombus mass occurs within the first three months of treatment (69).

Many clinicians perform baseline imaging at a predefined period of time after acute deep vein thrombosis, either at the end of treatment or after a period of months. These images can then be compared with future imaging in the event of new symptoms suspicious for DVT. This strategy is most helpful if no residual thrombosis was present on baseline imaging. However, if residual thrombosis is present, then the images can be compared to look for an increase in thrombus diameter. An increase in the length of thrombus of more than 5cm, or an increase in the diameter of the vein by more than 2mm, is suggestive of thrombus recurrence(70).

Ultrasound can usually distinguish acute from chronic thrombus. In acute thrombosis, the vein is distended by hypoechoic thrombus, and shows partial or no compressibility. In chronic thrombosis, the vein is not compressible, narrow and irregular, and shows echogenic thrombus attached to vessel walls with collateral vessels (66).

Studies have shown the inter-observer reliability for measurement of residual thrombosis in patients with a previous proximal DVT is high (71). There is very little data on inter-observer reliability in patients with distal DVT, but it is likely to be higher than for proximal DVT, given the lower sensitivity of diagnostic USS in this population (65). The combination of an equivocal recurrence on USS with a D-dimer is helpful if the D-dimer is positive, however a negative D-dimer is less reassuring in the IDVT population, due to the aforementioned lower sensitivity of the test. However, it is likely that the deep calf veins are cleared more rapidly and completely than the more proximal segments, making the presence of residual thrombosis less of an issue (72).

1.7 Thromboembolic Risk Relating to IDVT

The proportion of distal DVT which subsequently propagate to the proximal veins is not known, although estimates range widely, from 0-44% in the literature (31). Historical data from retrospective series suggested that distal DVT may be dangerous. Lagerstedt et al published a randomised trial in 1985(73) that included 51 patients with asymptomatic distal DVT diagnosed by venography. VTE recurrence rates at three months were 28% (8/28; 95% CI 13.2-48.6%) in untreated patients versus 0% (0/23; 95% CI 0.0-20.6%) in anticoagulated patients ($p<0.01$). Five of eight patients had recurrence with proximal extension, and one with PE. However, five of eight (63%) of these patients had a history of previous VTE and were therefore at high risk of recurrence. In addition, DVT extension was diagnosed by a combination of physical examination and serial isotope testing, which is far less sensitive than modern ultrasonography. Most other historical studies of distal DVT have similar issues, including a heterogeneous mix of patients, with symptomatic and non-symptomatic IDVT, and patients with concomitant PE(74).

More recent studies show that the three month thromboembolic risk in patients with serial negative CUS assessing proximal veins only is low (64, 75-77). Recent randomised data strongly suggest that only a small proportion of DVT extend proximally over one week, and of the distal DVT that do not extend over one week, that most resolve spontaneously without anticoagulant therapy, as evidenced by the low rate of recurrence in those who have two serial negative CUS (**table 1**). Risk factors for extension include positive D-dimer, thrombosis that is extensive or close to proximal veins, involvement of multiple veins, no reversible provoking factor for DVT, active cancer, history of VTE, and inpatient status(67).

PE at diagnosis must be distinguished from PE developing during the surveillance period after distal DVT has been diagnosed. There is a low incidence of PE reported during surveillance (78, 79). In contrast, reports of PE at presentation are higher and range from 5-56% (79). This is likely due to the more proximal portion of the DVT breaking off and embolising to the lungs, with only distal DVT found on subsequent ultrasound.

Table 1: Three Month Recurrence Rates in Those with C-CUS Versus Serial CUS

Study	Patients	3-Month VTE Recurrence Rate	3-Month VTE Recurrence	Calf Vein Thrombi
	(n)	Rate C-CUS % (95% CI)	Rate Serial CUS % (95% CI)	(% of total DVT)
Bernardi et al 2008	2098	1.2 (0.5-2.2)	0.9 (0.3-1.8)	23
Righini & Bounemeaux 2008	9116	0.3 (0.1-0.6)	0.6 (0.4-0.9)	50
Kearon et al 2005	810	2.1 (0.9-4.0)*	1.3 (0.4-2.9)	24#
Gibson et al 2009	521	1.2 (0.2-4.3)	2 (0.6-5.1)	15

C-CUS: single whole leg USS; Serial CUS: using two-point proximal USS only

*Venography performed in those with negative CUS and positive D-dimer.

Based on fact that 70 patients with initial DVT on USS not randomised, therefore assume 35 in each group with DVT, giving a denominator of 54 in the D-dimer group. Of these, 13 had distal DVT diagnosed on venography.

1.7.1 Thromboembolic Risk Relating To Distal DVT: Studies Comparing 2-Point C-CUS vs C-CUS

The best illustration of this is provided by Bernardi et al., (64), in their randomised study evaluating 2098 patients with suspected DVT. This study compared complete compression ultrasonography (examination of both proximal and distal leg veins; C-CUS) with 2-point ultrasonography (CUS) for diagnosis of suspected DVT. The group receiving C-CUS received anticoagulation if there was DVT (proximal and distal) on ultrasound at presentation. The 2-point ultrasonography group were also anticoagulated if there was (proximal) DVT on CUS at presentation, or on repeat CUS 7 days later. All patients with a negative single C-CUS or serial CUS had anticoagulation withheld.

The 3-month thromboembolic risk was 0.9% in the serial CUS group and 1.2% in the single C-CUS group. There was a significantly higher initial prevalence of DVT in the complete CUS group (278 cases) compared with 217 cases of proximal DVT in the 2-point group (absolute difference 4.3%, CI 0.5-8.1%). Interestingly, this higher initial prevalence was due to 65 initial cases of isolated calf DVT identified by complete CUS. It is likely there were similar numbers in the serial CUS group, which were not detected and treated. Despite this, the long-term outcome of the patients in each group was equivalent. Of the 256 patients

randomised to the 2-point strategy with abnormal D-dimers, 14 had “abnormal” repeat CUS within the next seven days (12 on the scheduled day 7 CUS, and 2 on CUS performed prior to day 7 due to symptoms). This equates to a 5.5% rate of proximal vein extension in the group where distal DVT were left untreated, which is somewhat lower than the 10-20% rates of extension for untreated IDVT often quoted in the literature.

Similarly, Gibson et al., (77) randomised 521 patients with a likely clinical probability for DVT, and those with a low probability and an abnormal D-dimer test result, to undergo either serial proximal CUS or a C-CUS examination. Of those with no DVT identified on the initial examination, the incidence of VTE during follow-up was 2% in the proximal CUS group and 1.2% in the C-CUS group ($P=0.69$, absolute difference 0.8%; 95% CI 1.8-3.4%).

Righini & Bounemeaux (75) performed a meta-analysis consisting of ten studies (pooling 9116 patients) using either only proximal vein CUS (serial in five studies, and single proximal CUS combined with D-dimer in one study) or single C-CUS (four studies). It should be noted that 50% of the DVTs found using C-CUS were distal DVT. Despite this, the two different strategies had very similar 3-month thromboembolic risks, amounting to 0.3% in the C-CUS group and 0.6% in the serial CUS group (**table 1**).

Finally, Palareti et al., (80), in their CALTHRO study, followed patients with a negative proximal CUS, with likely or unlikely pre-test clinical probability but abnormal D-dimer test, for three months. The patients all received a second proximal CUS after 5-7 days; they also received a complete, blinded CUS at study entry. Proximal extension occurred in 3% of patients within 5-7 days; over 90% of calf thrombi resolved without anticoagulant treatment.

1.7.2 Thromboembolic Risk Relating To Distal DVT: A Study Comparing 2-Point CUS vs Venography

Another randomised trial was performed by Kearon et al., (76), in which 810 outpatients with suspected DVT with initial normal proximal vein USS results were randomised to either venography based on the results of D-dimer testing, or repeat proximal CUS at one week. In the D-dimer group, those with a negative result had no further testing and had anticoagulant therapy withheld, whilst those with a positive result went on to venography. The 3 month VTE recurrence (proximal DVT/PE) rates were similar at 2% in each group for patients

deemed DVT negative by either strategy (negative D-dimer/negative venography or negative serial CUS of proximal leg veins).

1.7.3 Thromboembolic Risk Related to Isolated Muscular Calf Vein Thrombosis

Up to 40% of symptomatic calf vein thromboses appear to be isolated to veins draining the gastrocnemius and soleal muscles, without involvement of the venae comitantes of the tibial and peroneal veins (axial calf veins) (81). However, it is thought that many calf vein and thus proximal DVT may originate from an isolated muscular vein thrombosis. The natural history of these isolated gastrocnemius and soleal vein thromboses (IGSVT) is unknown, as the recommendations for management of distal vein DVT are almost exclusively based on the natural history of calf vein thrombi involving the axial calf veins.

There is, therefore, controversy regarding the management of IGSVT and no current guidelines exist. Various studies reporting on the outcome of treated and untreated IGSVT have been published with very different outcomes. Moreover, there is marked heterogeneity of patient characteristics and management across studies. Three studies below provide some insights into the natural history of IGSVT.

The first of these (82) was a prospective study which randomised patients with symptomatic, sonographically proven IGSVT to receive either ten days of therapeutic nadroparin and three months of compression therapy, or compression therapy alone. One hundred and seven patients were enrolled in the study. The rate of progression to DVT was the same in both groups (3.7%), however only one of the patients in each group progressed to the level of the popliteal vein or higher (1.9%). Thrombus recanalisation after three months was not significantly different between the two study groups.

The second study (81) was a prospective study looking at the natural history of untreated IGSVT. 135 limbs with IGSVT were studied for 3 months. Among those with IGSVT, 16.3% extended the thrombus to the level of the adjacent tibial or peroneal veins, or higher. However only 3% of the cases (4/135) propagated to the level of the popliteal vein (proximal extension), and 91% of the propagations occurred within 2 weeks of USS diagnosis. None of these propagated beyond the popliteal vein. IGSVT remained stable or resolved in 84% (113 of 135 limbs) without anticoagulant treatment during the 3-month follow-up. Gastrocnemius

vein thrombi appeared to be more prone to propagate than soleal vein thrombi (25.9% vs 14.3%), although this difference was not statistically significant ($p=0.15$). The presence of malignancy was the only recorded risk factor found to be predictive of IGSVT propagation ($p=0.02$).

Conversely, a third study reported a significant rate of extension of IGSVT to proximal veins (83). This was a prospective study evaluating the outcome of 128 patients presenting with IGSVT. Patients were followed clinically for up to 36 months. Full dose anticoagulant therapy was prescribed for one month, and extended to two additional months in case of incomplete recanalisation at one month (as seen on USS) or if risk factors for VTE were present. However this study was not confined to those with isolated IGSVT (patients with PE at baseline were included). The study also included seven patients with malignancy, in whom a limited course of anticoagulation is often not appropriate. Despite this, it would appear that one month of anticoagulant therapy was sufficient as there were no observed recurrences in the first three months of this study among those in whom anticoagulant therapy was restricted to one month (53/128 or 41.4%, 95% CI 0-0.07).

In summary, the low rate of proximal extension and recurrence at three months in both studies suggests that the rate of IGSVT propagation beyond the level of the calf veins is likely less than that of other calf vein thrombi.

1.8 Treatment of Venous Thromboembolism

1.8.1 Anticoagulation

The first anticoagulant to be used in clinical practice was heparin, a naturally occurring glycosaminoglycan. Heparin was first isolated from dog liver in 1916; this was found to demonstrate anticoagulant properties in vitro, and lead to excessive bleeding in animals (84). Heparin was first used in 1937 to prevent post-operative thrombosis, and in 1941 it was first used to treat arterial and venous thromboembolism.

UFH is removed by both saturable mechanisms (the reticuloendothelial system and endothelial cells), and non-saturable renal excretion. At low doses, UFH is mainly cleared by saturable mechanisms, whilst at high doses the non-saturable (renal) clearance becomes

important. UFH has a propensity to bind to plasma proteins, platelet factor 4, endothelial cells, macrophages and lipoproteins; all of which alter its bioavailability(85).

The advantages of unfractionated heparin (UFH) include its rapid onset and offset of action, its reversibility, and low renal clearance. However treatment doses of heparin need to be given by continuous IV infusion, and frequent monitoring of drug levels is required due to its narrow therapeutic window. Additionally, 2.6% of patients develop heparin-induced thrombocytopenia (HIT) (86), potentially leading to severe morbidity and mortality due to consumptive thrombocytopenia and thrombosis.

Heparin exerts its anticoagulant action via binding to antithrombin (AT), which converts the antithrombin from a slow to rapid inactivator of the coagulation factors thrombin (IIa) and Xa. The binding of heparin to AT is mediated via a pentasaccharide sequence. Low molecular weight heparins (LMWH) are derived from UFH, and are shorter polysaccharide sequences with a mean length of approximately 15 saccharide units, as opposed to 45 saccharide units with UFH. LMWH principally exerts its action via indirect activation of factor X (FX). Its advantages over UFH include greater bioavailability, subcutaneous administration, better correlation between dose and anticoagulant response (thus eliminating the need for routine monitoring), and lower risk of heparin-induced thrombocytopenia (HIT) and osteoporosis. Its disadvantages include lesser reversibility, prolonged half-life in patients with renal failure (the renal clearance of LMWH is 10-40%) and need for subcutaneous rather than oral administration, requiring once to twice daily injections.

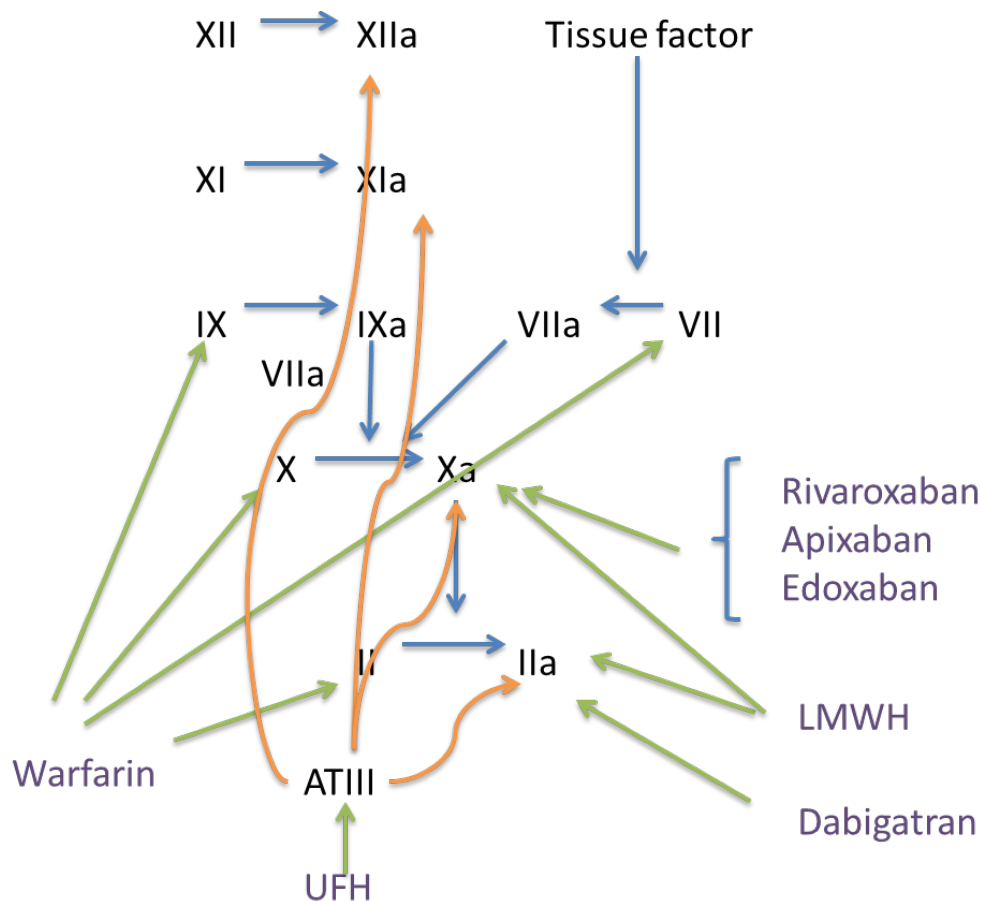


Figure 4: Site of action of anticoagulants

UFH = unfractionated heparin; ATIII degrades activated factors

The first oral anticoagulant (OAC) to be used in clinical practice was warfarin, which belongs to the vitamin K antagonist (VKA) class of drugs. Farmers first became aware of warfarin's effects in the 1920s, when previously healthy cattle in North America and Canada began dying of internal bleeding with no obvious precipitating cause (84). This was subsequently found to be due to moulds in damp hay, with the acquired coagulation disorder due to a prothrombin deficiency. The name warfarin is derived from the Wisconsin Alumni Research Foundation (WARF), the group that isolated the anticoagulation factor in 1941. Warfarin was initially used in 1948 to poison rats, leading to death from internal haemorrhage, and was subsequently used for anticoagulation in humans under the name 'coumadin'. Its high oral

bioavailability meant it was a more attractive option than heparin, and it soon became the widely used anticoagulant in the world.

Warfarin and other VKAs act by blocking the vitamin K epoxide reductase complex in the liver, leading to depletion of the vitamin K dependent clotting factors II, VII, IX and X, as well as the natural anticoagulant proteins, C and S. The epoxide reductase serves to recycle vitamin K between the reduced and epoxide forms (**figure 5**); it is the reduced form of vitamin K that is required as a cofactor for the gamma carboxylation of the vitamin-K dependent coagulation factors(87). Without gamma carboxylation, the vitamin-K dependent clotting factors are unable to bind calcium and phospholipid membranes.

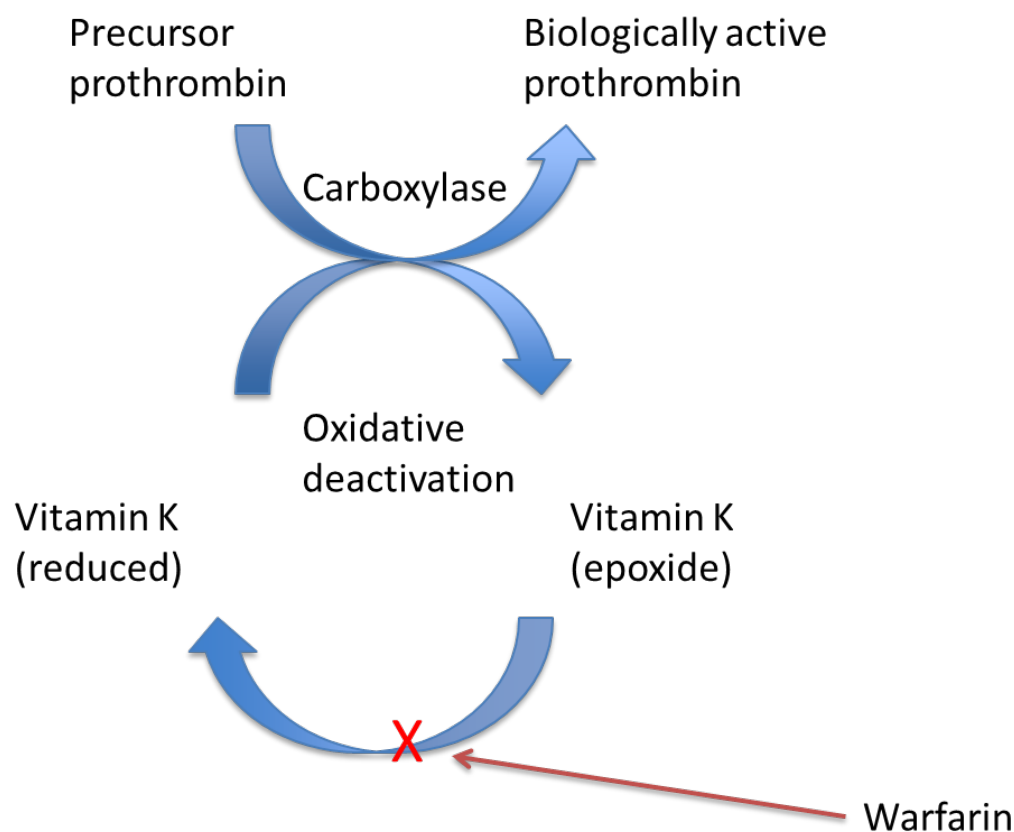


Figure 5: Warfarin Inhibition of Vitamin K

The effect of warfarin can be reversed by the oral or IV administration of vitamin K, with effects seen within six hours of administration. More rapid reversal (within minutes) can be achieved by the administration of prothrombin complex concentrates (PCCs), which are commercially available fractionated plasma products. PCCs are now widely used for the emergency reversal of VKAs in the setting of severe haemorrhage or need for urgent invasive procedures where adequate haemostasis is required.

The therapeutic effect of warfarin is measured by prolongation of the prothrombin time (PT), which measures the extrinsic pathway of anticoagulation. Initially, the lack of standardisation of the thromboplastins and methods of PT reporting lead to large discrepancies in the PT, and dosing of warfarin. The International Sensitivity Index (ISI) was therefore developed, which assesses the sensitivity of the thromboplastin and is used to calculate the International Normalised Ratio (INR). Reference materials for thromboplastins are available from the World Health Organization (WHO) and the European Commission.

The initial increase in INR during the first few days of warfarin therapy is not reflective of full anticoagulant effect, as the proteins with the shortest half-lives (VII, and the anticoagulant proteins C and S) are depleted first, leading to a transient procoagulant state. For this reason, patients must be treated with a heparin concurrently for a minimum of five days, and until the INR is therapeutic for two consecutive days(88).

Notable shortcomings of warfarin are the narrow therapeutic window, and the potential for multiple drug and food interactions, hence the need for frequent blood tests. The regular blood testing and clinician input for ongoing warfarin dosing is therefore resource intensive.

Unfortunately, the main risk of anticoagulation is bleeding. This entails an estimation of the risk-benefit balance for each patient when determining the length of anticoagulant therapy, in particular for those with unprovoked VTE, where the risk of VTE recurrence is substantial.

A meta-analysis of 33 studies (6) involving 4374 patient-years of VKA therapy showed that the rate of major bleeding was highest during the first 3 months of anticoagulation, 2.1% for the first three months, falling to 2.7 per 100 patient years thereafter. The case-fatality rate of major bleeding was 13.4% for the whole cohort, but fell to 9.1% when the sub-group of patients on VKA therapy for longer than 3 months was analysed. Similarly, the rates of intracranial bleeding were 1.48% during the first 3 months of VKA therapy, but fell to 0.65 per 100 patient-years after the first 3 months. This has been termed the ‘front-loading’

phenomenon (6), and is consistent with the identification of a group of patients prone to bleeding, who are more likely to develop this complication soon after the initiation of oral anticoagulation. Conversely, this is useful in identifying patients who are less likely to bleed should extended anticoagulation be indicated.

Factors that have been shown to increase the risk of bleeding include increasing age (particularly 75 years or above), concomitant use of antiplatelet agents, a history of bleeding, cancer, hypertension, cerebrovascular disease, diabetes, renal insufficiency, alcoholism and liver disease (89). In one cohort of patients treated with warfarin for a range of indications, the risk of bleeding increased by 1.5 fold for every 10-year increment in age (90). A range of prognostic scores have been designed in an attempt to predict the risk of bleeding in elderly patients with VTE, however these perform poorly when used in clinical practice (91).

In recent years, a number of direct oral anticoagulants (DOACS) have been developed for the treatment and prevention of VTE, and for stroke prevention in non-valvular atrial fibrillation. The commercially available DOACS include the direct thrombin inhibitor dabigatran, and the Xa inhibitors, rivaroxaban and apixaban. The advantages of these agents are minimal drug and food interactions and predictable dose responses, thus eliminating the need for regular blood test monitoring and dose adjustments(92), as well as minimal need for LMWH bridging.

The DOACS have varying degrees of renal clearance, and are thus contraindicated in patients with severe renal failure (creatinine clearance less than 25-30ml/min). Clinical trials have shown DOACS to be non-inferior to VKAs for treatment of VTE, with a significant reduction in the risk of intracranial and fatal bleeding; RR 0.37(95%CI0.45-0.83) and RR 0.36 (95%CI 0.15-0.84), respectively(92). There is no published data on the use of DOACs in patients with IDVT, as they were not included in the VTE trials, but it is reasonable to assume that patients with IDVT benefit similarly when treated with DOACS.

The effect of dabigatran can be reversed by the humanised monoclonal antibody fragment, idarucizumab, which binds dabigatran with high affinity and rapidly reverses its anticoagulant activity(93). Similarly, the recombinant modified human factor Xa decoy protein, Andexanet, binds and sequesters Xa inhibitors in the vascular space, leading to restoration of endogenous factor Xa activity and reduced levels of anticoagulant activity(94). This was shown to result in 'excellent or good' haemostasis in 79% of patients with acute major bleeding on a treatment trial (95). However it was associated with thrombotic events in

18% of patients during 30-day follow-up (96), and has been FDA-approved with a black box warning for thromboembolic events, including cardiac arrest and sudden death. More work is required in this field, with results from ongoing trials on alternative Xa-reversal agents awaited.

1.8.2: Duration of Treatment

The duration of anticoagulant therapy for VTE depends on the risk of recurrence versus the risk of bleeding. The risk of VTE recurrence is very low when provoked by surgery or trauma (less than 1% per year), but increases to approximately 4% annually for VTE provoked by risk factors such as pregnancy and the puerperium, and hormonal treatment (97). For those with permanent risk factors (e.g. prolonged immobilisation) and unprovoked proximal DVT and segmental PE, the risk of recurrence is approximately 11% after one year, 29% after five years and 40% after ten years (97) (98). For those with active cancer, the risk of recurrent thromboembolism is much higher, with a 12-month cumulative incidence of VTE of 20.7% (95% CI 15.6%-25.8%) (99). The risk of VTE recurrence after IDVT is approximately 2-4 fold lower than for proximal DVT (100) (101), with two recent studies showing annual recurrence rates of 2-3% (100).

A short course of anticoagulation (three to six months) is therefore indicated for proximal DVT and PE provoked by transient risk factors, as the risk of recurrent VTE is lower than the risk of bleeding on anticoagulation. For those with permanent risk factors such as active cancer, indefinite anticoagulation is generally recommended, unless the risk of bleeding is high enough to outweigh the risk of recurrence. Similarly, for a first idiopathic proximal DVT and PE, the 2016 American College of Chest Physician (ACCP) guidelines recommend extended anticoagulant therapy over a 3-month course for those with a low to moderate risk of bleeding (67).

For those with IDVT, the 2016 ACCP guidelines recommend treatment with a minimum of three months of anticoagulation, although it is also acknowledged that patients at high risk for bleeding, without serious symptoms or risk factors for extension, may instead benefit from serial imaging and withholding of anticoagulation unless the DVT extends. Suggested risk factors for extension were positive D-dimer, extensive thrombosis (>5cm in length, multiple vein involvement, >7mm maximum diameter), thrombosis close to proximal veins, no

reversible provoking factor, active cancer, history of VTE and inpatient status. Conversely, the 2013 International Consensus Statement (International Union of Angiology) advises a three-month course of oral anticoagulation for all patients with symptomatic IDVT (102).

However, given the risk of bleeding appears to outweigh the thromboembolic risk of IDVT, three months appears to be an excessive duration of treatment for patients with IDVT in the absence of ongoing risk factors, such as malignancy, immobilisation and pregnancy. Shorter durations of treatment have been examined in a number of studies, as outlined below.

The Treatment of Isolated Calf Thrombosis (TICT) study (103) treated patients with isolated distal deep vein thrombosis with twice daily subcutaneous (sc) administration of a full-dose of weight-adjusted low molecular weight heparin (LMWH) for one week, followed by a half dose of LMWH given once daily for three weeks. Only 2.9% of patients showed progression of thrombosis to proximal deep veins, and the majority of these occurred in patients with unprovoked (idiopathic) distal DVT.

Similarly, the DOTVAK (Dure'e Optimale Traitement AntiVitamines K) study (104) randomised patients with isolated calf vein thrombosis to six or twelve weeks of anticoagulation. The study was powered for equivalence. Permanent risk factors were present in 56.7% of patients in the six-week treatment arm as opposed to 43.8% in the 12-week arm; these included obesity, varicosity, heart failure, bedridden status, malignancy and congenital or acquired thrombophilia. Temporary risk factors were present in a similar proportion of patients in the 6-week and 12-week anticoagulation arms, at 68.3% and 69.7%, respectively. Temporary risk factors included surgery, trauma, lower limb plaster casts, puerperium, and immobilisation for medical conditions.

Recurrence rates were lower in patients with temporary as opposed to permanent risk factors. However, the two treatment regimens were equivalent, with VTE recurrence rates (comprised of proximal and distal DVT/PE) of 2% and 3.4% in the 6 week and 12 week groups, respectively.

A review of six randomised controlled trials (RCTs) and 25 observational cohort studies or case series(79) showed that overall, calf DVT propagation was reduced with anticoagulation. In those treated with surveillance only (i.e. no anticoagulation), the rate of propagation to the

level of the popliteal vein or higher was 8%. Overall the rate of major bleeding from the six RCTs ranged from 0-6%, with a reduction in bleeding risk over time.

Another meta-analysis assessing the efficacy and safety of anticoagulation therapy for adult patients with isolated calf vein thrombosis included two RCTs and six cohorts (126 patients treated with anticoagulation and 328 controls) (105). This showed that rates of PE (OR 0.12; 95% CI 0.02-0.77; $p=0.03$) and thrombus propagation (OR 0.29; 95% CI 0.14-0.62; $p=0.04$) were significantly lower in those receiving anticoagulation. Interestingly, when studies treating patients for >6 weeks versus <6 weeks were compared, there were no changes in pooled effect for PE, thrombus propagation or death. When only randomised trials were included, thrombus propagation was still significantly reduced by anticoagulation but PE rates were no longer significantly different between those receiving anticoagulation and those left untreated. All of these findings need to be interpreted with caution, however, due to small numbers of included subjects and poor methodologic quality of included studies.

The most recent randomised data comes from the Calf Vein Thrombosis Diagnosed by Ultrasonography (CACTUS) trial. This placebo-controlled trial randomised patients with a first isolated distal DVT to receive subcutaneous (sc) nadroparin 170 U/kg ($n=126$) or placebo ($n=133$) once daily for 42 days (106). The patients enrolled were low-risk outpatients, without active cancer or previous history of VTE. Patients with ongoing immobility (including lower limb plaster casts) were excluded. The primary efficacy outcome was a composite measure of extension of calf DVT to proximal veins, contralateral proximal DVT, and symptomatic pulmonary embolism at day 42 in the modified intention-to-treat population. Patients in both arms were prescribed compression stockings. Patients had a complete compression ultrasound performed at days 3-7 and 42, along with a clinical assessment, and then received 90 day telephone follow-up (**figure 6**).

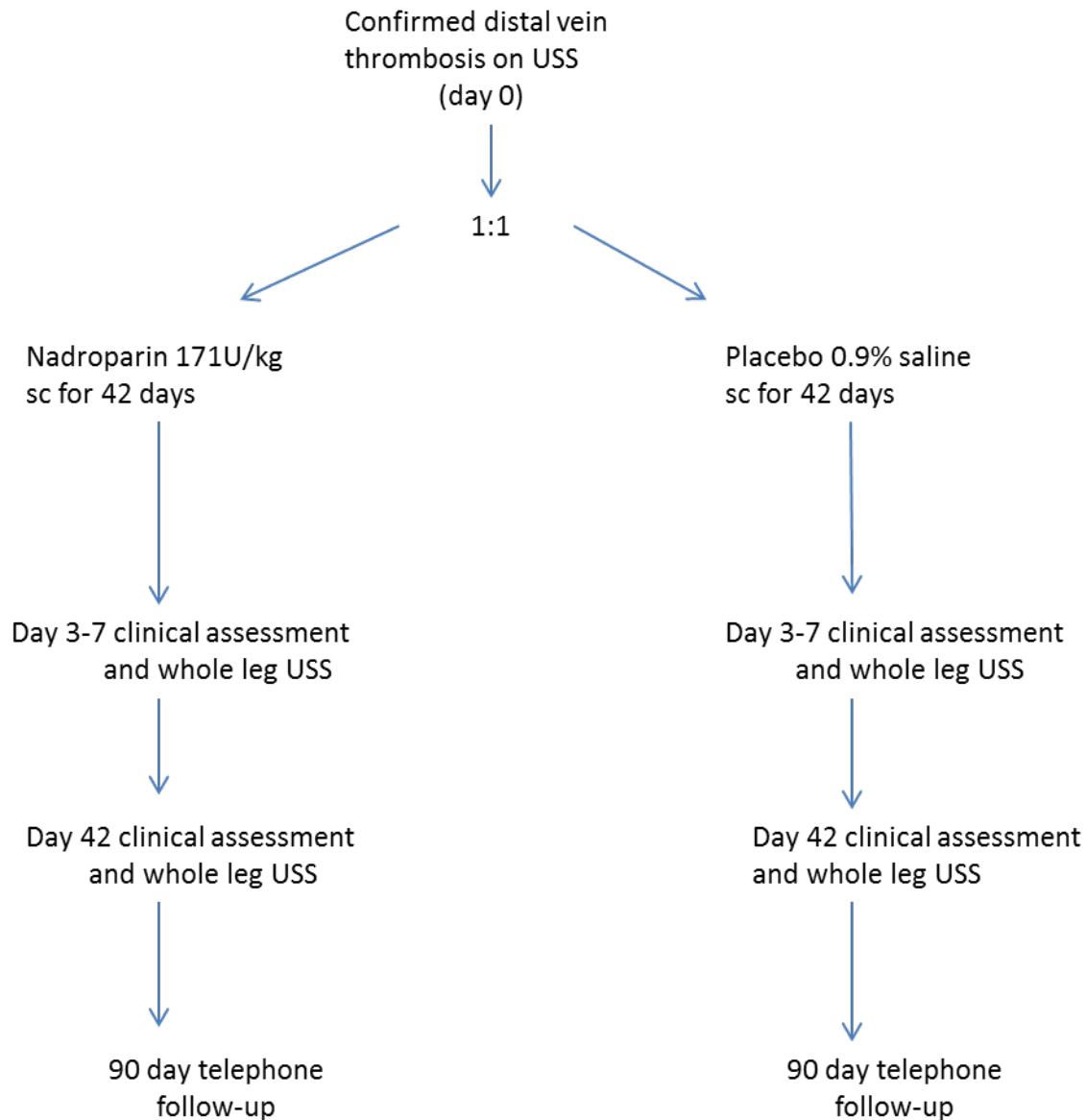


Figure 6: CACTUS study flow

No significant difference was seen between the nadroparin and placebo arms for the primary outcome by day 42 in the intention to treat analysis population (122 in the nadroparin group, 130 in the placebo group), with the composite primary outcome occurring in four patients (3%) in the nadroparin group and seven (5%) in the placebo group (risk difference -2.1%, 95% CI -7.8 to 3.5; $p=0.54$). However, nadroparin was associated with a significantly greater rate of major or clinically relevant non-major bleeding (4% vs 0.0%, $p=0.0255$).

Between day 42 and day 90 on the CACTUS study, there were no additional VTE events in the nadroparin group but one non-fatal PE in the placebo group, for a 90-day composite primary outcome of 3.3% in the nadroparin group and 6.2% in the placebo group. This difference was not statistically significant (absolute risk difference 2.9% [95% CI -7.8 to 3.5]); $p=0.28$), however this is possibly due to under-powering of the study to detect this difference.

Although not included in the composite primary outcome (CACTUS), there were also five (4%) patients in the placebo group diagnosed with an asymptomatic calf vein DVT on the study-mandated day 42 USS. A further two patients, one in each group, developed a new symptomatic calf vein DVT after completing their allocated treatment out to day 42.

The risk-benefit ratio appeared to favour observation in this low-risk group, although the rates of proximal extension and VTE recurrence are relatively high (3-5%), and it is possible that this study was underpowered to show a difference between groups. The numbers enrolled in each arm of the CACTUS study were relatively small at 122 in the nadroparin group and 130 in the placebo group, comprising only 50% of the pre-specified sample size.

Time to resolution of symptoms in each group was not reported, and it is possible that a shorter duration of treatment, in the order of 10-14 days, could be beneficial to shorten the duration of symptoms.

1.9 Post-Thrombotic Syndrome (PTS)

Post-thrombotic syndrome (PTS) is a chronic condition that develops in 20-50% of patients after deep vein thrombosis (107). Symptoms include pain, heaviness, swelling, cramps, itching or tingling of the affected limb. Signs include oedema, hyperpigmentation, venous eczema, telangiectasia, varicose collateral veins, lipodermatosclerosis and ulceration. It is likely secondary to damage to venous valves causing valvular reflux and residual venous obstruction by thrombus leading to impaired venous return. Both of these lead to increased venous pressure, resulting in reduced calf muscle perfusion and increased tissue permeability. The diagnosis is made clinically and is aided by clinical scales such as the Villalta PTS scale (108), a clinical scoring system based on PTS symptoms and signs (appendix 2a, 2b).

The VEINES-QOL/sym is a patient-reported questionnaire designed to assess quality of life and symptoms in patients with acute DVT. This consists of 26 items, covering symptoms (10 items), limitations in daily activities (9 items), time of day of greatest intensity (1 item), change over the past year (1 item), and psychological impact (5 items); with separate summary scores generated for symptoms (Veines-sym) and quality of life (Veines-QOL). This has been shown to be valid and reliable for use, and is a useful tool to allow rigorous, comprehensive evaluation of outcomes in clinical trials and epidemiological studies(109).

Risk factors for the development of PTS include increased body mass index (BMI), previous ipsilateral thrombosis, obesity, and older age(110, 111). Thrombophilic defects have not been found to be associated with PTS (112, 113) and one study (110) even showed a reduced risk of PTS with the presence of the FV Leiden or prothrombin mutations. There has also been an inconsistent association with patient-sex, with some studies showing an increased incidence in women. For example, Tick et al, in their large cohort of 1668 patients with DVT (112), showed that women had a 1.5-fold higher risk of developing PTS than men (95% CI 1.1-1.9). Conversely, a later study by the same author showed a higher risk of PTS in men (RR 1.4; 95% CI 0.98-2.2)(114). (Tick et al JTH 2010). Kahn et al (110) did not find an effect of gender on the development of PTS, although it should be noted that this cohort was substantially smaller (n=147, of whom 55 had evidence of PTS).

Other studies have also shown an association between sub-therapeutic anticoagulation with vitamin K antagonists and the development of PTS, with the REVERSE study showing that patients with a sub-therapeutic INR (less than 2.0 more than 20% of the time) in the first 3 months after a proximal DVT had an adjusted OR for developing PTS of 1.84 (95% CI 1.13–3.01) (115).

VTE recurrence has been shown to be higher in patients with PTS, with one study showing a cumulative probability of recurrent VTE of 7.4% in patients with PTS (at ≥ 18 months after diagnosis of DVT) as opposed to 1.6% in patients without PTS (RR 2.6, 95% CI 1.2-5.9).(116).

For many years, it was standard to prescribe below-knee elastic compression stockings (ECS) for two years after proximal DVT. This was based on the results of two randomised controlled trials showing that such a strategy reduced the rate of PTS by 50-60% (117, 118).

However, a more recent double-blind RCT (the SOX trial) challenged this, showing that graduated elastic compression stockings did not reduce PTS when compared with placebo stockings, and ECS are no longer considered standard treatment for PTS prevention(119).

Only a few studies have documented the subsequent development of PTS after distal DVT. Masuda et al., (78) followed 23 patients with IDDVT, and found that only 4% had developed hyperpigmentation and/or varicose veins at 3 years post diagnosis. Similarly, McLafferty et al (120) followed 37 patients with calf vein DVT for a mean of 3.4 years, and found few to have significant venous symptoms. Finally, Tick et al., (114) found that patients with proximal vein thrombosis had an increased risk of PTS when compared with patients with IDDVT (RR 2.3%, 95% CI 1.0-5.6). The rates of PTS in the IDDVT patients in the latter study (Tick et al, 2010) were surprisingly high (23%, compared with 55% in those with proximal DVT), however there were only 17 patients with IDDVT included in this study. This suggests that patients with distal DVT are less likely to develop PTS than patients who have had proximal DVT.

1.10 Conclusion

The above data suggest that the rate of development of proximal DVT in patients with isolated distal DVT is likely to be low regardless of whether no anticoagulation, or a short duration of anticoagulation of 4-6 weeks, is administered. Despite this, many patients in Australasia receive treatment for 3 months or longer. It is important to determine a safe duration of treatment for distal DVT, due to the ongoing risk of haemorrhage in those patients on anticoagulant therapy. This is of particular importance in Australia and New Zealand, where whole-leg CUS is employed, leading to the diagnosis of a significant number of IDDVT.

Anticoagulation therapy in patients with distal DVT may have benefits other than preventing early proximal extension, such as reducing duration of symptoms. The few studies that have documented the development of PTS following IDDVT suggest that this patient group is less likely to develop PTS than patients with proximal DVT (Masuda et al, McLafferty et al).

1.11 Research Proposal

We propose that a short duration of therapeutic dose anticoagulation for two weeks, thereby limiting the risk of haemorrhagic complications, may be of potential benefit in reducing symptoms and longer-term sequelae in patients with distal DVT, and will be associated with a low risk of progression or recurrence of DVT and the development of PE. To examine this hypothesis, we designed a prospective observational study of patients of distal DVT where the planned standard therapy was two weeks of therapeutic dose anticoagulant therapy. We also collected data to examine possible predictors of thrombus extension. An additional secondary outcome was the rate of post-thrombotic syndrome at 3 and 6 months.

Chapter 2: Materials and Methods

2.1 The TWISTER Study

The TWISTER (Two Weeks of Low Molecular Weight Heparin for Isolated Distal Vein DVT) study was a multicentre prospective observational study carried out at eleven sites in Australia and New Zealand. The primary objective was to determine the incidence of recurrent symptomatic venous thrombosis at 3 months in patients with isolated symptomatic distal DVT receiving initial treatment with 2 weeks of anticoagulant therapy.

Secondary objectives were to (1) determine the rate of resolution of symptoms in patients in isolated DVT, including the proportion of patients with complete resolution of symptoms after a two-week treatment period, (2) to determine the rate of post thrombotic syndrome at 3 and 6 months in individuals with isolated calf vein thrombosis; (3) to determine the incidence of proximal progression of distal DVT after 2 weeks of anticoagulant therapy; (4) to examine clinical predictors of extension or recurrence in patients with isolated distal DVT; and (5) to determine all-cause mortality.

The study was approved by the Ethics Committees of the participating centres.

2.2 Eligibility

2.2.1 Inclusion Criteria

We included consecutive patients presenting with symptomatic (up to 14 days since symptom onset), sonographically proven, provoked or unprovoked unilateral distal vein thrombosis (axial or muscular veins but not involving trifurcation or distal popliteal vein), with absence of symptomatic pulmonary embolism.

2.2.2 Exclusion criteria

Exclusion criteria were: age less than 18 years, more than 72 hours since diagnosis of DVT, DVT involving trifurcation or more proximal leg veins on imaging, prior DVT, active malignancy ie present at time of diagnosis, or on treatment, or treatment completed within 3 months, ongoing risk factors for propagation e.g. immobility, other indication for therapeutic anticoagulation (e.g. AF), active gastro-oesophageal ulceration or bleeding, other high risk for bleeding, platelet count $<80 \times 10^9/L$, severe renal impairment ($CrCl <30\text{ml/min}$), and pregnancy or within 6 weeks post-partum, concomitant superficial vein thrombosis, and bilateral distal DVT.

2.3 Treatment

The treatment consisted of therapeutic anticoagulation LMWH (enoxaparin) at a weight adjusted dose for an initial two week period. Rivaroxaban, a direct factor Xa inhibitor, became licensed for VTE treatment in Australia during the course of this study (from the end of 2012), and a subset of patients received a therapeutic dose of rivaroxaban instead for the same duration. The rivaroxaban dosing was as per treatment guidelines for proximal DVT/PE i.e. 15mg twice daily for two weeks, or 15mg twice daily for three weeks then 20mg once daily for those receiving longer durations of treatment.

2.4 Endpoints

The primary outcome was the symptomatic recurrence of venous thrombosis (DVT, non-fatal and fatal pulmonary embolism) within 3 months. Secondary outcomes were: asymptomatic proximal thrombus extension at 2 weeks; time course of symptom resolution including time to complete resolution of symptoms, and the proportion of patients with complete resolution at two weeks; all-cause mortality; post-thrombotic syndrome at 6 months, and predictors of recurrent or progressive DVT or new PE. Safety outcomes were bleeding (fatal, major, clinically relevant minor, according to ISTH definitions) and adverse effects of any study interventions which led to cessation of therapy.

2.5 Definitions

Distal vein thrombosis was defined as incompressible thrombus on ultrasound in infrapopliteal veins, i.e. involving one or more of: posterior tibial veins, peroneal veins, anterior tibial veins and muscular calf veins (gastrocnemius and soleal veins). *Extension of distal vein thrombosis* was defined as ultrasonographically-proven extension to involve the popliteal, femoral or iliac vein; or appearance of a proximal DVT in the contralateral leg. *Pulmonary embolism* was diagnosed in clinically suspected patients using widely accepted clinical criteria and either a high probability V-Q scan, positive CTPA, or invasive pulmonary angiogram demonstrating emboli. *Recurrent DVT* was defined as reappearance of thrombus in a previously involved vein, that was demonstrated to have resolved on subsequent compression ultrasound (C-CUS), or new thrombus in an anatomically different calf vein. The presence of *post-thrombotic syndrome* was assessed using the Villalta PTS scale (121).

Bleeding was classified as per ISTH criteria(122, 123), where major bleeding was defined as fatal bleeding, and/or symptomatic bleeding in a critical area or organ, such as intracranial, intraspinal, intraocular, retroperitoneal, intra-articular or pericardial, or intramuscular with compartment syndrome, and/or bleeding causing a fall in hemoglobin level of 20 g L^{-1} or more, or leading to transfusion of two or more units of whole blood or red cells. Clinically relevant non-major bleeding (CRNMB) was defined as bleeding that did not meet the criteria for major bleeding, but that required nonsurgical, medical intervention by a healthcare professional. All other bleeding was classified as minor.

2.6 Study Flow (figure 7)

The study opened for recruitment on 29 October 2010, and will close to recruitment after 31 December 2018. Patients were enrolled in the study as soon as possible after the diagnosis of isolated distal DVT. Informed consent was obtained in all patients prior to the initiation of any study procedures. All patients were treated with therapeutic anticoagulation in the form of therapeutic dose enoxaparin (1.5mg/kg once daily or 1mg/kg twice daily) or oral rivaroxaban (15mg twice daily) for an initial two week period. Compression stockings were able to be used (and their use recorded) at the discretion of the investigator. Patients were

asked to record symptoms of DVT, namely pain and limitation of activity, daily in a diary for each day they were on anticoagulation. Pain was assessed using a visual analogue scale (VAS) and functional impairment was documented using an adapted version of the VEINES-sym scale (**Appendix 4**). Symptoms were documented until complete resolution. Patients were instructed to return for review if at any stage they experience worsening or new symptoms.

Potential prognostic factors for proximal extension of thrombus were collected at the baseline visit. These included age, weight and height, location of thrombus, whether the thrombus was provoked or non-provoked, risk factors, and family history of VTE. Patients enrolled in the study had a D-dimer performed at diagnosis. A proportion of patients had their D-dimer recorded at 2 weeks, and then at 6 weeks if they received 6 weeks of anticoagulation (D-dimer sub-study).

Patients were reviewed by study investigators at 2 weeks, and undergo a repeat C-CUS of the affected limb. Patients were then managed according to ultrasound appearance and symptom resolution as follows:

- (a) If there was thrombus extension into the proximal veins on the 2 week C-CUS, patients continued anticoagulation with the duration at the discretion of the investigator;

- (b) If there were ongoing symptoms with unchanged appearances on C-CUS, patients received anticoagulation for a further 4 weeks. Patients continued to record symptoms of DVT daily until complete resolution. Patients were re-evaluated at the 6-week mark, and a repeat ultrasound was performed. If symptoms had completely resolved, anticoagulation was stopped. Patients with persistent symptoms or proximal extension on USS were managed independently of the study;

- (c) If symptoms had completely resolved at 2 weeks and there was no change or regression of thrombus on C-CUS, then anticoagulation was stopped.

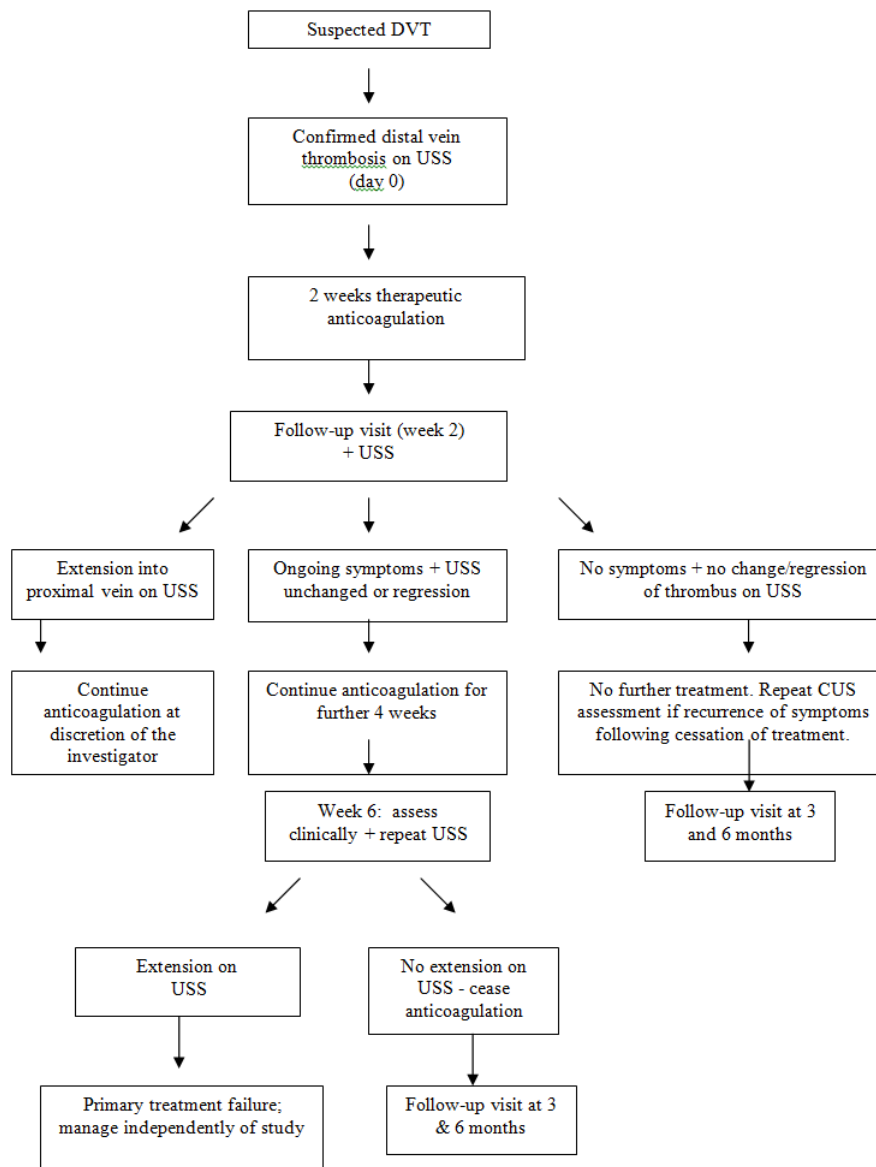


Figure 7: Study Flow

All patients completing 2 or 6 weeks of therapy were followed for objectively proven recurrent symptomatic DVT or PE for 6 months. Patients were reviewed at 3 and 6 months to confirm that there have been no episodes of recurrent VTE during this time period, and to assess for post-thrombotic syndrome using the Villalta PTS scale (**Appendix 2a**). Functional impairment was documented using the adapted version of the VEINES-sym scale.

2.7 Recruiting Centres

Over the course of the study, eleven centres opened for recruitment; four in New Zealand and seven in Australia. The Australian sites were: Monash Medical Centre (Victoria), Box Hill Hospital (Victoria), Frankston Hospital (Victoria), Prince of Wales Hospital (NSW), Concord Hospital (NSW), St George's Hospital (NWH), and Westmead Hospital (NSW). The New Zealand sites were: North Shore Hospital (Auckland), Middlemore Hospital (Auckland), Auckland Hospital and Christchurch Hospital.

2.8 Statistical Analysis

We pre-determined a 1% rate of recurrence out to 3 months of follow-up amongst those patients who received 2 weeks of treatment, and who then became symptom free and had no evidence of extension on ultrasound at 2 weeks. We predicted that 65% of patients would go into the experimental (2-week) treatment arm, 20% into the 6-week arm, and that 15% would have asymptomatic proximal vein extension on the 2-week ultrasound. On the basis of this, a sample size of 330 patients was calculated, estimated to exclude a 4% upper limit on the 95% confidence interval (CI) for VTE recurrence at 3 month follow-up.

This sample size was based on the assumption that the patients would fall into three groups as below:

- (a) Extension group - 15% of all the enrolled patients will experience proximal extension on USS at 2 weeks (as reported in the literature)
- (b) Asymptomatic group - 65% will be asymptomatic at 2 weeks. 10% of this arm is anticipated to be lost to follow-up or need anticoagulant therapy in the study arm.
- (c) Symptomatic group - the remaining 20% will therefore require ongoing therapy

This would give us an estimated sample size of 200 patients in the **asymptomatic group** with no symptoms and no change/regression of thrombus on USS. The sample size calculation for this arm with proportions and 95% CI are provided below.

Sample size	Event rate	Proportion and 95% CI	99%CI
100	1/100	1.0 (0.03-5.5%)	(0.06-7.2%)
	2/100	2.0 (0.2-7%)	(0.1-8.9%)
	3/100	3.0 (0.6-8.5%%	(0.3-10.6%
150	1/150	0.6 (0.02-3.7%)	(0.006-4.9)
	2/150	1.3 (0.16-4.7)	(0.07-6%)
	3/150	2.0 (0.5-5.7%)	(0.2-7.1%)
	4/150	2.6 (0.7-6.7)	(0.4-8.2%)
175	1/175	0.6 (0.02-3.1%)	(0.0006-4.2)
	2/175	1.1 (0.1-4.1)	(0.05-5.2)
	3/175	1.7 (0.4-4.9%)	(0.2-6.1)
	4/175	2.3 (0.6-5.7%)	(0.3-7.0%)
200	1/200	0.5 (0.02-2.8%)	(0.006-3.7%)
	2/200	1.0 (0.12-3.6)	(0.05-4.6%)
	3/200	1.5 (0.3-4.3%)	(0.1-5.4%)
	4/200	2.0 (0.5-5.0%)	0.3-6.2%)
	5/200	2.5 (0.8-5.7%)	(0.5-6.9%)
250	2/250	0.8 (0.09-2.9%)	(0.04-3.6)
	3/250	1.2 (0.2-3.5%)	(0.1-4.3)
	4/250	1.6 (0.4-4.1)	
	5/250		

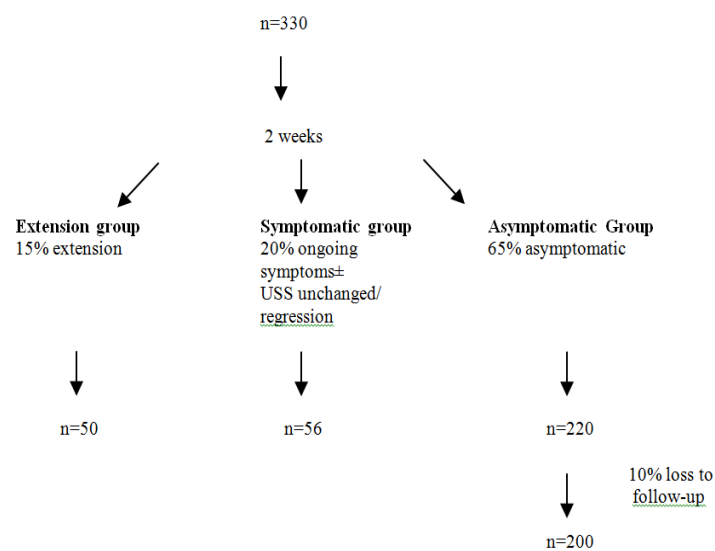


Figure 8: Sample size calculation for Study Arm

2.9 Adjudication of VTE recurrence/extensions

Ultrasounds were adjudicated centrally for all reported extensions (within the distal venous system, or extensions into proximal venous system) and VTE recurrences within 3 months of enrolment on the study. In an effort to reduce bias, de-identified ultrasounds from 10% of patients without reported events were also sent for adjudication.

Three adjudicators were asked to review the de-identified scans and reported independently of each other. The three adjudicators were also blinded as to which ultrasounds were from patients deemed to have had “events” and which were from the control patients. The adjudicators then supplied reports to the steering committee. These reports were collated and sent to the Data Safety Monitoring Board (DSMB), comprised of three members.

It should be noted that although it was intended that all ultrasounds were de-identified, not all centres were able to do this. However adjudicators were blinded to patients. Some Australian centres could not supply ultrasounds for the event adjudication for the second interim analysis, due to difficulty obtaining these from private providers. In addition, only one adjudicator was able to supply a report for the second analysis (second 100 patients).

Chapter 3: Interim Analysis

3.1 Pre-adjudication

An interim analysis was performed once the first 100 patients had been enrolled, and the results are summarised in the consort diagram below. The ultrasounds from subjects who were deemed to have had events (proximal or distal thrombus extension, VTE recurrence) were sent for adjudication to three independent adjudicators. The adjudicators were also sent a random sample of ultrasounds from 10% of those subjects deemed not to have had events. Where agreement could not be reached by all three adjudicators, then the consensus decision of two of the adjudicators was recorded.

The events sent for adjudication were: 2 thrombus extensions into the proximal lower leg veins (occurring on treatment), and 8 thrombus extensions within the distal leg veins, with all but one (a distal extension) occurring whilst patients were receiving their initial two weeks of full dose anticoagulation. There were also two cases of VTE recurrence within 3 months, both distal DVT.

3.2 Post-adjudication

There was agreement on the two distal DVT recurrences and two proximal extensions occurring on treatment (i.e. occurring on full dose anticoagulation); however it was adjudicated that three of the distal extensions on treatment (anticoagulation) had either unchanged/resolved thrombus on ultrasound or partial resolution. One of the control ultrasounds was also deemed to have distal extension at the 2-week time point, and no change at the 6-week time point. On review of the original report, this was indeed the case, and it appears the investigator had documented this as ‘partial resolution’ rather than the radiologist. This patient had reduced symptoms at the time of the 2-week ultrasound, and received a further 4 weeks of treatment as per protocol due to ongoing symptoms (group B).

Subsequent to the analysis, it was discovered that one of the ‘distal extensions’ sent for adjudication was a mistake, with the report showing unchanged thrombus; this was one of the ultrasounds that was adjudicated as showing unchanged thrombus at week 2.

Of the remaining 9 control scans, there was agreement between at least two of the three adjudicators that the original scan reports were correct.

Therefore there were six distal extensions on treatment, and two proximal extensions on treatment (**figure 9**)

The distal DVT recurrence rate was 2/77 (2.6%; 95%CI 0.3-9.1%) in those stopping anticoagulation at 2 weeks (group C). There were no episodes of proximal DVT or PE recurrence.

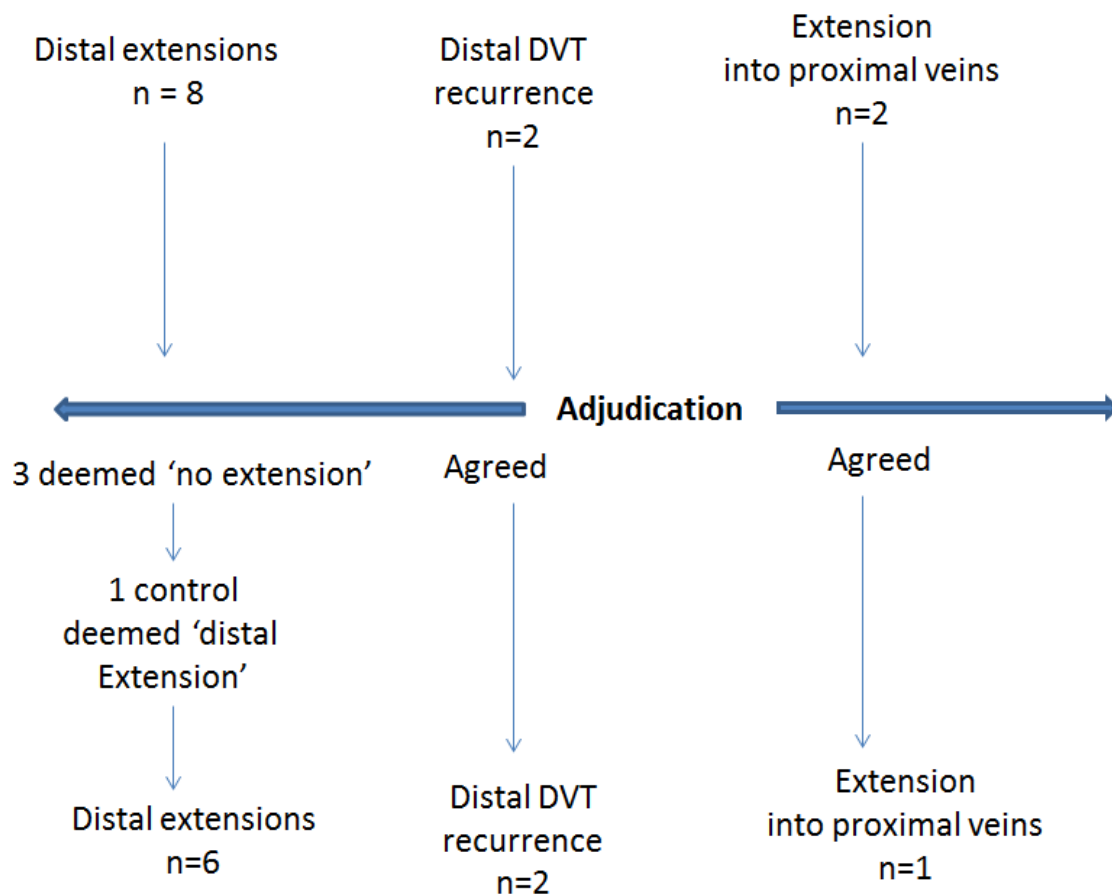


Figure 9: Adjudication for Interim Analysis 1

*CUS: complete compression ultrasound

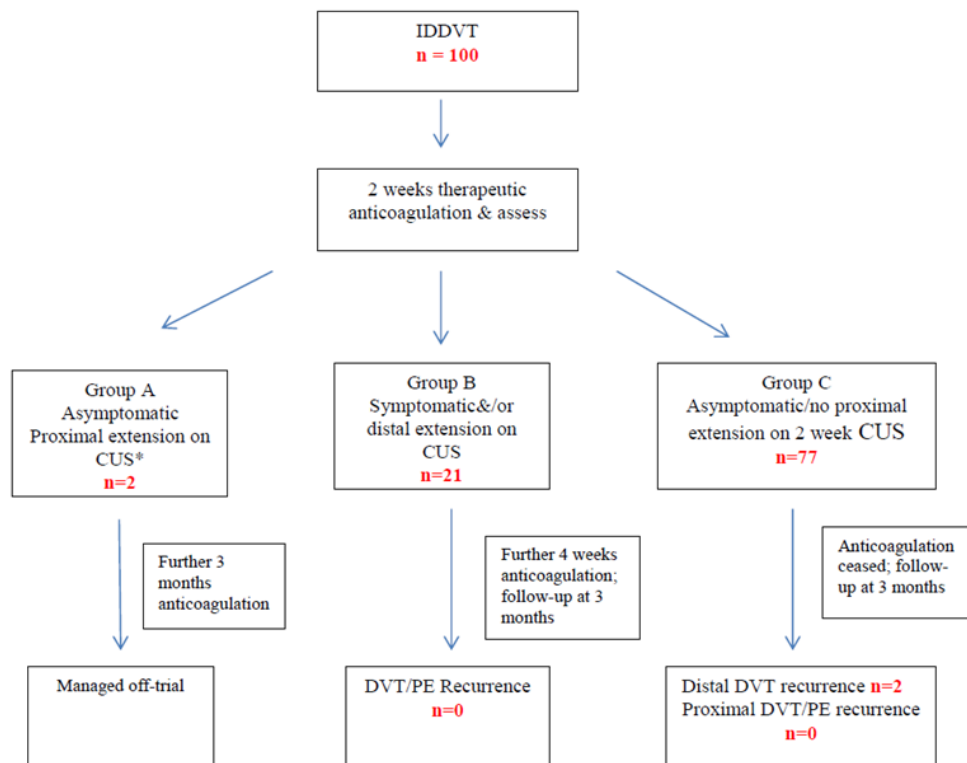


Figure 10: Consort Diagram for First 100 Patients

3.3 Post-Thrombotic Syndrome (PTS)

Six month follow-up data was available for 95 patients. The proportion of patients with PTS was 9/95 (9.5%), (95% CI 5-18%); all cases were mild on the Villalta scale. The incidence of PTS increased over time, present in five patients at 3 months and seven at 6 months. Sixty-seven percent of patients with PTS were males, compared with 45% of the total cohort (p-value NS). Fifty-six percent (5/9) of those with PTS had unprovoked DVT, compared with 35% of patients without PTS (NS). Residual thrombosis was present in 6/9 (67%) PTS patients compared with 83% in the total cohort.

3.4 Bleeding events

There were fourteen episodes of bleeding on treatment, twelve minor and two clinically relevant non-major bleeds (CRNMBs). One of the CRNMBs (a 7 cm diameter abdominal

wall haematoma at the site of enoxaparin subcutaneous injection) necessitated cessation of treatment; the other CRNMB (a 10cm possibly traumatic chest wall haematoma) necessitated halving of the enoxaparin dose. There were no episodes of major bleeding.

3.5 All-cause mortality

There were no deaths reported within the six month follow-up period.

3.6 Conclusion

77% of patients were able to be treated with 2 weeks of anticoagulation, with a 2.6% rate of distal VTE recurrence, and no proximal DVT/PE recurrences within 3 months.

3.7 Data safety monitoring board

The outcome of the interim analysis was supplied to the DSMB, comprised of three members. No concerns were identified, and continuation of the trial was approved.

3.8 Post-interim analysis

After the interim analysis and discussion with members of the TWISTER steering group and study statistician, it was decided to amend the sample size due to 77% of patients going into the experimental arm (2 weeks of anticoagulation, group C) rather than the predicted 65% at study initiation. Therefore recruitment can cease when 200 patients have been enrolled in the experimental arm (2 weeks therapeutic anticoagulation i.e. group C) or when a total of 330 patients have been recruited (as initially predicted). A protocol amendment was approved by Ethics Boards in Australia and NZ in 2015.

We also reviewed the definition of the primary outcome of recurrent VTE, that is, symptomatic recurrence of **proximal** venous thrombosis (**proximal** DVT, non-fatal and fatal pulmonary embolism) within 3 months, rather than venous thrombosis inclusive of calf vein DVT, as the former is accepted as having greater clinical significance. After discussion with

the statistician, it was decided that the primary outcome should not be amended. However, this will be included as an additional analysis when the data is analysed.

Chapter 4: Results

A second interim analysis was commenced in March 2017; at this time a further 102 patients had completed six-month follow-up. One patient from the first 100 has been removed from the final analysis, as this patient was discovered to have been enrolled with a thrombus involving the superficial veins only (subject 3004), with no involvement of the deep calf veins.

4.1 Baseline Demographics

Baseline demographics were available for 200 patients (**table 2**). Records were not available on one patient lost to follow-up prior to the two-week visit.

Table 2. Baseline demographics for the first 200 patients

Age; median (IQR)	51 (27)
BMI; median (IQR)	28.5 (7.3)
Men	99 (50%)
Ethnicity	
White	168 (84%)
Asian	11 (5.5%)
Black	1 (0.5%)
Maori	3 (1.5%)
Pacific Island	2 (1%)
Other	8 (4%)
Not stated/recorded	7 (3.5%)
Unprovoked	63 (31%)
Risk Factors	
Family history VTE	23 (12%)
Systemic estrogen therapy	25 (13%)
Immobilisation	43 (22%)
Air Travel >4 hours	46 (23%)
History venous insufficiency/ varicose veins	29 (15%)

IQR = interquartile range

4.2 Distribution/Characteristics of IDVT

Table 3 shows clinical characteristics and details of treatment. Forty-six percent of patients had thrombus confined to the muscular veins only, with the remainder having thrombus involving axial +/- muscular veins (**figure 11; table 3**). One patient did not have the site of thrombus recorded.

Table 3: Clinical characteristics of the first 200 patients

Characteristic	n (%)
Left leg	106 (53)
Bilateral	2 (1)
Veins involved	
Muscular vein only	91 (45.5)
Gastrocnemius	64 (32)
Soleal	23 (11.5)
Gastrocnemius + soleal	4 (2)
Axial +/- muscular vein	108 (54)
Unknown	1 (0.5)
Signs/symptoms	
Swelling	52 (26)
Pain	80 (40)
Erythema	16 (8)
Treatment	
Rivaroxaban	67 (33.5)
LMWH	132 (66)
LMWH/warfarin	1 (0.5)
Concomitant aspirin	18 (9)

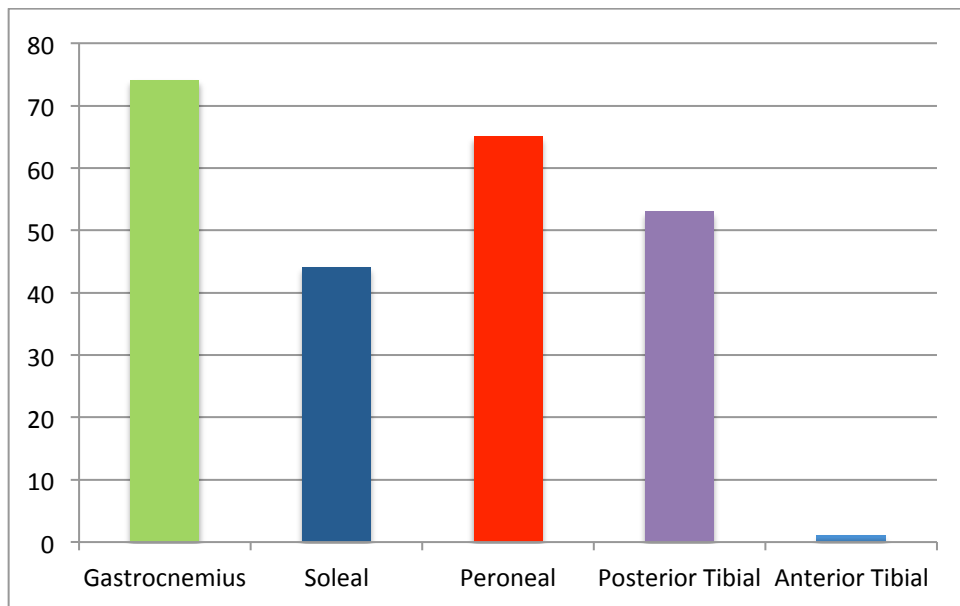


Figure 11: Distribution of IDDVT

4.3 Compression Stockings

The use of compression stockings was recorded for 106 of 199 patients (**table 4**).

Compression stocking use was only recorded for one of three patients in group A; therefore statistical comparisons were only attempted for differences between groups B and C, as any comparisons with group A were unlikely to be meaningful.

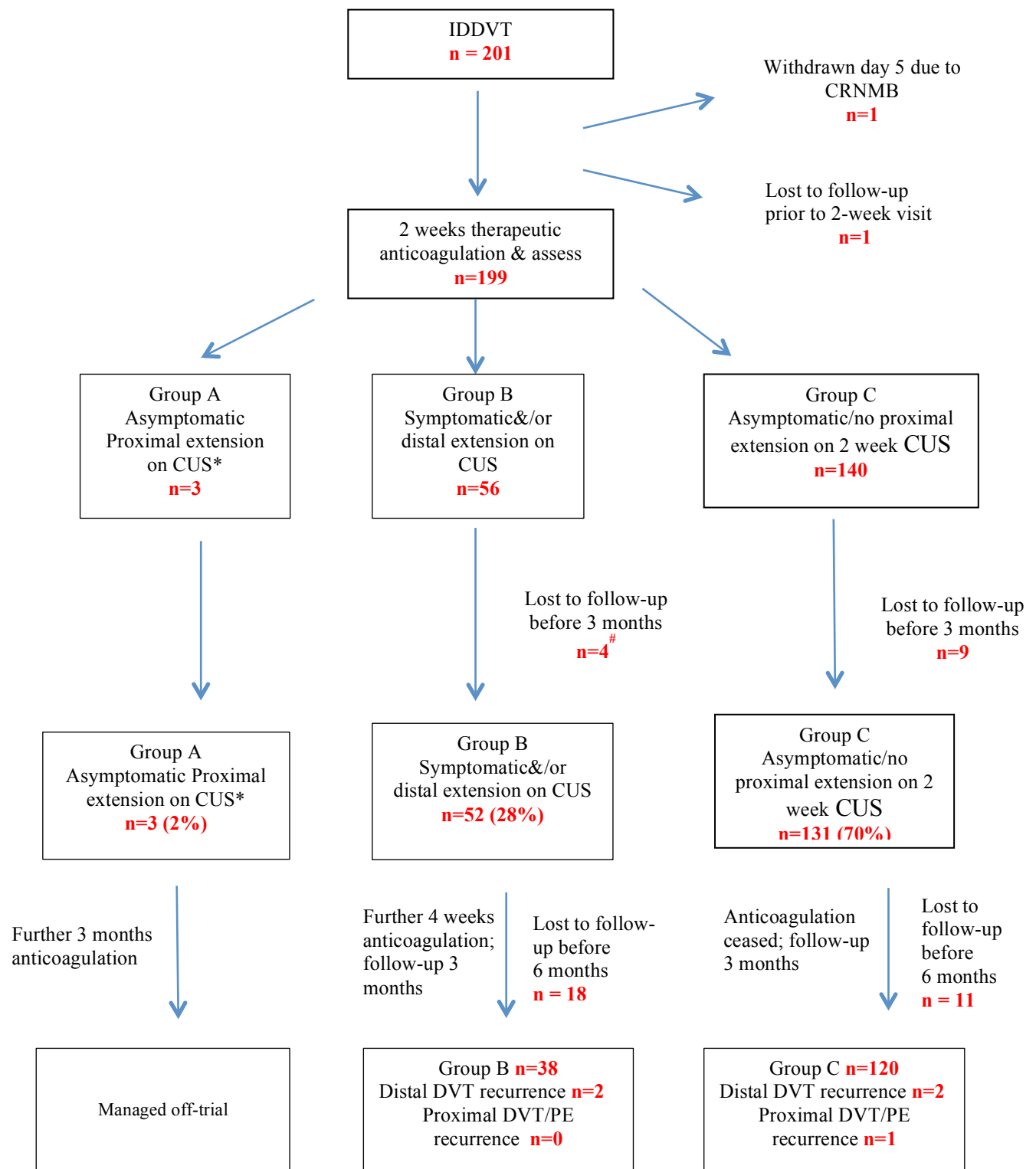
There were no significant differences in stocking use between treatment groups B and C at baseline, two weeks or three months. There was a difference in compression stocking use between groups B and C at six months (40% and 12%, respectively), which only just reached significance ($p=0.049$).

Table 4: Compression Stocking Use Over Course of Study

Compression Stocking	n (%)	p
Baseline, n=106	22 (21)	
Group C, n=77	13 (17)	0.205
Group B, n=28	9 (32)	
Two Weeks, n=105	25 (24)	
Group C, n=73	11 (15)	
Group B, n=32	14 (44)	
3 Months, n=72	16 (22)	
Group C, n=50	9 (18)	0.226
Group B, n=22	7 (32)	
6 months, n=49	10 (20)	
Group C, n=34	4 (12)	0.049
Group B, n=15	6 (40)	

4.4 Two-Week Visit and Treatment Allocation

The consort diagram on the following page summarises the allocation of patients to the three treatment groups, and outcomes at three months.



*CUS: complete compression ultrasound

#: Includes one distal extension incorrectly assigned to group A

Figure 12: Consort Diagram

One of the patients was withdrawn on day 5 due to CRNMB, and one was lost to follow-up before the two-week visit. Of the remainder, 140 were allocated to 2 weeks anticoagulation (**group C**), 56 to 6 weeks anticoagulation (**group B**) and three had treatment extended to three months or longer due to extension into the popliteal vein (proximal extension) on the 2-week ultrasound (**group A**). One patient with distal extension on the two-week ultrasound was also incorrectly assigned to group A and discharged from the study by the investigator.

Thirteen patients were lost to follow-up before three months, including the patient incorrectly assigned to group A. Of the remaining 186 patients, 131 patients received two weeks of anticoagulation (**group C, 70%**); 52 received six weeks anticoagulation (**group B, 28%**) and three (**group A; 2%**) received at least 3 months anticoagulation due to proximal extension on the 2-week ultrasound.

Figure 13 shows treatment allocation by site. Sites enrolling higher numbers of patients (27-50 patients per site) tended to allocate more patients to the two-week anticoagulation arm ($\geq 70\%$), compared with sites enrolling lower numbers of patients.

There was no significant difference in the proportions of subjects allocated to the two-week treatment arm (**group C**) when comparing the LMWH versus rivaroxaban groups, 97/131 (74%) and 43/67 (64%) in the LMWH and rivaroxaban groups, respectively ($p=0.1866$).

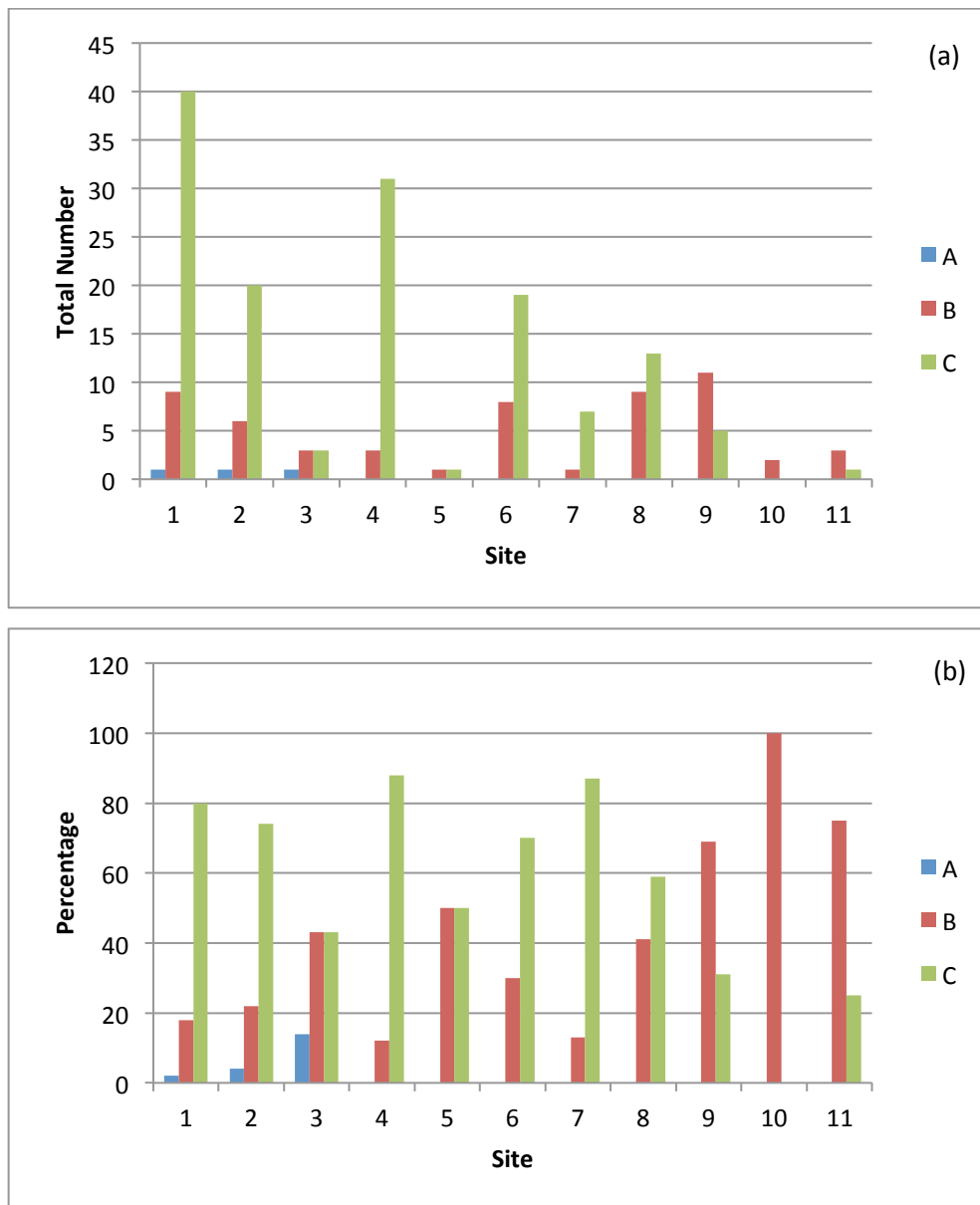


Figure 13: Treatment Arm by Site (a) Total Numbers (b) Percentage in Each Arm

4.5 Adjudication (Second analysis)

The events reported in the second 100 patients are as summarised in **figure 14**. Nine scans on patients deemed not to have events (control scans) were also requested.

Ultrasonounds could not be provided for five distal extensions and a recurrent distal DVT/superficial vein thrombus; the latter was diagnosed at the three month visit. In addition, three of the ‘control’ scans requested could not be provided. Therefore scans were provided

on four distal extensions, one distal extension with subsequent distal recurrence, and one proximal extension; as well as seven control scans.

The adjudicator deemed that two distal extensions in fact showed partial thrombus resolution but was in agreement with all other reports. Of the control scans, the adjudicator deemed that one USS deemed as showing partial resolution actually showed thrombus extension with the distal veins. As in the first interim analysis, this subject was therefore added into the group of patients with distal extension. Of the remainder, the adjudicator agreed that there was no evidence of distal or proximal extension.

For the USS scans that could not be provided, the final result was recorded as per the ultrasound report.

Therefore for the second adjudication, there were eight distal extensions on treatment (one of whom also had a distal recurrence), one proximal extension on treatment, two distal recurrences and one asymptomatic proximal DVT recurrence on an unscheduled ultrasound. The combined results of adjudication 1 and 2 are summarised in **figure 15**.

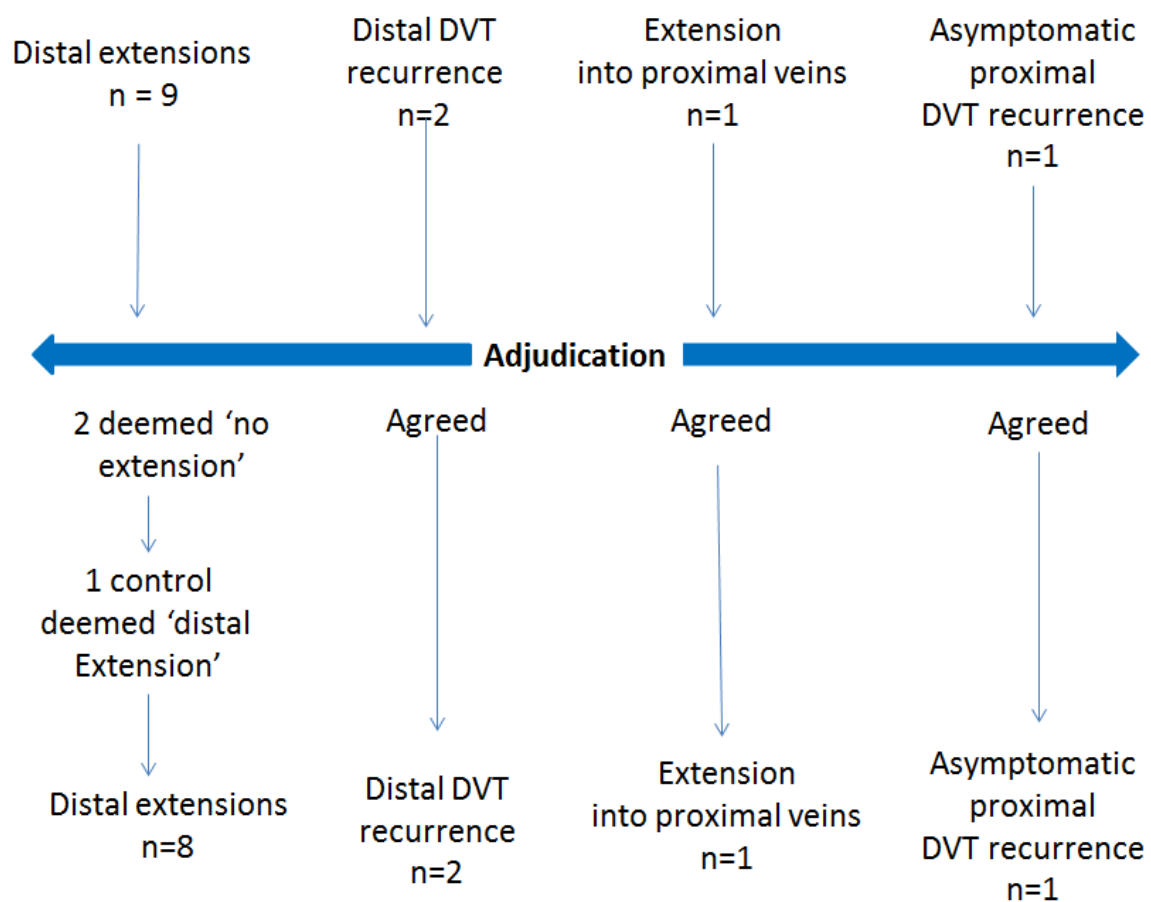


Figure 14: Adjudication for Second Interim Analysis

*For ultrasounds performed at 2 weeks, unless otherwise stated

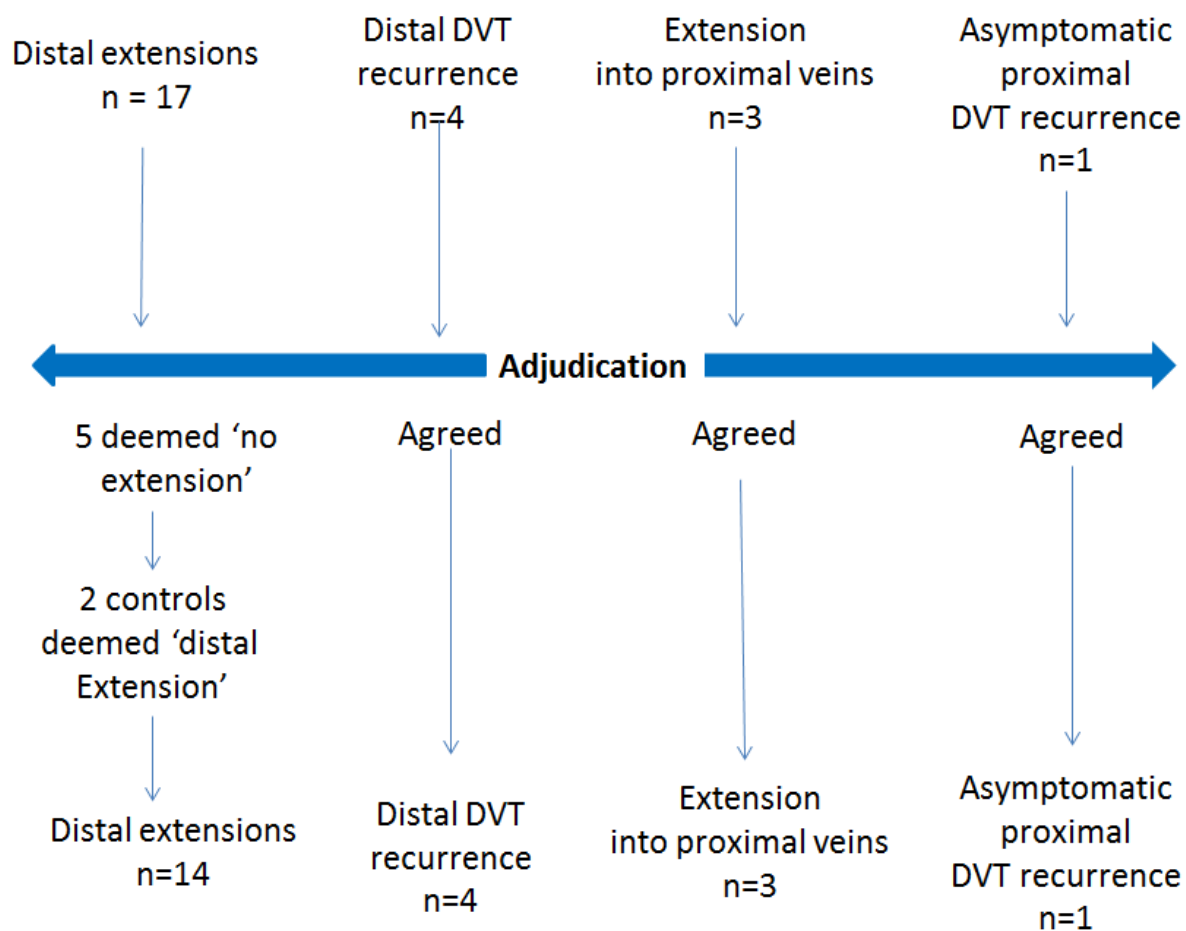


Figure 15: Adjudication for First 200 Patients

*For ultrasounds performed at 2 weeks, unless otherwise stated

4.6 Primary Outcome

The primary outcome was the symptomatic recurrence of venous thrombosis (DVT, non-fatal and fatal pulmonary embolism) within 3 months. Two patients in the experimental arm (those receiving two weeks of anticoagulation, **group C**) had symptomatic VTE recurrence. Both of these were distal DVT. One patient in the experimental arm also experienced an asymptomatic proximal recurrence, detected on an unscheduled ultrasound two months after stopping enoxaparin at the 2-week follow-up visit (i.e. within the 3-month follow-up period). This does not meet the primary outcome definition (as it was not symptomatic) and is therefore not included in the primary outcome. The rate of VTE recurrence is therefore 2/131, or 1.5% (95% CI 0.19-5.41%).

4.6.1 Symptomatic Distal DVT Recurrences in Experimental Group (C)

- (1) The first patient had a provoked peroneal and posterior tibial DVT in the context of a recent tibial plateau fracture. The patient received two weeks of Clexane; the 2-week ultrasound showed partial resolution of thrombus and treatment was stopped. The patient developed new symptoms in the ipsilateral leg four weeks later and a repeat ultrasound showed new thrombus in the soleal and gastrocnemius veins. The patient was reported to have been mobile at the time of distal progression.
- (2) The second patient was treated with two weeks of enoxaparin for an unprovoked gastrocnemius vein DVT. The 2-week ultrasound showed partial resolution of thrombus. Two months later, the patient developed new symptoms in the contralateral leg, and an ultrasound showed a gastrocnemius vein thrombus.

4.6.2 Asymptomatic Proximal DVT Recurrence

This subject was diagnosed with an unprovoked left peroneal vein DVT, and was treated with enoxaparin for two weeks. The subject was asymptomatic at the two week visit, and the enoxaparin was stopped. The ultrasound at this time point showed partial resolution of the peroneal vein thrombus. The investigator elected to repeat the ultrasound of the leg four weeks after enoxaparin was stopped to assess for thrombus resolution, which is not mandated by protocol. The patient was asymptomatic at this time. The repeat ultrasound showed that

the peroneal vein thrombus had resolved, but a non-occlusive thrombus was now present in the left posterior tibial vein.

The subject was left off anticoagulation. A repeat ultrasound five weeks later showed non-occlusive echogenic thrombus in the posterior tibial, peroneal and popliteal veins, with no acute thrombus seen. The subject was still asymptomatic at this time point. The subject remained off anticoagulation, did not suffer any further recurrences, and remained asymptomatic at the six-month follow-up.

4.7 Secondary Outcomes

4.7.1 Asymptomatic Proximal Thrombus Extension at Two Weeks

Three patients had extension of thrombus into proximal veins on the two-week ultrasound, out of a total of 199 patients, giving a rate of $3/199 = 1.5\%$ (95% CI 0.31-4.34%).

- (1) The first patient had a minimally provoked gastrocnemius and soleal vein thrombus, in the context of reduced mobility over the preceding six weeks due to depression. The patient was commenced on rivaroxaban. The ultrasound at two weeks showed occlusive thrombus in the gastrocnemius and soleal veins as previously, but with extension into the **popliteal and distal superficial femoral veins**. There is no documentation on whether the patient was still symptomatic at this time point. The patient was commenced on warfarin, and was lost to follow-up after this time point.
- (2) The second patient presented with an idiopathic posterior tibial vein DVT, and was commenced on enoxaparin. An ultrasound at two weeks showed thrombus in the posterior tibial, peroneal and **popliteal veins**, along with short segments of thrombus in the gastrocnemius and soleal veins. Despite this, the patient reported that the swelling in her leg had reduced, and the pain had completely resolved. She was commenced on warfarin, with a plan for indefinite anticoagulation.
- (3) The third patient was enrolled after diagnosis of a minimally provoked gastrocnemius vein thrombus in the context of long distance air travel, along with thrombus in

several varicose veins attached to the long saphenous vein. The patient was commenced on enoxaparin. The two-week ultrasound showed propagation of the thrombus into the **distal popliteal vein**. Despite this, the patient reported that their pain had completely resolved, apart from mild aching in the calf after standing for long periods. The patient was treated with warfarin for six months.

4.7.2 Time Course to Symptom Resolution

Days to symptom resolution was recorded for 165 patients. For 35 patients this was not recorded. The majority of patients, 159 (80%), had complete resolution of symptoms at two weeks; 137 in the two-week treatment group (C), two in the proximal extension group (A), and twenty in the six-week treatment arm (B).

Four patients had ongoing symptoms at the time of the six month follow-up visit, of whom three had documented PTS. In the remaining 165 patients, the mean days to symptom resolution was 16. This is likely to be an overestimate, as many patients did not have their exact day of symptom resolution recorded and it was therefore documented as follows: e.g. if the patient was recorded as having symptoms present at day 14, but was then recorded as being symptom-free at day 42, the day of symptom resolution was recorded as day 42.

The mean VAS pain score at enrolment was 4 out of 10; the mean Veines-sym score was 10 (out of a maximum of 20 for patients with no functional impairment i.e., the higher the Veines-sym score, the better the function for an individual patient).

Figure 16 shows scores by treatment group over the course of the study.

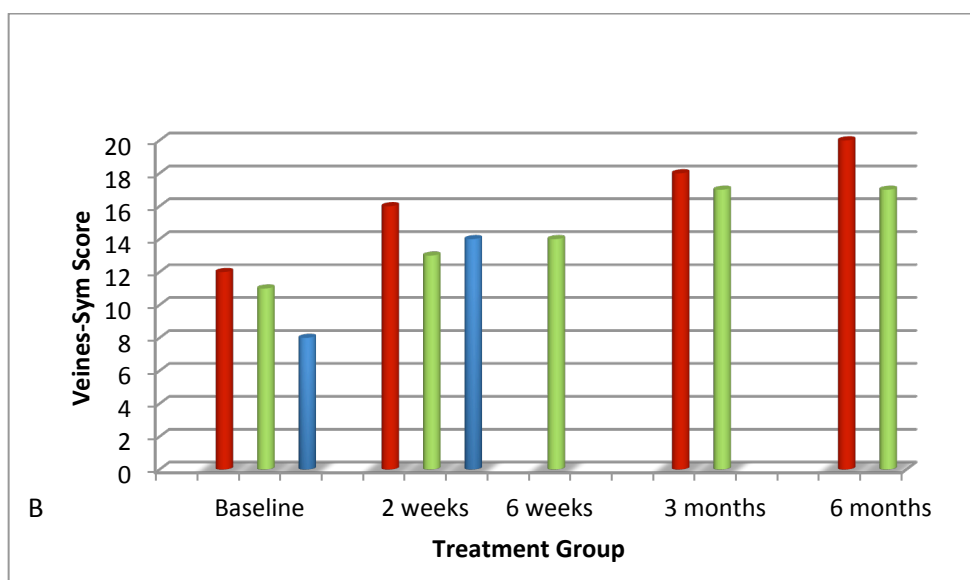
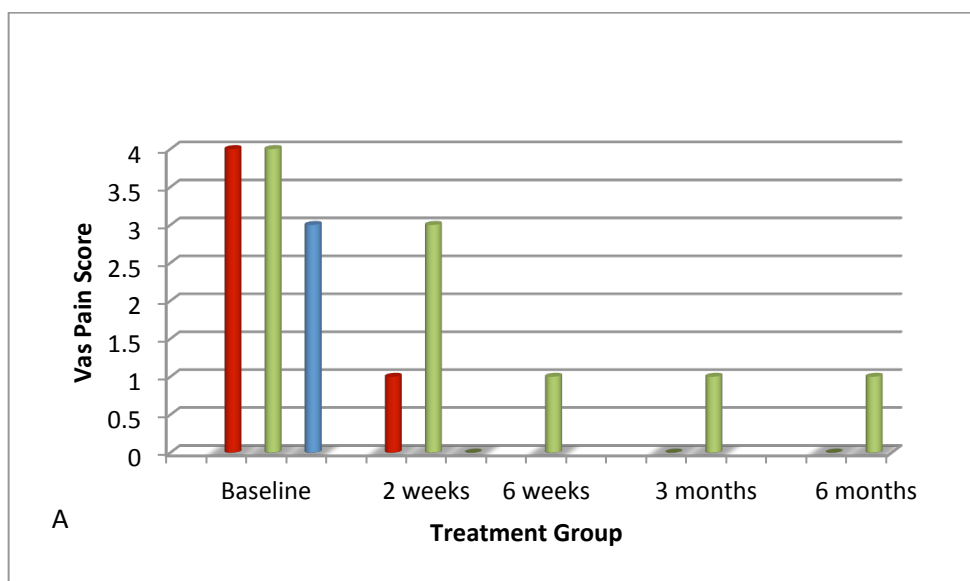


Figure 16. VAS pain scores (A) and Veines-sym scores (B). Group C = red; Group B= green; Group A = blue.

4.7.3 Post-Thrombotic Syndrome (PTS)

Characteristics of patients with and without PTS are as shown in **table 5**. Six month follow-up data was available for 158 patients. Of these, seven patients (4.3%, 95% CI 1.7-8.6%) were recorded as having symptoms and signs compatible with PTS, as assessed by the Villalta PTS scale. All cases were mild on the Villalta Scale, with a mean score of 5 at three months and 6 at six months. Another patient had symptoms compatible with PTS at 3

months, with a Villalta score of 6, but the score had reduced to 4 (indicating no PTS) by six months and their data is therefore not included in table 6.

Because the numbers of patients with PTS were so small, statistical analysis to look for differences between groups have not been performed, as this is unlikely to be accurate. However, there was a trend towards increased rates of PTS in subjects of male sex (57% of all subjects with PTS), and in those with unprovoked IDVT (43% of those with PTS, compared with 27% in the subjects without PTS).

Table 5: Characteristics of Patients with Post-Thrombotic Syndrome

Characteristic	PTS (n=7)	No PTS (n=151)
Male sex	4 (57%)	70 (46%)
Age (median)	65	52
BMI (mean)	30	29
Unprovoked	3 (43%)	44 (29%)
Muscular vein only	3 (43%)	44 (29%)
Group C (2 weeks anticoagulation)	5 (71%)	110 (75%)
Residual thrombus at 2 weeks	4 (57%)	110 (75%)
Venous Insufficiency	1 (14%)	121 (80%)
Anticoagulant		
LMWH	6 (86%)	107 (71%)
Rivaroxaban	1	43
Warfarin	0	1
D-dimer (mean)		
Baseline	1366*	1118^
2 weeks	447#	484>
Ethnicity		
White	5 (71%)	129 (85%)
Maori	0	2
Pacific Islander	1	1
Asian	1	8
Black	0	1
Other	0	4

*recorded for 4 patients; # recorded for 3 patients; ^ recorded for 80 patients; > recorded for 56 patients

4.7.4 All-Cause Mortality

No deaths were reported during the six-month follow-up period of the study.

4.7.5 Predictors of Recurrent or Progressive DVT/PE

Of the three symptomatic VTE recurrences, three occurred in subjects with provoked IDDVT, and one occurred in a subject with an unprovoked IDDVT. This gives a rate of VTE recurrence of 1/63 (1.6%) unprovoked versus 3/136 (2.2%) provoked, which is not significantly different ($p = 1$). Similarly, there was no significant difference in symptomatic VTE recurrence between the LMWH and rivaroxaban groups, at 4/132 versus 0/67, respectively ($p=0.303$).

Overall, there were 14 distal extensions on treatment, three proximal extensions on treatment, four recurrent (distal) DVT, and one asymptomatic proximal DVT recurrence i.e. 21 patients with 22 events (one patient had both a distal extension on the 2-week ultrasound and then a distal recurrence at one month after stopping LMWH). No predictors of thrombus extension or recurrence were identified (**table 6**), including axial vein involvement, provoked versus unprovoked thrombus, choice of anticoagulant and D-dimer at baseline or 2 weeks.

Table 6: Characteristics of Subjects with DVT Recurrence or Extension

Column1	Event (n=21)	No Event (n=179)	p
Age (median)	55	52	0.829
BMI (mean)	31.2	29.3	0.238
Axial vein involvement	5	60	0.369
Unprovoked IDDVT	6	54	0.88
Baseline D-dimer* (mean) [†]	1508.2	998.6	0.143
2-week D-dimer* (mean)	542.5	411.5	0.12
Rivaroxaban therapy	6	62	0.579
LMWH	15	117	0.579

[†] D-dimers measured for 105 patients at baseline and 69 patients at 2 weeks

* p-value for log transformation of D-dimer value

4.8 Treatment Group Characteristics

At two weeks, 78% of patients had residual thrombus on ultrasound. The presence of residual thrombus was significantly different between the asymptomatic group (C) and group with ongoing symptoms (B), at 72% and 93%, respectively ($p=0.0001$). Therefore it appears that the presence of ongoing symptoms strongly correlated with the presence of residual thrombus in group B patients.

Table 7: Ultrasound Results at Two Weeks in Asymptomatic vs Symptomatic Patients

Ultrasound Result	Overall	Asymptomatic	Symptomatic	p
	n=199	Group C	Group B	
		n = 140	n=56	
Complete Resolution	43 (22%)	39 (28%)	4 (7%)	0.0001
Residual Thrombus	156 (78%)	101(72%)	52 (93%)	
Partial Resolution	87 (44%)	64 (46%)	24 (42%)	
No Change	53 (26%)	33 (23%)	19 (34%)	
Extension within distal veins	12 (6%)	3 (2%)	9 (16%)	
Extension to proximal veins	31 (1.5%)	0 (0%)	0 (0%)	
Complete resolution but new superficial vein thrombus	1 (0.5%)	1 (1%)	0 (0%)	

Thrombus extension within the distal venous system occurred in 12 (6%) of patients overall. Of these, four patients were allocated to the asymptomatic (two-week) treatment arm (C), seven patients to the six-week treatment arm (B), and one patient was incorrectly assigned to the proximal extension arm (A). Overall, nine of the twelve (75%) had no or improved symptoms at two weeks, despite the ultrasound showing distal extension of thrombus. Of the remaining three, two had ongoing (unchanged) symptoms, and one had worsening symptoms leading to an unscheduled ultrasound showing distal extension at day two (but was asymptomatic by day 14).

In those allocated to the two-week treatment arm despite distal extension, there were no VTE recurrences, and there was no evidence of post-thrombotic syndrome in two of four patients assessed at the six month visit (two patients were lost to follow-up at six months).

4.9 Six-Week Visit (Symptomatic Arm) - Group B

As per study protocol, patients in the symptomatic arm (Group B, n=56) received a further four weeks of enoxaparin or rivaroxaban and were seen again at the six-week time point with a repeat ultrasound. Three patients in this group were lost to follow-up prior to the six week visit.

The pain scores and Veines-sym functional scores were very similar across groups, and do not appear to correlate with resolution of thrombus on ultrasound.

Table 8: Six-Week Ultrasounds in Symptomatic Arm (Group B)

Ultrasound Result n=53	n (%)	VAS pain (mean)	Veines-sym (mean)
Complete Resolution	17 (32)	2	14
No Change Thrombus	13 (24)	1	13
Partial Resolution Thrombus	18 (34)	1	15
Extension Within Distal Veins	1 (2)	not done	not done
Not done/not recorded	4 (8)	N/A	N/A

4.10 D-dimer Sub-Study

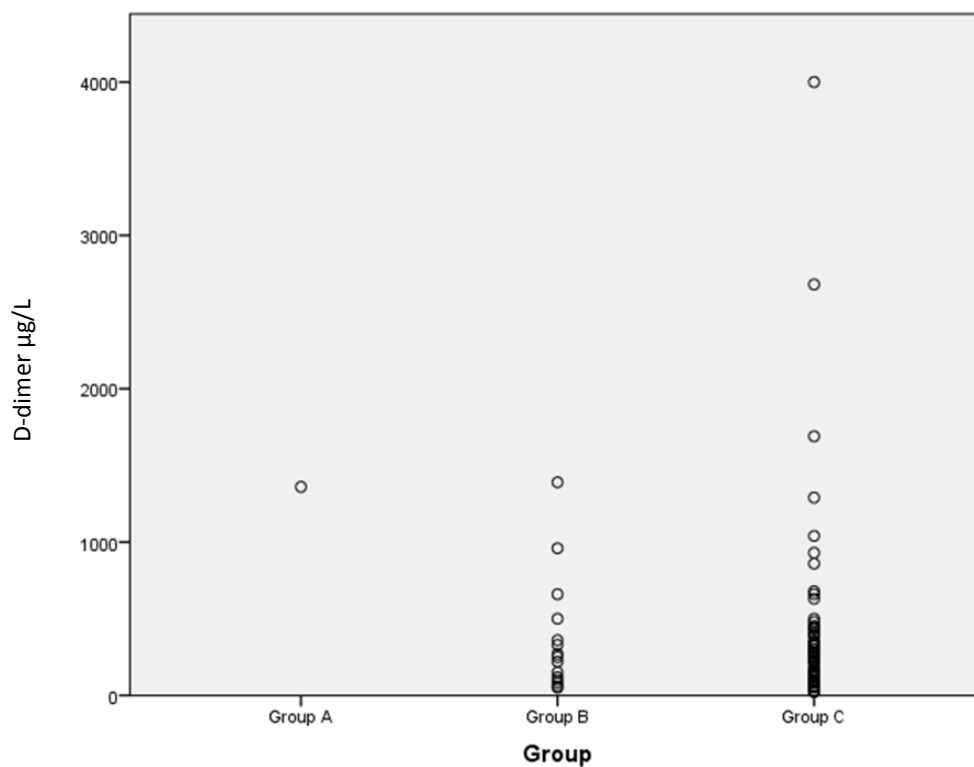
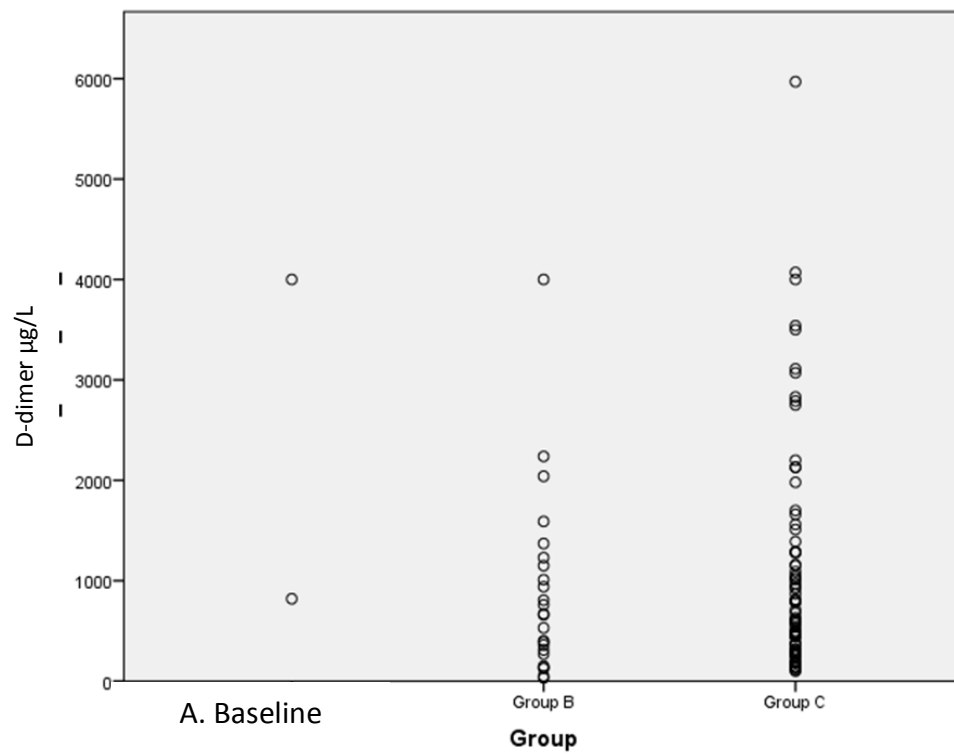
Eight centres participated in the D-dimer sub-study. D-dimers were measured at baseline and two weeks in all patients, and at six weeks in patients receiving six weeks of anticoagulation.

There was no difference in D-dimers between groups A (proximal extension at two weeks), B (symptomatic at 2 weeks) and C (asymptomatic at 2 weeks) at either the baseline or two week time points.

As only one patient in group A had D-dimer measurements, this patient was removed from further analysis. This patient had a D-dimer of 4000 μ g/L at baseline, reducing to 1360 μ g/L on two-week follow-up.

Of the 105 patients with a baseline D-dimer score, 69 had a repeat D-dimer at 2 weeks. D-dimers reduced over time in the cohort as a whole, and in all treatment groups, with the lowest D-dimers seen in the 6-week treatment group.

Log transformations of D-dimers were performed prior to analysis as the D-dimers were not distributed normally. The log reduction in D-dimer over two weeks was statistically significantly different in both groups B and C (**table 9**). The reduction in D-dimer was also significantly different between groups B and C ($p=0.021$), with a greater reduction in group C when compared with group B. This difference remained when a single outlier with a particularly large drop in D-dimer from baseline to two weeks (subject 1035) was removed from the analysis, $p=0.016$.



B. Two Weeks

Figure 17: D-dimers According to Treatment Group at (A) baseline and (B) two weeks

Table 9: Change in D-dimers over time

Group	D-dimer Baseline (µg/L; 95% CI)	D-dimer 2 weeks (µg/L; 95% CI)	p
Group B	737.9 (200.9-1274.9)	357.1 (152.5-561.7)	0.12
Group C	1039.1 (766.9-1311.1)	381.0 (227.6-534.4)	0

4.11 Bleeding

Twenty-five patients (12.5%) developed bleeding during the course of the study (**tables 10 & 11**). The majority of bleeding events (22; 88%) occurred within the first two weeks. Five of these were clinically relevant non-major bleeds (CRNMB; 2.5% of patients overall). The remainder were minor bleeds, with no episodes of major bleeding reported. There was no significant difference in bleeding between patients on LMWH and rivaroxaban, although there was a trend towards more bleeding in patients on LMWH (19/133; 14.4%) than in patients on rivaroxaban (5/67; 7.5%).

The only factor that was significantly different between bleeding and non-bleeding groups was an elevated BMI, with a mean of 33.2 in the bleeding group and 28.8 in the no bleed group. The reasons for this are unclear.

Table 10: Characteristics of Bleeding Patients

Column1	Bleed n=25	No Bleed n=176	p
Age (median)	51	52	0.681
BMI (mean)	33.2	28.8	0.014
Axial vein involvement	4	61	0.062
Unprovoked IDVT	6	54	0.494
Baseline D-dimer (mean)	944.7	1075.5	0.718
Two-week D-dimer (mean)	353.3	436.7	0.798
Rivaroxaban therapy	5	63	0.118
LMWH	20	113	0.118

* p-value for log transformation of D-dimer value

Table 11: Clinically Relevant Non-Major Bleeds

Index DVT	Location	Time	Anticoagulant	Management
Gastrocnemius + soleal	10cm bruise chest ?traumatic	Week 1	LMWH	LMWH reduced to 50% dose day 5
Gastrocnemius	25cm haematoma LMWH injection site	Week 1	LMWH	LMWH stopped day 7
Soleal	Menorrhagia	First 2 weeks	Rivaroxaban	Iron infusion
Gastrocnemius	Haematoma complicating muscle tear	First 2 weeks	Rivaroxaban	Rivaroxaban stopped week2
Posterior tibial	18cm haematoma at site of plantaris tendon rupture	Week 1	LMWH	LMWH stopped day 5

4.12 Muscular Vein Sub-analysis

Outcomes were compared between patients with thrombus confined to muscular (soleal and gastrocnemius) veins only compared with patients with axial vein involvement at baseline (**table 12**). There were no significant differences between groups in all assessed parameters, including the proportion allocated to the two-week treatment (asymptomatic) arm, VTE recurrence and thrombus resolution at two weeks. Similarly, there were no significant differences between those with thrombus confined to the soleal vein versus those with thrombus confined to the gastrocnemius veins for the same parameters (**table 13**).

Table 12: Characteristics of patients with muscular vein only versus axial vein thrombus

Characteristic	Muscular vein only n=91	Axial vein n=108	p
Proximal recurrence	0	1	1
Proximal extension	2	1	0.594
VTE recurrence	1	2	1
Distal extension	4	8	0.552
Thrombus resolution at 2 weeks	18	25	0.607
Asymptomatic at 2 weeks (group C)	67	73	0.436
Post-thrombotic syndrome	3	4	1
CRNMB*	4	1	0.181

*Clinically relevant non-major bleeding

Table 13: Characteristics of patients with soleal vein versus gastrocnemius vein thrombosis[#]

Characteristic	Soleal Vein Only ^ n=23	Gastrocnemius vein only n=64	p
Proximal extension	0	1	1
Distal extension	1	4	1
VTE recurrence	0	2	1
Thrombus resolution at 2 weeks	7	9	0.115
Asymptomatic at 2 weeks (Group C)	17	47	1
Post-thrombotic syndrome	0	3	0.563
CRNMB*	1	2	1

^ Patients with concomitant soleal and gastrocnemius thrombus excluded from analysis

*Clinically relevant non-major bleeding

Chapter 5: Discussion

5.1 Choice of Study Design

The protocol for the TWISTER trial was developed in 2010. At this time, there was no data from prospective randomised controlled trials to guide clinicians on the optimal duration of treatment for IDVT. The CACTUS trial(106), the first randomised controlled trial for management of IDVT was still enrolling patients at this time. Many centres in Australia and New Zealand were treating patients with a minimum of 6-12 weeks anticoagulation. Previous studies had shown that 4 weeks of LMWH (full dose for one week, followed by half dose LMWH for 3 weeks) (103) and 6 weeks of vitamin K antagonist therapy (104) were safe, with low rates of proximal extension and VTE recurrence. However, there were no studies examining a short duration (2 weeks) of treatment for IDVT. In addition, no studies have previously examined the rate of resolution of symptoms in this group, nor have they examined the incidence of PTS in a prospective, systematic manner

We opted for a prospective cohort study to examine this question, with patients stratified into one of three groups based on their signs/symptoms and two-week follow-up whole leg ultrasound. A randomised controlled trial comparing 2 weeks of treatment with standard of care (6 or 12 weeks anticoagulation) was another possibility, but this would have been costly and resource intensive, especially as a substantial sample size would have been required (at least double). For instance, the randomised, double-blind, placebo-controlled trial for treatment of IDVT (CACTUS) (106) estimated that they would need 286 patients in each arm (placebo versus six weeks of anticoagulation) to give the study 90% power to detect a 70% risk reduction in the rate of the primary efficacy outcome. The investigators, however, only managed to recruit 259 patients in total, illustrating the difficulties in recruiting patients to studies of this nature.

A VTE recurrence rate of $\leq 2\%$ within three months was deemed acceptable for this trial, as serial proximal CUS over one week shows a rate of proximal DVT of 0.6-2% in patients left off anticoagulation during this period (64, 75, 77, 124), **table 1**. Similarly, historical studies in patients using venography show a rate of VTE recurrence or proximal extension during 3-month follow-up of 1.3% (95% CI 0.15 - 4.4%) in those not treated based on a normal

venogram(125). More recently, the CACTUS study (106) showed a recurrence rate of 5% in the placebo group, and 3% in the group receiving six weeks of LMWH therapy. Therefore a VTE recurrence rate of $\leq 2\%$ is well within acceptable limits.

In retrospect, our primary outcome should have been symptomatic recurrence of **proximal** venous thrombosis (**proximal** DVT, non-fatal and fatal pulmonary embolism) within 3 months, rather than recurrence of VTE inclusive of distal DVT, as the former is accepted as having greater clinical significance.

5.2 Baseline Demographics

The majority of patients enrolled on study were of European ancestry (84%), which reflects the higher incidence of VTE in this ethnic group (34), as well as the demographics of enrolling centres. The median age was 52 years, compared with a higher mean age of 61 years in a recent New Zealand study that included both proximal and distal DVT (34). This is in accordance with previous data showing IDVT is less prevalent in the elderly (46, 126). There was an approximately equal distribution in baseline thrombosis between the left and right legs, as has been previously reported (29). Similarly, there was no difference in distribution according to sex, consistent with previous studies.

As is typical for IDVT, 69% of events were provoked, often in the setting of recent surgery or trauma. This, in part, likely accounts for the lower VTE recurrence rate after IDVT compared with proximal DVT, and previous IDVT studies have also demonstrated lower VTE recurrence rates in those with provoked versus unprovoked events(104). There was no significant difference in VTE recurrence rates between subjects with provoked and unprovoked IDVT in our study, however there were only four VTE recurrences, making such comparisons difficult.

5.3 Primary Outcome

We have demonstrated that 70% of patients can be treated with 2 weeks of anticoagulation, with a low rate of VTE recurrence (2/131, or 1.5%; 95% CI 0.19-5.41%), if they are asymptomatic at the two-week time point, with no evidence of extension of the distal thrombus into the popliteal vein or beyond. The rate of symptomatic proximal DVT or PE

recurrence, which is the clinically important outcome, was 0/140 or 0% (95% CI 0.00-2.60). As discussed above, the episode of asymptomatic proximal ‘recurrence’ was questionable, given this was a finding of non-occlusive proximal DVT found on a non-mandated USS in an asymptomatic patient, and our primary outcome was ‘the *symptomatic* recurrence of venous thrombosis within two months’.

The largest management study evaluating IDVT is the CACTUS study (106), a randomised, double-blind placebo-controlled trial, as described in the background above. The primary efficacy outcome in the CACTUS study was a composite measure of extension of calf DVT to proximal veins, contralateral proximal DVT, and symptomatic pulmonary embolism at day 42. In total, there were four events (3.3%) in the nadroparin arm, comprised of two non-fatal PE, one proximal extension on the day 3-7 ultrasound, and one proximal extension during the 6-week follow-up period. In the placebo group, there were seven events (5.4%), comprised of three proximal extensions on the day 3-7 USS, and four proximal extensions during the 6 week (42 day) follow-up period.

This compares with three subjects with proximal extension (1.5%) on the scheduled two-week ultrasound in the TWISTER study. None of our subjects were diagnosed with proximal extension beyond two weeks (despite all patients in the six-week treatment arm having a second routine USS at the six-week time point), and none of our subjects were diagnosed with recurrent symptomatic proximal DVT or PE.

If we compare outcomes between TWISTER patients and CACTUS patients (i.e. removing the asymptomatic proximal extensions within the first 2 weeks in both studies), then the rates of proximal VTE recurrence, including fatal/non-fatal PE are not significantly different between the six-week nadroparin arm of CACTUS (3/121, 2.5%; 95% CI 0.51-7.1%) and TWISTER (0/128, 0%; 95% CI 0.0-2.8%), $p = 0.113$. However, the proximal VTE recurrence rates are significantly different between the placebo arm of CACTUS and TWISTER, at 4% (5/124) in the CACTUS study placebo arm and 0% (0/128) in the TWISTER study ($p=0.027$). The overall VTE rates were also significantly different between the placebo arm of CACTUS and TWISTER, at 9.3% and 1.5%, respectively ($p=0.0047$); **table 13.**

Table 13: Proximal VTE Recurrence Rates in CACTUS and TWISTER studies

VTE*	Nadroparin 6 weeks CACTUS (n=122)	LMWH or Rivaroxaban 2 weeks TWISTER (n=131)	Placebo 6 weeks CACTUS (n=130)
Proximal DVT	1	0	4
PE	2	0	1
Distal DVT	1	2	6
Total VTE	4 (3.3%)	2 (1.5%)	12 (9.3%)

*Excluding proximal DVT extension within 2 weeks

Table 14: VTE Recurrence Rates in CACTUS and TWISTER studies With Statistical Comparisons

Treatment Arm	Proximal DVT/PE*	Total VTE*
Nadroparin 6 weeks (CACTUS)	3	4
LMWH or rivaroxaban 2 weeks (TWISTER)	0	3
p-value	0.1133	0.7143
Placebo 6 weeks (CACTUS)	5	12
LMWH or rivaroxaban 2 weeks (TWISTER)	0	2
p-value	0.0250	0.0047

*Excluding proximal DVT extension within 2 weeks

This suggests that in those IDVT patients with symptom resolution at 2 weeks, with no evidence of extension into the proximal veins (i.e., above trifurcation) on 2-week USS, that two weeks of anticoagulation should be the standard of care, as this significantly lowers VTE recurrence rates when compared with placebo, with low rates of bleeding (2.5% CRNMB on TWISTER), and possibly faster resolution of symptoms.

Eighty percent of patients on the TWISTER study reported complete resolution of symptoms at two weeks. Days to resolution of symptoms were not recorded in the CACTUS study; therefore it is unknown whether there was a significant difference in time to symptom resolution between the anticoagulant and placebo arms. A randomised study comparing two weeks of anticoagulation versus placebo would be required to definitively answer this question.

The slow rate of recruitment on the CACTUS study led to speculation regarding possible inclusion bias, due to the reluctance of some investigators to expose patients to the ‘no treatment’ arm(127). Conversely, the TWISTER study was pragmatically designed to give all

patients a minimum of two weeks of anticoagulation, with a two-week USS, and to only cease anticoagulation in those patients who were asymptomatic. In hindsight, this is likely to have identified a lower risk group, but the two-week duration of anticoagulation/repeat USS, and extension of anticoagulation to six weeks for those with ongoing symptoms, were design features to encourage clinicians to enrol all eligible patients into the study. Therefore one would expect the inclusion bias to be lower in the TWISTER study.

5.4 Asymptomatic Proximal DVT Recurrence

There was one asymptomatic proximal DVT recurrence in a subject in the two-week treatment group. Although we have included this patient in the primary outcome, they did not fit our definition, which was ‘the symptomatic recurrence of venous thrombosis (DVT, non-fatal and fatal pulmonary embolism) within three months’. The baseline ultrasound was reported as showing a thrombus in the left peroneal vein. The two-week ultrasound showed partial resolution of the peroneal vein thrombus. A ‘recurrent’ distal DVT (thrombus in left posterior tibial vein, with no residual thrombus in peroneal vein) was found on an unscheduled ultrasound performed four weeks after enoxaparin was ceased merely to assess for thrombus resolution. The patient was asymptomatic at this time point.

A further ultrasound five weeks later (i.e. nine weeks after enoxaparin was ceased) showed non-occlusive thrombus within the posterior tibial, peroneal and popliteal veins; the subject was asymptomatic at this time point also. It was noted on this last ultrasound that views were ‘poor’. The patient stayed off antithrombotic treatment with no adverse outcomes recorded over the six-month follow-up period of the study. It is difficult to ascertain in this case whether the proximal DVT was present at the time of the first ultrasound, which seems more likely given the non-occlusive (chronic) appearances of the thrombi on the ultrasounds obtained at the six-week and eleven-week time points.

Our study protocol was specifically designed to limit the number of scans in our cohort, and this case illustrates how our strategy should minimise the chances of obtaining a false positive scan. For a cohort of 200 patients, CUS has a sensitivity of 97% and a specificity of 94% for proximal DVT(65), but we would expect that CUS may miss six proximal DVT in this cohort, and similarly that 6% may be falsely labelled as proximal DVT when they are not.

5.5 Symptom Resolution

This was often not recorded well, and was difficult to assess retrospectively from patient diaries, as patients often recorded pain due to their underlying provoking event rather than pain solely due to the DVT. This was also the case for the functional scoring system. This improved once the CRFs were amended to include a field for ‘days to symptom resolution’, and investigators were instructed to ask patients to specifically focus on scoring pain/functional impairment due to the DVT. However in some cases this was not possible, due to the nature of the underlying injury e.g., calf muscle tear.

However, in general, VAS pain scores reduced over time, and functional status as assessed by Veines-sym scores tended to normalise over time (**figure 16**).

5.6 Post-Thrombotic Syndrome

We have reported six-month PTS outcomes for 164 patients, which is the largest cohort of IDDTV PTS data reported in the literature. Rates of PTS at 6 months were low at 4.3% (95% CI 1.7-8.65); all were graded as mild. There were no patients with moderate or severe PTS on our study. This compares with PTS rates of 20-50% following proximal DVT, with severe PTS comprising 5-10% of such cases (111). PTS rates for patients with IDDTV vary widely in the literature, from 4-43% (78) to 23% (114) although the number of patients with distal DVT in these studies is low, from 17 to 60 (79, 114, 128).

Our PTS follow-up was only out to six months, and it is known that most cases of PTS occur within 24 months of the acute thrombotic event (118). Therefore we cannot exclude the possibility that at least one of the patients with mild PTS may have progressed over the next several months to severe PTS. However the cumulative risk of PTS has been demonstrated to be lower in patients with distal as opposed to proximal DVT(129), with the same study showing that quality of life tended to improve rather than worsen over time in patients with PTS (with most of the improvement occurring between one and four months after the DVT). Therefore it seems unlikely that our patients with mild PTS would have progressed to severe PTS over the next several months.

The PTS study enrolling the largest number of distal DVT patients (n=60) reported rates of 43.3%, not significantly different from PTS rates in the proximal DVT cohort (59.7%), although it is likely this was limited by statistical power (130). It is difficult to ascertain why PTS rates were so high in this study, however the patient cohort was quite different from the low-risk population enrolled on TWISTER, and included patients immobilised in plaster casts and pregnant patients.

Although the numbers of patients with PTS in this cohort were too small to make any statistical comparisons, there was a trend towards higher rates of PTS in males (57% of those with PTS were male compared with 46% males in the non-PTS cohort), and in those with unprovoked IDVT (43% compared with 29% in the non-PTS group). There is no consistent evidence that either patient sex or unprovoked events increase the rates of PTS (111), however larger numbers would be required to determine if there was a statistically significant difference between these groups.

Similarly, the presence of residual thrombus at 2 weeks was not predictive of PTS development in this cohort. However two weeks is a short time frame for residual thrombus assessment, and it is possible that assessment at a later time point may have shown an association between residual thrombosis and PTS. Previous studies have shown that more proximal DVT (e.g., iliofemoral as opposed to popliteal DVT) confer a higher risk of DVT recurrence, which may account for some of the increase in risk of PTS in this same patient group (131). Improved rates of thrombus resolution and recanalisation have also been reported for distal vein DVT, due to reduced associated damage to venous valves and resultant valvular reflux (131). There were no VTE recurrences in the subjects with PTS in this study.

With regards to PTS scoring on this study, for several patients this was assessed via telephone rather than by a clinic visit as some patients were reluctant to return for assessment at three and six months. Patients were asked if they had any signs or symptoms consistent with PTS, and were scored as 'no PTS' if they denied these symptoms. It seems unlikely these patients had significant PTS if they reported that their leg had returned to normal, and therefore the validity of the results is likely to be quite sound, although it is recognised this method of assessment is not ideal. However we believe our low PTS rates are valid, as we used sensitive tools (Villalta scale and adapted Veines-sym tool), both of which have been well validated.

Therefore we are extremely confident that PTS rates in this population are $\leq 10\%$. Similarly, we are confident that severe PTS is highly unlikely after a single first episode of IDVT.

5.7 D-dimer Sub-study

A subset of patients had D-dimers measured at baseline and two weeks, and in six weeks in group B patients (symptomatic group). As expected, D-dimers reduced over time in the cohort as a whole and in all treatment groups. The log reduction in D-dimer was significantly different between groups B and C ($p=0.021$), although this is unlikely to be clinically significant. This finding was probably because there were some patients in group C with high D-dimers at baseline. However the mean D-dimers were similar at 2 weeks between groups B and C, with mean D-dimers in both groups less than the cut-off value of <500 mcg/L (**table 9**).

D-dimer levels were not significantly different in patients with events (proximal or distal VTE extension or recurrence) when compared with patients who did not have events. In summary, D-dimer levels were not predictive for outcomes for patients with IDVT in the patients included in this sub-study.

5.8 Bleeding

Data from the REITE Registry showed that IDVT patients have a lower risk of bleeding on anticoagulation than those with proximal DVT, with 2.2% vs 1% major bleeding in the patients with proximal and distal DVT, respectively (46). Consistent with this, there were no episodes of major bleeding in our cohort, and a low rate of CRNMB (2.5%). This is likely because the IDVT patients are younger, with less comorbidities, and because they have received anticoagulation for a shorter period of time than patients with proximal DVT, who require a minimum of three months anticoagulation. Of note, major or clinically relevant non-major bleeding occurred in 4% of patients in the six-week nadroparin arm in the CACTUS study (one major and four CRNMB), slightly higher than in our cohort.

Three of the CRNMB necessitated early cessation of treatment (prior to two weeks in two patients allocated to the two-week treatment arm, and prior to six weeks in one subject in the six-week treatment arm). Another subject with CRNMB required reduction of LMWH to

50% of the total dose. The remaining two subjects with CRNMB did not require any alteration to the treatment plan.

The mean BMI was significantly higher in the patients who bled (33.2) versus the non-bleeding cohort (28.8), $p=0.014$. The reason for this is unclear, and this finding has not previously been reported in the literature. It is possible that a weight-based dose of LMWH was inaccurate for those with a higher BMI, leading to over-anticoagulation in this group. However, usually weight-based dosing of LMWH correlates quite closely with anticoagulant response, even at extremes of body weight (132).

We did not find any other factors predictive for bleeding, including age, axial vein versus muscular vein only involvement, unprovoked event, D-dimers or choice of anticoagulant (i.e., rivaroxaban versus LMWH). However there was a trend towards more bleeding in patients on LMWH (19/133; 14.4%) than in patients on rivaroxaban (5/67; 7.5%). In accordance with this, the majority of 'bleeds' were bruises or haematomas at the site of LMWH injection (10/25, 40%). Conversely, three of four of the menorrhagia cases were on rivaroxaban. This is consistent with reports of heavy menstrual bleeding (HMB) on rivaroxaban, more so than with other anticoagulants (133, 134); further studies are awaited to investigate the true incidence and mechanisms of this observation. Options to reduce HMB in women on anticoagulants include continuing estrogen-containing contraception until the anticoagulant course is finished, which has not been shown to increase rates of VTE recurrence(135), introduction of high dose progesterone or use of progesterone-secreting IUDs.

As is typical for bleeding on anticoagulation (6), the majority of the bleeds occurred early, with 88% occurring in the first two weeks. There was a trend towards higher rates of bleeding in patients receiving concomitant aspirin (3/18; 17% (95% CI 3.6-41.4%)) than in patients receiving anticoagulation alone (12%); this difference did not reach statistical significance ($p=0.48$). The trend towards higher rates of bleeding on an antiplatelet/anticoagulant combination is consistent with that reported in the literature, with a large cohort study of over 82,000 patients showing a hazard ratio for bleeding of 1.83 (95% CI 1.72-1.96) for a warfarin-aspirin combination compared with warfarin alone (136).

This highlights the advisability of reviewing the need for concomitant antiplatelet therapy when commencing a patient on anticoagulation, as in many cases it is safe for the patient to

withhold the antiplatelet therapy during the period of anticoagulation. In patients who have an absolute requirement to remain on their antiplatelet agent (e.g. coronary stents), the case is even more compelling to provide an abbreviated course of anticoagulation for IDVT.

There was no increase in bleeding in female patients in our cohort, although this has previously been reported as a risk factor for bleeding (137).

5.9 Predictors of Recurrent or Progressive DVT/PE

No predictors for recurrent or progressive VTE were identified, including for variables such as axial vein versus muscular vein involvement, unprovoked DVT, baseline or two-week D-dimer, or choice of anticoagulant (rivaroxaban versus enoxaparin).

The high number of distal extensions on treatment, along with three proximal extensions on treatment, is unexpected in light of how effective LMWH and rivaroxaban were in the pivotal studies in treatment of proximal DVT and PE (138, 139). It is likely that many of the extensions on treatment were due to diagnostic bias in the mandatory 2-week USS rather than true treatment failure. As noted previously, the specificity of CUS for proximal DVT is 94%(65), and it is possible that these were all false positives. One way of further investigating this would have been to consider venography in these patients, as this is the reference standard, to further clarify this issue. This was not mandated in the study protocol, but could be considered for further such studies.

As discussed previously, inter-observer variability in the evaluation of distal veins via compression ultrasound is high, with a low sensitivity (65). Therefore it is not surprising that the ‘distal extension’ group was the group in which the ultrasound finding was most likely to change on adjudication, with five ‘distal extensions’ at two weeks deemed not to have extension. Conversely, two controls deemed not to have extension were deemed to have extension by the adjudicators. In such cases, where a repeat USS shows ‘distal extension’, it seems pragmatic to stop treatment if the patient’s symptoms have resolved.

5.10 Treatment Group Allocation

An interesting observation is that the proportions of patients allocated to group C (2 weeks anticoagulation) versus group B (6 weeks anticoagulation) at the two-week visit differed widely between sites. Site 4 had the highest proportion of patients in the two-week treatment arm at 91% (31/34), compared with 25% at site 10 (1/4). The three sites enrolling the highest numbers of patients (27-50 patients per site) tended to allocate more patients to the two-week treatment arm ($\geq 70\%$), compared with the three sites enrolling the lowest number of patients (0-50% allocated to the two-week arm). When examining the CRFs between sites, it is apparent that the variability in assignment of patients to treatment groups depended on two main factors.

(1) Patient reporting of symptoms. In some instances, patients were still reporting pain (as documented in the patient diaries) at week 2, however on questioning many reported that the pain due to the *calf vein clot* had resolved. In these instances, the persistent pain was due to the underlying injury and/or surgical trauma that had provoked the IDVT. Some investigators were better at differentiating this than others. Those who did not were more likely to assign patients to the 6-week treatment arm. This has also led to bias in reporting of 'days to symptom resolution'. In some instances, it is impossible to distinguish pain due to calf vein clot from pain due to injury and if in doubt, patients should receive six weeks rather than two weeks of anticoagulation.

(2) The result of the repeat CUS at 2 weeks. In many instances, the repeat CUS was performed at a different centre from the initial diagnostic scan. It was relatively common for the repeat CUS to show that the DVT had extended within a distal vein (12/199 patients at 2 weeks). On one occasion, the DVT had resolved but there was now a 'new DVT' in a different distal vein. In four instances, including the case of the 'new DVT in a different distal vein', investigators felt comfortable with discontinuing treatment as the patient was symptom-free at this point. Others allocated the patient to the six-week treatment arm, regardless of whether the patient had ongoing symptoms or not. Overall, 77% of patients had no or improved symptoms despite the USS report of distal extension.

This reflects the recognition by some investigators that USS imaging of the distal veins is problematic, with a lower sensitivity and specificity than in the proximal veins. This is also reflected in the adjudication process, where 5/17 (29.4%) of the 'distal extensions' on

treatment were instead adjudicated to have either unchanged, partial or complete resolution of thrombus. It should also be noted that all three adjudicators reached agreement in only 55% of reviewed cases in interim analysis 1.

In terms of wider applicability, it seems reasonable to stop anticoagulation at two weeks if the patient is symptom-free unless the repeat ultrasound scan shows evidence of extension into the proximal veins. It also appears safe to stop treatment after two weeks in asymptomatic patients if the two-week ultrasound report shows extension confined to the distal veins, or a thrombus in a different distal vein. As noted above, four patients had their treatment stopped despite the ultrasound finding of a 'new DVT' in a different distal vein' (1) or DVT extension within a distal vein (3); none of these patients had recurrent DVT or PE during the 3-month follow-up period of the study

In view of this, the utility of the 2-week USS in an asymptomatic patient is questionable. However three patients in this study had 'proximal extension' on the 2-week scan, at least two of whom were asymptomatic (with no information available on the third patient). When proximal extension was detected on the 2-week scan, then all investigators continued anticoagulation for a minimum of three months, as is appropriate. The risk of VTE recurrence in patients with proximal DVT receiving less than three months of anticoagulation is high (4), therefore the 2-week USS is an important safety measure.

Of note, if we had stopped treatment based purely on resolution of symptoms (with no scheduled 2-week USS), then any repeat USS for recurrent symptoms would have demonstrated a proximal DVT, which would have been assumed at that point to represent 'proximal DVT recurrence'. This would have elevated our incident of proximal DVT recurrence to rates very similar to those seen in the placebo arm of the CACTUS study.

5.11 Muscular Vein Sub-analysis

Ninety-one patients in our cohort (46%) had thrombus confined to muscular veins only at baseline. The literature on the natural history of isolated gastrocnemius and soleal vein thromboses (IGSVT), and appropriate treatment of these, shows widely different rates of propagation to proximal veins and PE, depending on which patient groups are included in studies. Despite this, the evidence appears to favour a short course of, or no, anticoagulation

treatment for this group of patients if high risk patients are excluded (e.g. those with active malignancy, pregnancy/post-partum, or evidence of concomitant PE) (81-83).

In contrast to previous studies, we did not find any significant difference between patients with IGSVT and those with axial vein thrombus with respect to rates of proximal vein extension or VTE recurrence. There was also no difference between groups with regards to proportions allocated to the two-week treatment group, thrombus resolution at two weeks, thrombus extension on treatment (within distal veins or into proximal veins), or rates of PTS and bleeding (**table 12**). Similarly, when patients with muscular vein thrombosis were divided into those with soleal versus gastrocnemius vein thrombi, there were no differences between the two sub-groups for any of the above parameters (**table 13**). In accordance with this, the CACTUS study (106) did not find a difference in the proportion of patients with the primary outcome when examining the IGSVT and axial vein IDVT groups separately, despite patients with IGSVT making up approximately half of all patients on study.

In summary, patients with muscular vein thrombosis appeared to derive a similar net clinical benefit to patients with thrombus involving the axial distal veins, and should be considered equally when considering a limited course (two weeks) of anticoagulation.

5.12 *Compression Stockings*

Compression stockings were used at the investigator's discretion, with use of compression stockings recorded for 106 patients. The only time point where a significant difference in compression stocking use was noted was at six months, with a higher use in group B (32%) than in group C (18%), $p=0.049$. This is likely due to ongoing symptoms in group B. It is unlikely that the use of compression stockings altered patient outcomes in any way, in particular with regards to PTS. This is especially in light of recent trial findings showing that graduated compression stockings do not reduce PTS when compared with placebo stockings(119). In accordance with this, there were similar proportions of patients in the asymptomatic group (group C; two weeks anticoagulation) in both the PTS and non-PTS cohorts, at 71% and 75%, respectively (**table 5**).

5.13 *Rivaroxaban Sub-Analysis*

One-third of our cohort received rivaroxaban, with nearly two-thirds receiving LMWH and one patient receiving warfarin. There were no significant differences between the LMWH and rivaroxaban cohorts with respect to the primary outcome, overall events (including VTE recurrence, distal thrombus extension and asymptomatic proximal thrombus extension), PTS, bleeding, or treatment group allocation. Patients with IDVT were not included in the DOAC treatment studies. However on the basis of our data, rivaroxaban appears to be similar in efficacy to LMWH for treatment of IDVT.

5.14 *Challenges in Conducting Clinical Trials*

This was an investigator-led trial, with very limited funding. There was no resource for clinical monitoring or face-to-face meetings with recruiting sites. I have therefore had to carry out my own monitoring of the study, and this has meant that problems have not always been picked up in a timely fashion. The trial commenced in late 2010, and for the first interim analysis (conducted in 2014), there were no issues with obtaining ultrasounds for adjudication from public and private providers. However, obtaining ultrasounds from some Australian sites has been much more difficult this time, with some sites (a) refusing to provide images due to 'legal' reasons and (b) some private providers having no record of ultrasounds performed more than two years ago. In view of this difficulty, we have requested that all sites provide deidentified copies of ultrasounds at the time of the event, rather than retrospectively, which may help with the second issue.

The lack of dedicated clinical monitors meant that systematic problems at some sites were not picked up for several months. For example, one investigator was recording 'not applicable' on the CRF for 'time to symptom resolution' for all patients, even when it was apparent that the symptoms had clearly resolved, so this data was not recorded for many of their patients. This was pointed out to the investigator after the first interim analysis, and data recording has improved at this site.

5.14.1 Recruitment

The recruitment rate to this study was slow, for a number of reasons:

- (1) The patients of interest were a low risk group of mobile outpatients without malignancy or prior VTE (many with distal DVT were screened but did not fit the criteria).
- (2) The inclusion criterion dictating a 72-hour window from time of diagnosis to recruitment excluded some patients, who were not keen to return to hospital to be enrolled on the study once treatment had already been commenced (especially in New Zealand, where the oral anticoagulant option, rivaroxaban, was not available until very recently i.e., 1st August 2018).
- (3) Use of injections only in NZ group, especially once the oral anticoagulant dabigatran became funded for VTE treatment in New Zealand in 2014. Rivaroxaban was registered but not funded for VTE treatment in New Zealand at that time. The introduction of a dabigatran treatment arm was discussed. However the treatment trials for VTE treatment with dabigatran treated the patients with 5 days of treatment-dose LMWH first, so this would not remove the requirement for a short period of injections, and it was decided it was not worth introducing a third anticoagulant into the study. The introduction of rivaroxaban early in the study has potentially stratified outcomes, although there do not appear to have been in differences in outcomes (primary or secondary) in the patients on this study, apart from a trend towards reduced bleeding in the rivaroxaban group. In addition to this, the vast majority of patients with IDVT are now treated with direct oral anticoagulants, therefore the use of rivaroxaban on study once this become funded was important to reflect current practice.
- (4) The more widespread use of direct oral anticoagulants has led to treatment of many IDVT in the community rather than being referred to hospitals.
- (5) This was an investigator-led study with a limited source of funding, \$100 per patient on enrolment, and \$100 on completion of six-month follow-up. This increased to \$400 total payment per patient from 1st September 2017. It did not appear that the funding was a major limitation to enrolment in this study for most sites, with most sites commenting that the major barrier was due to the first three factors as listed above. However, one of the highest recruiting sites in New Zealand (Auckland City Hospital) needed extra reimbursement to allow them to fund the second (week 2)

ultrasound scan, as this was not deemed 'standard of care' by their DHB. This slowed recruitment at this site for some months until we became aware of the issue and provided the extra funding (an extra \$250/patient).

5.14.2 Loss To Follow-Up

Thirteen subjects were lost to follow-up before three months. This potentially has impact on the primary outcome, which is 'the symptomatic recurrence of venous thrombosis (DVT, non-fatal and fatal pulmonary embolism) *within 3 months*'. Of those lost to follow-up, one was lost before two weeks, and therefore was not allocated to two versus six weeks of treatment. Of the remaining twelve subjects, nine were in the two-week (experimental) treatment arm and three were in the six-week treatment arm. Loss to follow-up at three months overall was therefore 6.4% for the primary outcome, and 6.8% in the experimental arm.

It is perhaps not surprising that the majority of those lost to follow-up were in the two-week treatment arm, as this cohort were by definition asymptomatic at two weeks, were no longer on anticoagulation, and therefore less likely to feel the need to be reassessed at the three-month time point. The 'best case' scenario is that these patients remained free of VTE recurrence/death from pulmonary embolism. The 'worst case' scenario is that all of the patients lost to follow-up have died due to fatal pulmonary embolism.

With the best-case scenario in mind, the VTE recurrence rate in the experimental (two-week) treatment arm remains 2.3%. With the worst-case scenario in mind, the VTE recurrence rate in the experimental (two-week) treatment arm becomes $12/132 = 9.1\%$ (95% CI 4.79-15.34%), and the mortality rate would be $9/132 = 6.8\%$ (95% CI 3.2-12.6%). If we took the worst-case scenario to be correct, then this would mandate stopping the trial (as per the stopping rules), and the conclusion should be that two weeks of anticoagulation for isolated distal vein DVT is not safe, with a high rate of VTE recurrence and death.

The LOST-IT trial (140), published in the British Medical Journal in 2012, was a systematic review examining the effect of loss to follow-up in randomised controlled trials. The investigators performed a Medline search of five top medical journals from 2005-2007; 235 randomised controlled trials reporting significant patient binary important outcomes were

included. They found that 13% of these trials did not report whether loss to follow-up occurred. In those studies that did report loss to follow-up, the median percentage of subjects lost to follow-up was 6%. The method by which loss to follow-up was handled was unclear in 19% of studies, however the most commonly used method was survival analysis (65%). When data from these studies was analysed under various assumptions, 17% of trials lost significance if those lost to follow-up were assumed to have the event of interest, 19% lost significance if the participants were assumed not to have the event of interest, and 58% lost significance under the worst-case scenario (i.e. amongst those lost to follow-up, all in the treatment group and none in the control group had the event). Under the more likely assumption that the event was higher amongst those lost to follow-up in the treatment group than in the control group, then 0-33% of trials were no longer significant for the primary outcome measure. The authors concluded that 'plausible assumptions regarding outcomes of patients lost to follow-up could change the interpretation of results of randomised controlled trials published in top medical journals'.

Consistent with this, a survey of over 300 RCTs (141) published in four leading medical journals which used Kaplan-Meier plots showed that less than half of the studies presented assessable and consistent information on loss to follow-up. They concluded that the validity of the results in many studies, and the assumptions one can draw from these, is questionable.

With this in mind, we have been very transparent about the loss to follow-up in this study, which at 6.4% at three months, is consistent with the median loss to follow-up in the RCTs included in the LOST-IT trial (140). However, applying a 'worst-case' scenario to our primary outcome is also unlikely to give us a true estimate of the VTE recurrence rate in this low-risk population with a first isolated symptomatic distal DVT. We have therefore chosen to present the results using as a denominator the subjects who have completed three month follow-up, but have included a consort diagram to show loss to follow-up, and when this has occurred. This will allow reviewers and clinicians to draw their own conclusions when interpreting the results of this study.

A further twenty-nine patients were lost to follow-up between three and six months, of which eighteen were in group B (symptomatic arm) and eleven in group C (asymptomatic arm). Overall, 19% of patients were lost to follow-up by six months, which is important in the context of interpreting the data on post-thrombotic syndrome. It is likely that most of those

lost to follow-up at this point were asymptomatic, meaning that rates of post-thrombotic syndrome rates may have been lower if these patients were included in the denominator.

5.14.3 Adjudication

For the first interim analysis, USS scans were obtained on all patients with events (distal or proximal vein extension or recurrence), and sent to three adjudicators for adjudication. However only one adjudicator was able to perform the second interim analysis, despite the other two adjudicators agreeing to perform this task and receiving copies of the ultrasound scans for this purpose. In addition to this, some Australian centres could not supply ultrasounds for the event adjudication for the second interim analysis, due to difficulty obtaining these from private providers. Therefore only seven of the eleven 'events' in the second analysis were able to be adjudicated, by one independent adjudicator.

Conclusion

A two-week course of anticoagulation (LWMH or rivaroxaban) is efficacious and safe for the majority of patients with isolated symptomatic DVT (70%), with no proximal DVT or PE recurrences, a low rate of distal DVT recurrence (1.5%), and a low rate of bleeding; and should replace 6-12 weeks of anticoagulation as the standard of care in this group.

References

1. Palareti G, Schellong S. Isolated distal deep vein thrombosis: what we know and what we are doing. *Journal of thrombosis and haemostasis : JTH*. 2012;10(1):11-9. Epub 2011/11/16.
2. Kearon C. Natural history of venous thromboembolism. *Circulation*. 2003;107(23 Suppl 1):I22-30. Epub 2003/06/20.
3. Douketis JD, Gu CS, Schulman S, Ghirarduzzi A, Pengo V, Prandoni P. The risk for fatal pulmonary embolism after discontinuing anticoagulant therapy for venous thromboembolism. *Annals of internal medicine*. 2007;147(11):766-74. Epub 2007/12/07.
4. Bouitte F, Pinede L, Schulman S, Agnelli G, Raskob G, Julian J, et al. Influence of preceding length of anticoagulant treatment and initial presentation of venous thromboembolism on risk of recurrence after stopping treatment: analysis of individual participants' data from seven trials. *BMJ*. 2011;342:d3036. Epub 2011/05/26.
5. Tran HA, Chunilal SD, Harper PL, Tran H, Wood EM, Gallus AS. An update of consensus guidelines for warfarin reversal. *The Medical journal of Australia*. 2013;198(4):198-9. Epub 2013/03/05.
6. Linkins LA, Choi PT, Douketis JD. Clinical impact of bleeding in patients taking oral anticoagulant therapy for venous thromboembolism: a meta-analysis. *Annals of internal medicine*. 2003;139(11):893-900. Epub 2003/12/04.
7. Bates SM, Kearon C, Crowther M, Linkins L, O'Donnell M, Douketis J, et al. A diagnostic strategy involving a quantitative latex D-dimer assay reliably excludes deep venous thrombosis. *Annals of internal medicine*. 2003;138(10):787-94. Epub 2003/05/21.
8. Douketis JD, Foster GA, Crowther MA, Prins MH, Ginsberg JS. Clinical risk factors and timing of recurrent venous thromboembolism during the initial 3 months of anticoagulant therapy. *Archives of internal medicine*. 2000;160(22):3431-6. Epub 2000/12/09.
9. R V. Phlogose und Thrombose im Gefäßsystem. *Gesammelte Abhandlungen zur Wissenschaftlichen Medizin Frankfurt: Staatsdruckerei*. 1856.
10. Esmon CT. Basic mechanisms and pathogenesis of venous thrombosis. *Blood reviews*. 2009;23(5):225-9. Epub 2009/08/18.
11. Berger G, Hartwell DW, Wagner DD. P-Selectin and platelet clearance. *Blood*. 1998;92(11):4446-52. Epub 1998/12/03.
12. Myers DD, Hawley AE, Farris DM, Wroblewski SK, Thanaporn P, Schaub RG, et al. P-selectin and leukocyte microparticles are associated with venous thrombogenesis. *Journal of vascular surgery*. 2003;38(5):1075-89. Epub 2003/11/07.
13. Martinod K, Wagner DD. Thrombosis: tangled up in NETs. *Blood*. 2014;123(18):2768-76. Epub 2013/12/25.
14. Sen I. Plasma DNA and Neutrophil Extracellular Traps (NET): A Novel Biomarker in Lower Limb Venous Thrombosis *Journal of Thrombosis and Circulation: Open Access*. 2016;10:2572-9462.
15. Kimball AS, Obi AT, Diaz JA, Henke PK. The Emerging Role of NETs in Venous Thrombosis and Immunothrombosis. *Frontiers in immunology*. 2016;7:236. Epub 2016/07/23.
16. Turpie AG, Chin BS, Lip GY. Venous thromboembolism: pathophysiology, clinical features, and prevention. *BMJ*. 2002;325(7369):887-90. Epub 2002/10/19.
17. Sanchez-Luceros A, Meschengieser SS, Marchese C, Votta R, Casais P, Woods AI, et al. Factor VIII and von Willebrand factor changes during normal pregnancy and puerperium. *Blood coagulation & fibrinolysis : an international journal in haemostasis and thrombosis*. 2003;14(7):647-51. Epub 2003/10/01.

18. Bremme KA. Haemostatic changes in pregnancy. *Best practice & research Clinical haematology*. 2003;16(2):153-68. Epub 2003/05/24.
19. Tchaikovski SN, Rosing J. Mechanisms of estrogen-induced venous thromboembolism. *Thrombosis research*. 2010;126(1):5-11. Epub 2010/02/19.
20. Sousou T, Khorana AA. New insights into cancer-associated thrombosis. *Arteriosclerosis, thrombosis, and vascular biology*. 2009;29(3):316-20. Epub 2009/02/21.
21. Timp JF, Braekkan SK, Versteeg HH, Cannegieter SC. Epidemiology of cancer-associated venous thrombosis. *Blood*. 2013;122(10):1712-23. Epub 2013/08/03.
22. Sheth RA, Niekamp A, Quencer KB, Shamoun F, Knuttinen MG, Naidu S, et al. Thrombosis in cancer patients: etiology, incidence, and management. *Cardiovascular diagnosis and therapy*. 2017;7(Suppl 3):S178-S85. Epub 2018/02/06.
23. Selby R, Geerts W, Ofosu FA, Craven S, Dewar L, Phillips A, et al. Hypercoagulability after trauma: hemostatic changes and relationship to venous thromboembolism. *Thrombosis research*. 2009;124(3):281-7. Epub 2008/12/02.
24. McLintock C, Brighton T, Chunilal S, Dekker G, McDonnell N, McRae S, et al. Recommendations for the prevention of pregnancy-associated venous thromboembolism. *The Australian & New Zealand journal of obstetrics & gynaecology*. 2012;52(1):3-13. Epub 2011/09/29.
25. Giannakopoulos B, Krilis SA. The pathogenesis of the antiphospholipid syndrome. *The New England journal of medicine*. 2013;368(11):1033-44. Epub 2013/03/15.
26. Meroni PL, Borghi MO, Raschi E, Tedesco F. Pathogenesis of antiphospholipid syndrome: understanding the antibodies. *Nature reviews Rheumatology*. 2011;7(6):330-9. Epub 2011/05/11.
27. Kauffmann RH, Velkamp JJ, Van Tilburg NH, Van Es LA. Acquired antithrombin III deficiency and thrombosis in the nephrotic syndrome. *The American journal of medicine*. 1978;65(4):607-13. Epub 1978/10/01.
28. Schulman S. Distal deep vein thrombosis--a benign disease? *Thrombosis research*. 2014;134(1):5-6. Epub 2014/05/02.
29. Ouriel K, Green RM, Greenberg RK, Clair DG. The anatomy of deep venous thrombosis of the lower extremity. *Journal of vascular surgery*. 2000;31(5):895-900. Epub 2000/05/11.
30. Schellong SM. Distal DVT: worth diagnosing? Yes. *Journal of thrombosis and haemostasis : JTH*. 2007;5 Suppl 1:51-4. Epub 2007/08/01.
31. Righini M. Is it worth diagnosing and treating distal deep vein thrombosis? No. *Journal of thrombosis and haemostasis : JTH*. 2007;5 Suppl 1:55-9. Epub 2007/08/01.
32. Garry J, Duke A, Labropoulos N. Systematic review of the complications following isolated calf deep vein thrombosis. *The British journal of surgery*. 2016;103(7):789-96. Epub 2016/04/10.
33. Righini M, Paris S, Le Gal G, Laroche JP, Perrier A, Bounameaux H. Clinical relevance of distal deep vein thrombosis. Review of literature data. *Thrombosis and haemostasis*. 2006;95(1):56-64. Epub 2006/03/18.
34. Liao S, Woulfe T, Hyder S, Merriman E, Simpson D, Chunilal S. Incidence of venous thromboembolism in different ethnic groups: a regional direct comparison study. *Journal of thrombosis and haemostasis : JTH*. 2014;12(2):214-9. Epub 2013/11/29.
35. Heit JA. Epidemiology of venous thromboembolism. *Nature reviews Cardiology*. 2015;12(8):464-74. Epub 2015/06/17.
36. Wendelboe AM, Raskob GE. Global Burden of Thrombosis: Epidemiologic Aspects. *Circulation research*. 2016;118(9):1340-7. Epub 2016/04/30.
37. White RH. The epidemiology of venous thromboembolism. *Circulation*. 2003;107(23 Suppl 1):I4-8. Epub 2003/06/20.

38. Heit JA. Venous thromboembolism: disease burden, outcomes and risk factors. *Journal of thrombosis and haemostasis : JTH*. 2005;3(8):1611-7. Epub 2005/08/17.
39. Goldhaber SZ. Risk factors for venous thromboembolism. *Journal of the American College of Cardiology*. 2010;56(1):1-7. Epub 2010/07/14.
40. Zazos P, Kouklakis G, Saibil F. Inflammatory bowel disease and thromboembolism. *World journal of gastroenterology*. 2014;20(38):13863-78. Epub 2014/10/17.
41. Anderson FA, Jr., Wheeler HB, Goldberg RJ, Hosmer DW, Patwardhan NA, Jovanovic B, et al. A population-based perspective of the hospital incidence and case-fatality rates of deep vein thrombosis and pulmonary embolism. The Worcester DVT Study. *Archives of internal medicine*. 1991;151(5):933-8. Epub 1991/05/01.
42. Cohen A, Jeyaindran S, Kim JY, Park K, Sompradeekul S, Tambunan KL, et al. Treating pulmonary embolism in Pacific Asia with direct oral anticoagulants. *Thrombosis research*. 2015;136(2):196-207. Epub 2015/07/04.
43. Philbrick JT, Shumate R, Siadaty MS, Becker DM. Air travel and venous thromboembolism: a systematic review. *Journal of general internal medicine*. 2007;22(1):107-14. Epub 2007/03/14.
44. Schwarz T, Siegert G, Oettler W, Halbritter K, Beyer J, Frommhold R, et al. Venous thrombosis after long-haul flights. *Archives of internal medicine*. 2003;163(22):2759-64. Epub 2003/12/10.
45. Dalen JE. Economy class syndrome: too much flying or too much sitting? *Archives of internal medicine*. 2003;163(22):2674-6. Epub 2003/12/10.
46. Galanaud JP, Sevestre-Pietri MA, Bosson JL, Laroche JP, Righini M, Brisot D, et al. Comparative study on risk factors and early outcome of symptomatic distal versus proximal deep vein thrombosis: results from the OPTIMEV study. *Thrombosis and haemostasis*. 2009;102(3):493-500. Epub 2009/09/01.
47. Chan WS, Spencer FA, Ginsberg JS. Anatomic distribution of deep vein thrombosis in pregnancy. *CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne*. 2010;182(7):657-60. Epub 2010/03/31.
48. Imberti D, Agnelli G, Ageno W, Moia M, Palareti G, Pistelli R, et al. Clinical characteristics and management of cancer-associated acute venous thromboembolism: findings from the MASTER Registry. *Haematologica*. 2008;93(2):273-8. Epub 2008/01/29.
49. Di Nisio M, van Es N, Buller HR. Deep vein thrombosis and pulmonary embolism. *Lancet*. 2016;388(10063):3060-73. Epub 2016/07/05.
50. Bounameaux H, Perrier A, Righini M. Diagnosis of venous thromboembolism: an update. *Vasc Med*. 2010;15(5):399-406. Epub 2010/10/12.
51. Wells PS, Anderson DR, Rodger M, Forgie M, Kearon C, Dreyer J, et al. Evaluation of D-dimer in the diagnosis of suspected deep-vein thrombosis. *The New England journal of medicine*. 2003;349(13):1227-35. Epub 2003/09/26.
52. Righini M, Perrier A, De Moerloose P, Bounameaux H. D-Dimer for venous thromboembolism diagnosis: 20 years later. *Journal of thrombosis and haemostasis : JTH*. 2008;6(7):1059-71.
53. Robert-Ebadi H, Righini M. Management of distal deep vein thrombosis. *Thrombosis research*. 2016;149:48-55. Epub 2016/11/28.
54. Goodacre S, Sampson FC, Sutton AJ, Mason S, Morris F. Variation in the diagnostic performance of D-dimer for suspected deep vein thrombosis. *QJM : monthly journal of the Association of Physicians*. 2005;98(7):513-27. Epub 2005/06/16.
55. Carrier M, Righini M, Djurabi RK, Huisman MV, Perrier A, Wells PS, et al. VIDAS D-dimer in combination with clinical pre-test probability to rule out pulmonary embolism. A systematic review of management outcome studies. *Thrombosis and haemostasis*. 2009;101(5):886-92. Epub 2009/05/01.

56. Robert-Ebadi H, Righini M. Management of distal deep vein thrombosis. *Thrombosis research*. 2017;149:48-55. Epub 2016/11/28.
57. Fraser DG, Moody AR, Morgan PS, Martel AL, Davidson I. Diagnosis of lower-limb deep venous thrombosis: a prospective blinded study of magnetic resonance direct thrombus imaging. *Annals of internal medicine*. 2002;136(2):89-98. Epub 2002/01/16.
58. Hull R, van Aken WG, Hirsh J, Gallus AS, Hoicka G, Turpie AG, et al. Impedance plethysmography using the occlusive cuff technique in the diagnosis of venous thrombosis. *Circulation*. 1976;53(4):696-700. Epub 1976/04/01.
59. Cruickshank MK, Levine MN, Hirsh J, Turpie AG, Powers P, Jay R, et al. An evaluation of impedance plethysmography and 125I-fibrinogen leg scanning in patients following hip surgery. *Thrombosis and haemostasis*. 1989;62(3):830-4. Epub 1989/11/24.
60. Zierler BK. Ultrasonography and diagnosis of venous thromboembolism. *Circulation*. 2004;109(12 Suppl 1):19-14. Epub 2004/03/31.
61. Ginsberg JS, Kearon C, Douketis J, Turpie AG, Brill-Edwards P, Stevens P, et al. The use of D-dimer testing and impedance plethysmographic examination in patients with clinical indications of deep vein thrombosis. *Archives of internal medicine*. 1997;157(10):1077-81. Epub 1997/05/26.
62. Wells PS, Brill-Edwards P, Stevens P, Panju A, Patel A, Douketis J, et al. A novel and rapid whole-blood assay for D-dimer in patients with clinically suspected deep vein thrombosis. *Circulation*. 1995;91(8):2184-7. Epub 1995/04/15.
63. Kearon C, Ginsberg JS, Hirsh J. The role of venous ultrasonography in the diagnosis of suspected deep venous thrombosis and pulmonary embolism. *Annals of internal medicine*. 1998;129(12):1044-9. Epub 1998/12/29.
64. Bernardi E, Camporese G, Buller HR, Siragusa S, Imberti D, Berchio A, et al. Serial 2-point ultrasonography plus D-dimer vs whole-leg color-coded Doppler ultrasonography for diagnosing suspected symptomatic deep vein thrombosis: a randomized controlled trial. *Jama*. 2008;300(14):1653-9.
65. Goodacre S, Sampson F, Thomas S, van Beek E, Sutton A. Systematic review and meta-analysis of the diagnostic accuracy of ultrasonography for deep vein thrombosis. *BMC medical imaging*. 2005;5:6. Epub 2005/10/06.
66. Karande GY, Hedgire SS, Sanchez Y, Baliyan V, Mishra V, Ganguli S, et al. Advanced imaging in acute and chronic deep vein thrombosis. *Cardiovascular diagnosis and therapy*. 2016;6(6):493-507. Epub 2017/01/27.
67. Kearon C, Akl EA, Ornelas J, Blaivas A, Jimenez D, Bounameaux H, et al. Antithrombotic Therapy for VTE Disease: CHEST Guideline and Expert Panel Report. *Chest*. 2016;149(2):315-52. Epub 2016/02/13.
68. Prandoni P, Lensing AW, Prins MH, Bernardi E, Marchiori A, Bagatella P, et al. Residual venous thrombosis as a predictive factor of recurrent venous thromboembolism. *Annals of internal medicine*. 2002;137(12):955-60. Epub 2002/12/18.
69. Prandoni P, Cogo A, Bernardi E, Villalta S, Polistena P, Simioni P, et al. A simple ultrasound approach for detection of recurrent proximal-vein thrombosis. *Circulation*. 1993;88(4 Pt 1):1730-5. Epub 1993/10/01.
70. Gibbs H. The diagnosis of recurrent deep venous thrombosis. *Australian Prescriber*. 2007;30:38-40.
71. Tan M, Bornais C, Rodger M. Interobserver reliability of compression ultrasound for residual thrombosis after first unprovoked deep vein thrombosis. *Journal of thrombosis and haemostasis : JTH*. 2012;10(9):1775-82. Epub 2012/06/26.
72. Schellong SM. Diagnosis of recurrent deep vein thrombosis. *Hamostaseologie*. 2013;33(3):195-200. Epub 2013/07/03.

73. Lagerstedt CI, Olsson CG, Fagher BO, Oqvist BW, Albrechtsson U. Need for long-term anticoagulant treatment in symptomatic calf-vein thrombosis. *Lancet*. 1985;2(8454):515-8. Epub 1985/09/07.
74. Galanaud JP, Quenet S, Rivron-Guillot K, Quere I, Sanchez Munoz-Torrero JF, Tolosa C, et al. Comparison of the clinical history of symptomatic isolated distal deep-vein thrombosis vs. proximal deep vein thrombosis in 11 086 patients. *Journal of thrombosis and haemostasis : JTH*. 2009;7(12):2028-34. Epub 2009/10/02.
75. Righini M, Bounameaux H. Clinical relevance of distal deep vein thrombosis. *Current opinion in pulmonary medicine*. 2008;14(5):408-13. Epub 2008/07/31.
76. Kearon C, Ginsberg JS, Douketis J, Crowther MA, Turpie AG, Bates SM, et al. A randomized trial of diagnostic strategies after normal proximal vein ultrasonography for suspected deep venous thrombosis: D-dimer testing compared with repeated ultrasonography. *Annals of internal medicine*. 2005;142(7):490-6. Epub 2005/04/06.
77. Gibson NS, Schellong SM, Kheir DY, Beyer-Westendorf J, Gallus AS, McRae S, et al. Safety and sensitivity of two ultrasound strategies in patients with clinically suspected deep venous thrombosis: a prospective management study. *Journal of thrombosis and haemostasis : JTH*. 2009;7(12):2035-41.
78. Masuda EM, Kessler DM, Kistner RL, Eklof B, Sato DT. The natural history of calf vein thrombosis: lysis of thrombi and development of reflux. *Journal of vascular surgery*. 1998;28(1):67-73; discussion -4. Epub 1998/07/31.
79. Masuda EM, Kistner RL, Musikasinthorn C, Liquido F, Geling O, He Q. The controversy of managing calf vein thrombosis. *Journal of vascular surgery*. 2012;55(2):550-61. Epub 2011/10/29.
80. Palareti G, Cosmi B, Lessiani G, Rodorigo G, Guazzaloca G, Brusi C, et al. Evolution of untreated calf deep-vein thrombosis in high risk symptomatic outpatients: the blind, prospective CALTHRO study. *Thrombosis and haemostasis*. 2010;104(5):1063-70. Epub 2010/08/10.
81. Macdonald PS, Kahn SR, Miller N, Obrand D. Short-term natural history of isolated gastrocnemius and soleal vein thrombosis. *Journal of vascular surgery*. 2003;37(3):523-7. Epub 2003/03/06.
82. Schwarz T, Buschmann L, Beyer J, Halbritter K, Rastan A, Schellong S. Therapy of isolated calf muscle vein thrombosis: a randomized, controlled study. *Journal of vascular surgery*. 2010;52(5):1246-50. Epub 2010/07/16.
83. Gillet JL, Perrin MR, Allaert FA. Short-term and mid-term outcome of isolated symptomatic muscular calf vein thrombosis. *Journal of vascular surgery*. 2007;46(3):513-9; discussion 9. Epub 2007/08/08.
84. Wardrop D, Keeling D. The story of the discovery of heparin and warfarin. *British journal of haematology*. 2008;141(6):757-63. Epub 2008/03/22.
85. Tahir R. A Review of Unfractionated Heparin and Its Monitoring. *US Pharmacist*. 2007;32(7):HS-26-HS-36.
86. Martel N, Lee J, Wells PS. Risk for heparin-induced thrombocytopenia with unfractionated and low-molecular-weight heparin thromboprophylaxis: a meta-analysis. *Blood*. 2005;106(8):2710-5. Epub 2005/06/30.
87. Ansell J, Hirsh J, Hylek E, Jacobson A, Crowther M, Palareti G. Pharmacology and management of the vitamin K antagonists: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). *Chest*. 2008;133(6 Suppl):160S-98S. Epub 2008/07/24.
88. O'Reilly RA, Aggeler PM. Studies on coumarin anticoagulant drugs. Initiation of warfarin therapy without a loading dose. *Circulation*. 1968;38(1):169-77. Epub 1968/07/01.

89. de Jong PG, Coppens M, Middeldorp S. Duration of anticoagulant therapy for venous thromboembolism: balancing benefits and harms on the long term. *British journal of haematology*. 2012;158(4):433-41. Epub 2012/06/28.
90. van der Meer FJ, Rosendaal FR, Vandenbroucke JP, Briet E. Bleeding complications in oral anticoagulant therapy. An analysis of risk factors. *Archives of internal medicine*. 1993;153(13):1557-62. Epub 1993/07/12.
91. Scherz N, Mean M, Limacher A, Righini M, Jaeger K, Beer HJ, et al. Prospective, multicenter validation of prediction scores for major bleeding in elderly patients with venous thromboembolism. *Journal of thrombosis and haemostasis : JTH*. 2013;11(3):435-43. Epub 2013/01/03.
92. van Es N, Coppens M, Schulman S, Middeldorp S, Buller HR. Direct oral anticoagulants compared with vitamin K antagonists for acute venous thromboembolism: evidence from phase 3 trials. *Blood*. 2014;124(12):1968-75. Epub 2014/06/26.
93. Pollack CV, Jr. Evidence supporting idarucizumab for the reversal of dabigatran. *The American journal of emergency medicine*. 2016;34(11S):33-8. Epub 2016/10/05.
94. Siegal DM, Curnutte JT, Connolly SJ, Lu G, Conley PB, Wiens BL, et al. Andexanet Alfa for the Reversal of Factor Xa Inhibitor Activity. *The New England journal of medicine*. 2015;373(25):2413-24. Epub 2015/11/13.
95. Connolly SJ, Milling TJ, Jr., Eikelboom JW, Gibson CM, Curnutte JT, Gold A, et al. Andexanet Alfa for Acute Major Bleeding Associated with Factor Xa Inhibitors. *The New England journal of medicine*. 2016;375(12):1131-41. Epub 2016/08/31.
96. Brighton CT, Black J, Friedenberg ZB, Esterhai JL, Day LJ, Connolly JF. A multicenter study of the treatment of non-union with constant direct current. *The Journal of bone and joint surgery American volume*. 1981;63(1):2-13. Epub 1981/01/01.
97. Prandoni P, Barbar S, Milan M, Campello E, Spiezia L, Piovela C, et al. Optimal duration of anticoagulation. Provoked versus unprovoked VTE and role of adjunctive thrombophilia and imaging tests. *Thrombosis and haemostasis*. 2015;113(6):1210-5. Epub 2014/08/15.
98. Marchiori A, Mosena L, Prins MH, Prandoni P. The risk of recurrent venous thromboembolism among heterozygous carriers of factor V Leiden or prothrombin G20210A mutation. A systematic review of prospective studies. *Haematologica*. 2007;92(8):1107-14. Epub 2007/07/26.
99. Prandoni P, Lensing AW, Piccioli A, Bernardi E, Simioni P, Girolami B, et al. Recurrent venous thromboembolism and bleeding complications during anticoagulant treatment in patients with cancer and venous thrombosis. *Blood*. 2002;100(10):3484-8. Epub 2002/10/24.
100. Barco S, Corti M, Trinchero A, Picchi C, Ambaglio C, Konstantinides SV, et al. Survival and recurrent venous thromboembolism in patients with first proximal or isolated distal deep vein thrombosis and no pulmonary embolism. *Journal of thrombosis and haemostasis : JTH*. 2017;15(7):1436-42. Epub 2017/04/26.
101. Baglin T, Douketis J, Tosetto A, Marcucci M, Cushman M, Kyrle P, et al. Does the clinical presentation and extent of venous thrombosis predict likelihood and type of recurrence? A patient-level meta-analysis. *Journal of thrombosis and haemostasis : JTH*. 2010;8(11):2436-42. Epub 2010/08/21.
102. Nicolaidis AN, Fareed J, Kakkar AK, Comerota AJ, Goldhaber SZ, Hull R, et al. Prevention and treatment of venous thromboembolism--International Consensus Statement. *International angiology : a journal of the International Union of Angiology*. 2013;32(2):111-260. Epub 2014/01/10.
103. Parisi R, Visona A, Camporese G, Verlato F, Lessiani G, Antignani PL, et al. Isolated distal deep vein thrombosis: efficacy and safety of a protocol of treatment. *Treatment of*

- Isolated Calf Thrombosis (TICT) Study. *International angiology : a journal of the International Union of Angiology*. 2009;28(1):68-72. Epub 2009/02/05.
104. Pinede L, Ninet J, Duhaut P, Chabaud S, Demolombe-Rague S, Durieu I, et al. Comparison of 3 and 6 months of oral anticoagulant therapy after a first episode of proximal deep vein thrombosis or pulmonary embolism and comparison of 6 and 12 weeks of therapy after isolated calf deep vein thrombosis. *Circulation*. 2001;103(20):2453-60. Epub 2001/05/23.
 105. De Martino RR, Wallaert JB, Rossi AP, Zbehlik AJ, Suckow B, Walsh DB. A meta-analysis of anticoagulation for calf deep venous thrombosis. *Journal of vascular surgery*. 2012;56(1):228-37 e1; discussion 36-7. Epub 2012/01/03.
 106. Righini M, Galanaud JP, Guenneveux H, Brisot D, Diard A, Faisse P, et al. Anticoagulant therapy for symptomatic calf deep vein thrombosis (CACTUS): a randomised, double-blind, placebo-controlled trial. *The Lancet Haematology*. 2016;3(12):e556-e62. Epub 2016/11/12.
 107. Kahn SR, Desmarais S, Ducruet T, Arsenault L, Ginsberg JS. Comparison of the Villalta and Ginsberg clinical scales to diagnose the post-thrombotic syndrome: correlation with patient-reported disease burden and venous valvular reflux. *Journal of thrombosis and haemostasis : JTH*. 2006;4(4):907-8. Epub 2006/04/26.
 108. Kahn SR. Measurement properties of the Villalta scale to define and classify the severity of the post-thrombotic syndrome. *Journal of thrombosis and haemostasis : JTH*. 2009;7(5):884-8. Epub 2009/03/27.
 109. Kahn SR, Lamping DL, Ducruet T, Arsenault L, Miron MJ, Roussin A, et al. VEINES-QOL/Sym questionnaire was a reliable and valid disease-specific quality of life measure for deep venous thrombosis. *J Clin Epidemiol*. 2006;59(10):1049-56.
 110. Kahn SR, Kearon C, Julian JA, Mackinnon B, Kovacs MJ, Wells P, et al. Predictors of the post-thrombotic syndrome during long-term treatment of proximal deep vein thrombosis. *Journal of thrombosis and haemostasis : JTH*. 2005;3(4):718-23. Epub 2005/03/01.
 111. Rabinovich A, Kahn SR. The postthrombotic syndrome: current evidence and future challenges. *Journal of thrombosis and haemostasis : JTH*. 2017;15(2):230-41. Epub 2016/11/20.
 112. Tick LW, Kramer MH, Rosendaal FR, Faber WR, Doggen CJ. Risk factors for post-thrombotic syndrome in patients with a first deep venous thrombosis. *Journal of thrombosis and haemostasis : JTH*. 2008;6(12):2075-81. Epub 2008/11/06.
 113. Kahn SR. How I treat postthrombotic syndrome. *Blood*. 2009;114(21):4624-31. Epub 2009/09/11.
 114. Tick LW, Doggen CJ, Rosendaal FR, Faber WR, Bousema MT, Mackaay AJ, et al. Predictors of the post-thrombotic syndrome with non-invasive venous examinations in patients 6 weeks after a first episode of deep vein thrombosis. *Journal of thrombosis and haemostasis : JTH*. 2010;8(12):2685-92. Epub 2010/09/24.
 115. Chitsike RS, Rodger MA, Kovacs MJ, Betancourt MT, Wells PS, Anderson DR, et al. Risk of post-thrombotic syndrome after subtherapeutic warfarin anticoagulation for a first unprovoked deep vein thrombosis: results from the REVERSE study. *Journal of thrombosis and haemostasis : JTH*. 2012;10(10):2039-44. Epub 2012/08/01.
 116. Stain M, Schonauer V, Minar E, Bialonczyk C, Hirschl M, Weltermann A, et al. The post-thrombotic syndrome: risk factors and impact on the course of thrombotic disease. *Journal of thrombosis and haemostasis : JTH*. 2005;3(12):2671-6. Epub 2005/12/20.
 117. Prandoni P, Lensing AW, Prins MH, Frulla M, Marchiori A, Bernardi E, et al. Below-knee elastic compression stockings to prevent the post-thrombotic syndrome: a randomized, controlled trial. *Annals of internal medicine*. 2004;141(4):249-56. Epub 2004/08/18.

118. Brandjes DP, Buller HR, Heijboer H, Huisman MV, de Rijk M, Jagt H, et al. Randomised trial of effect of compression stockings in patients with symptomatic proximal-vein thrombosis. *Lancet*. 1997;349(9054):759-62. Epub 1997/03/15.
119. Kahn SR, Shapiro S, Wells PS, Rodger MA, Kovacs MJ, Anderson DR, et al. Compression stockings to prevent post-thrombotic syndrome: a randomised placebo-controlled trial. *Lancet*. 2014;383(9920):880-8. Epub 2013/12/10.
120. McLafferty RB, Moneta GL, Passman MA, Brant BM, Taylor LM, Jr., Porter JM. Late clinical and hemodynamic sequelae of isolated calf vein thrombosis. *Journal of vascular surgery*. 1998;27(1):50-6; discussion 6-7. Epub 1998/02/25.
121. Villalta S BP, Piccioli A, Lensing A, Prandoni P. Assessment of validity and reproducibility of a clinical scale for the post-thrombotic syndrome (abstract). *Haemostasis*. 1994;24:158a.
122. Schulman S, Angeras U, Bergqvist D, Eriksson B, Lassen MR, Fisher W. Definition of major bleeding in clinical investigations of antihemostatic medicinal products in surgical patients. *Journal of thrombosis and haemostasis : JTH*. 2010;8(1):202-4. Epub 2009/11/03.
123. Kaatz S, Ahmad D, Spyropoulos AC, Schulman S. Definition of clinically relevant non-major bleeding in studies of anticoagulants in atrial fibrillation and venous thromboembolic disease in non-surgical patients: communication from the SSC of the ISTH. *Journal of thrombosis and haemostasis : JTH*. 2015;13(11):2119-26. Epub 2016/01/15.
124. Cogo A, Lensing AW, Koopman MM, Piovella F, Siragusa S, Wells PS, et al. Compression ultrasonography for diagnostic management of patients with clinically suspected deep vein thrombosis: prospective cohort study. *BMJ*. 1998;316(7124):17-20. Epub 1998/02/06.
125. Hull R, Hirsh J, Sackett DL, Taylor DW, Carter C, Turpie AG, et al. Clinical validity of a negative venogram in patients with clinically suspected venous thrombosis. *Circulation*. 1981;64(3):622-5. Epub 1981/09/01.
126. Palareti G, Agnelli G, Imberti D, Moia M, Ageno W, Pistelli R, et al. Do Italian vascular centers look for isolated calf deep vein thrombosis? Analysis of isolated calf deep vein thromboses included in the "Master" Registry. *International angiology : a journal of the International Union of Angiology*. 2008;27(6):482-8. Epub 2008/12/17.
127. Schellong SM. Low risk is not enough: the dilemma of calf vein thrombosis. *The Lancet Haematology*. 2016;3(12):e548-e9. Epub 2016/11/15.
128. Roberts LN, Patel RK, Donaldson N, Bonner L, Arya R. Post-thrombotic syndrome is an independent determinant of health-related quality of life following both first proximal and distal deep vein thrombosis. *Haematologica*. 2014;99(3):e41-3. Epub 2014/01/21.
129. Kahn SR, Shbaklo H, Lamping DL, Holcroft CA, Shrier I, Miron MJ, et al. Determinants of health-related quality of life during the 2 years following deep vein thrombosis. *Journal of thrombosis and haemostasis : JTH*. 2008;6(7):1105-12. Epub 2008/05/10.
130. Roberts LN, Patel RK, Chitongo PB, Bonner L, Arya R. Presenting D-dimer and early symptom severity are independent predictors for post-thrombotic syndrome following a first deep vein thrombosis. *British journal of haematology*. 2013;160(6):817-24. Epub 2013/01/09.
131. Baldwin MJ, Moore HM, Rudarakanchana N, Gohel M, Davies AH. Post-thrombotic syndrome: a clinical review. *Journal of thrombosis and haemostasis : JTH*. 2013;11(5):795-805. Epub 2013/02/26.
132. Patel JP, Roberts LN, Arya R. Anticoagulating obese patients in the modern era. *British journal of haematology*. 2011;155(2):137-49. Epub 2011/08/19.
133. Ferreira M, Barsam S, Patel JP, Czuprynska J, Roberts LN, Patel RK, et al. Heavy menstrual bleeding on rivaroxaban. *British journal of haematology*. 2016;173(2):314-5. Epub 2015/07/28.

134. De Crem N, Peerlinck K, Vanassche T, Vanheule K, Debaveye B, Middeldorp S, et al. Abnormal uterine bleeding in VTE patients treated with rivaroxaban compared to vitamin K antagonists. *Thrombosis research*. 2015;136(4):749-53. Epub 2015/08/15.
135. Martinelli I, Lensing AW, Middeldorp S, Levi M, Beyer-Westendorf J, van Bellen B, et al. Recurrent venous thromboembolism and abnormal uterine bleeding with anticoagulant and hormone therapy use. *Blood*. 2016;127(11):1417-25. Epub 2015/12/24.
136. Hansen ML, Sorensen R, Clausen MT, Fog-Petersen ML, Raunso J, Gadsboll N, et al. Risk of bleeding with single, dual, or triple therapy with warfarin, aspirin, and clopidogrel in patients with atrial fibrillation. *Archives of internal medicine*. 2010;170(16):1433-41. Epub 2010/09/15.
137. Alotaibi GS, Almodaimegh H, McMurtry MS, Wu C. Do women bleed more than men when prescribed novel oral anticoagulants for venous thromboembolism? A sex-based meta-analysis. *Thrombosis research*. 2013;132(2):185-9. Epub 2013/08/13.
138. Prins MH, Lensing AW, Bauersachs R, van Bellen B, Bounameaux H, Brighton TA, et al. Oral rivaroxaban versus standard therapy for the treatment of symptomatic venous thromboembolism: a pooled analysis of the EINSTEIN-DVT and PE randomized studies. *Thrombosis journal*. 2013;11(1):21. Epub 2013/09/24.
139. Iorio A, Agnelli G. Low-molecular-weight and unfractionated heparin for prevention of venous thromboembolism in neurosurgery: a meta-analysis. *Archives of internal medicine*. 2000;160(15):2327-32. Epub 2000/08/06.
140. Akl EA, Briel M, You JJ, Sun X, Johnston BC, Busse JW, et al. Potential impact on estimated treatment effects of information lost to follow-up in randomised controlled trials (LOST-IT): systematic review. *BMJ*. 2012;344:e2809. Epub 2012/05/23.
141. Vervolgyi E, Kromp M, Skipka G, Bender R, Kaiser T. Reporting of loss to follow-up information in randomised controlled trials with time-to-event outcomes: a literature survey. *BMC medical research methodology*. 2011;11:130. Epub 2011/09/23.
142. Baglin TP, Keeling DM, Watson HG, British Committee for Standards in H. Guidelines on oral anticoagulation (warfarin): third edition--2005 update. *British journal of haematology*. 2006;132(3):277-85.
143. Wells PS, Anderson DR, Bormanis J, Guy F, Mitchell M, Gray L, et al. Value of assessment of pretest probability of deep-vein thrombosis in clinical management. *Lancet*. 1997;350(9094):1795-8. Epub 1998/01/15.
144. Ten Cate-Hoek AJ, Prins MH. Management studies using a combination of D-dimer test result and clinical probability to rule out venous thromboembolism: a systematic review. *J Thromb Haemost*. 2005;3(11):2465-70. Epub 2005/09/10.

Appendices

Appendix 1: TWISTER Protocol

Two Weeks of Low Molecular Weight Heparin for Distal Vein Thrombosis (TWISTER)

Version 8 17.3.2016

Eileen Merriman, Huyen Tran, Simon McRae, Sanjeev Chunilal, Tim Brighton

2010

SUMMARY

Approximately 50% of symptomatic episodes of deep vein thrombosis (DVT) will be confined to the calf veins (distal DVT). The proportion of distal DVT that propagate to the proximal veins, increasing the risk of pulmonary embolism, is not known. The best treatment of isolated distal DVT is therefore controversial and options include no treatment, follow-up scanning and treatment of only those patients with thrombus propagating to proximal veins, and full anticoagulation for periods ranging from 2 weeks to 3 months.

There is good evidence that the 3-month thromboembolic risk in patients with a negative CUS that is limited to the proximal veins is low, in the order of 1%. Previous studies have demonstrated that patients treated with a short period of anticoagulation (4-6 weeks) have a low risk of developing recurrent DVT or PE. In addition, the specificity of CUS for distal DVT is lower than that for proximal DVT, increasing the proportion of false positive findings, making it likely that a proportion of patients diagnosed with distal DVT are treated unnecessarily, with the attendant risks of major and fatal haemorrhage.

The need for anticoagulation of patients with distal DVT to prevent recurrent DVT is therefore uncertain, however a survey of current practice suggested that most patients with this condition currently receive antithrombotic therapy. The impact of anticoagulation on initial patient symptoms, and the subsequent risk of the post-thrombotic syndrome are also unclear, and may be a possible alternative justification for antithrombotic therapy.

In this proposed multicentre, prospective, cohort study, we plan to determine if a shorter duration of anticoagulation (minimum 2 weeks) is a safe and effective treatment for isolated distal vein thrombosis.

STUDY HYPOTHESIS

That a limited 2 week duration of anticoagulation for the treatment of isolated distal vein thrombosis leads to complete resolution of symptoms, and a low rate of clinically significant recurrent venous thromboembolism (VTE) during 3 months of follow-up ($\leq 2\%$ clinical symptomatic recurrence).

PRIMARY OBJECTIVES

1. To determine the incidence of symptomatic venous thrombosis at 3 months in patients with isolated distal DVT (provoked and unprovoked) receiving initial treatment with 2 weeks of anticoagulant therapy.

SECONDARY OBJECTIVES

1. To determine the rate of resolution of symptoms in patients in isolated DVT, including the proportion of patients with complete resolution of symptoms after a two-week treatment period.
2. To determine the rate of post thrombotic syndrome at 3 and 6 months in individuals with isolated calf vein thrombosis.
3. To determine the incidence of proximal progression of distal DVT after 2 weeks of anticoagulant therapy.
4. To examine clinical predictors of extension or recurrence in patients with isolated distal DVT.
5. To determine all-cause mortality.

6. To determine the incidence of VTE recurrence at 3 months in those with persistently elevated versus normal D-dimers
7. To determine the time course to symptom resolution in those with persistently elevated D-dimers at 2 weeks.

BACKGROUND

The most widely used non-invasive tool for investigation of deep venous thrombosis (DVT) of the lower limb is venous compression ultrasonography (CUS). Historically, compression was applied to the common femoral vein at the groin and the popliteal vein at the popliteal fossa (2-point ultrasonography). This had the advantages of being rapid, broadly available and reproducible. The main disadvantage is the need to repeat the ultrasound after one week to detect distal DVT extending to proximal veins (serial 2-point ultrasonography). In recent years, colour-coded Doppler USS scans have been employed to evaluate the entire deep venous system, termed complete compression ultrasound (C-CUS). This requires an experienced operator, but has the advantage of requiring only a single test as the incidence of subsequent confirmed DVT during the 3 month period after an initial negative C-CUS has been shown to be low at less than 2%(64). However, this technique will mean that isolated episodes of distal DVT (thrombus involving the infrapopliteal vessels only) will be detected, with such events making up approximately 50% of all diagnosed DVT.

The best treatment of distal DVT is controversial and current options include (a) no anticoagulant treatment, with follow-up scanning and treatment of those which propagate to proximal veins; (b) short term anticoagulation, ranging from 2-6 weeks; and (c) full anticoagulation for 3 months. The BCSH guidelines on oral anticoagulation recommend at least 6 weeks anticoagulation after calf vein thrombosis (142).

The proportion of distal DVT which subsequently propagate to the proximal veins is not known, although estimates range widely, from 0-44% in the literature (31). However, the 3-month thromboembolic risk in patients with a negative CUS of the proximal veins only is low, as demonstrated by Bernadi et al (64) in a randomised study which evaluated 2465 patients with suspected DVT. The 3-month thromboembolic risk was 0.9% in the repeat

CUS group and 1.2% in the single complete CUS group. As a significant proportion of these patients were likely to have had undetected distal DVT, this would suggest that withholding treatment in such patients may be safe. The TICT (Treatment of Isolated Calf Thrombosis) study treated patients with isolated distal deep vein thrombosis with twice daily subcutaneous (sc) administration of a full dose of weight-adjusted low molecular weight heparin (LMWH) for one week, followed by a half dose LMWH given once daily for three weeks (103). Only 2.9% of patients showed progression of thrombosis to proximal deep veins, and the majority of these occurred in patients with unprovoked distal DVT. Similarly, the DOTAVK (Dure'e Optimale du Traitement AntiVitamines K) study (5) randomised patients with isolated calf vein thrombosis to six or twelve weeks of anticoagulation. Recurrence rates were lower in patients with temporary as opposed to permanent risk factors. However, the two treatment regimens were equally efficacious, with recurrence rates of 2% and 3.4% in the 6 week and 12 week groups, respectively.

Taken together, the above data would suggest that the rate of development of proximal DVT in patients with isolated distal DVT is likely to be low regardless of whether no anticoagulation, or short duration anticoagulation of 4 to 6 weeks, is administered. It is important to not overtreat patients with distal DVT, as the risk of haemorrhage with anticoagulation is not insubstantial, with the annual incidence of major haemorrhage estimated at 0.25-3% per year, and the incidence of minor haemorrhage at 6-14% per year (104). Concerns have also been raised regarding the specificity of ultrasound for distal DVT (75), suggesting that a proportion of patients treated for the condition will not actually have thrombus present. Despite these facts, a recent survey of current clinical practice in Australia has found that the majority of patients still receive anticoagulation, with a significant proportion receiving treatment for 3 months or longer (McRae private communication).

It is possible that anticoagulant therapy in patients with distal DVT may have other benefits other than preventing early proximal extension. The impact of anticoagulation on the rate of resolution of symptoms in patients with isolated DVT is unknown, with it being possible that in untreated patients symptoms may take longer to resolve. Similarly, the impact of anticoagulation on the subsequent risk of post-thrombotic syndrome in patients with isolated DVT is also unknown.

We propose that short duration anticoagulation for 2 weeks, therefore limiting the risk of haemorrhagic complications, may be of potential benefit in reducing symptoms and longer-

term sequelae in patients with distal DVT, while still being associated with a low risk of progression or recurrence of DVT and the development of PE. To further examine this theory we plan to perform a prospective observational study of patients with distal DVT where the planned standard therapy will be two weeks of anticoagulant therapy. We also plan to collect data to examine possible predictors of thrombus extension.

SCIENTIFIC QUESTIONS

In patients with distal vein thrombosis treated with short duration (2 weeks) anticoagulation, what is the risk of recurrent venous thrombosis after 3 months, and what proportion of patients will have complete resolution of symptoms at the end of the treatment period?

STUDY DESIGN

The proposal is a multicentre, prospective cohort study. Study patients will be derived from consecutive patients with isolated distal DVT presenting to participating centres.

STUDY POPULATION

Inclusion Criteria

- Patients aged 18 years or older with acute symptomatic provoked or unprovoked unilateral distal vein thrombosis (axial or muscular veins but not involving trifurcation or distal popliteal vein)
- Absence of symptomatic pulmonary embolism

Exclusion Criteria

- Duration of symptoms for >2 weeks
- >72 hours since diagnosis of DVT
- DVT involving trifurcation or more proximal leg veins on imaging
- Concomitant superficial vein thrombosis
- Prior DVT
- Active malignancy ie present at time of diagnosis, or on treatment, or treatment completed within 3 months
- Ongoing risk factors for propagation e.g. immobility (>50% of day in bed or ≥ 72 hours), plaster cast or non-weight bearing
- Other indication for therapeutic anticoagulation (e.g. AF)
- Active gastro-oesophageal ulceration or bleeding

- Other high risk for bleeding (e.g. recent neurosurgery, vascular retinopathy, coagulopathy)
- Platelet count $<80 \times 10^9/L$
- Renal impairment (CrCl $<30\text{ml/min}$)
- Pregnancy or within 6 weeks post-partum

TREATMENT

Therapeutic anticoagulation LMWH at a weight adjusted dose, or rivaroxaban therapeutic dose given for an initial two week period.

STUDY OUTCOMES

Primary Outcomes

- Symptomatic recurrence of venous thrombosis (DVT, non-fatal and fatal pulmonary embolism) within 3 months.

Secondary Outcomes

- Asymptomatic proximal thrombus extension at 2 weeks
- Time course of symptom resolution including time to complete resolution of symptoms, and the proportion of patients with complete resolution at two weeks.
- All-cause mortality
- Post-thrombotic syndrome at 6 months
- Predictors of recurrent or progressive DVT or new PE

Safety Outcomes

Bleeding (fatal, major, clinically relevant minor)

Adverse effects of any study interventions which lead to cessation of therapy.

DEFINITIONS & DIAGNOSTIC CRITERIA

Distal vein thrombosis:

Incompressible thrombus on ultrasound in **infrapopliteal veins**, ie involving one or more of: posterior tibial veins, peroneal veins, anterior tibial veins and muscular calf veins.

Exclusion of DVT:

Via combination of:

- (1) Low clinical probability and negative D-dimer (143, 144)
- (2) Low probability + abnormal D-dimer **or** likely clinical probability **and** negative C-CUS examination (77)
- (3) In the case of an inconclusive C-CUS, a repeat C-CUS will be performed at one week and if this is still inconclusive, then venography will be performed.

Extension of distal vein thrombosis:

Ultrasonographically-proven extension to the popliteal, femoral or iliac vein; or appearance of a proximal DVT in the contralateral leg.

Pulmonary embolism:

Diagnosed in clinically suspected patients using widely accepted clinical criteria and either high probability V-Q scan, positive CTPA, or pulmonary angiogram demonstrating emboli.

Recurrent DVT:

- (1) Reappearance of thrombus in a previously involved vein, that was demonstrated to have resolved on subsequent USS
- (2) New thrombus in a different anatomical calf vein

Major bleeding:

- (1) Fatal bleeding and/or
- (2) Symptomatic bleeding in a critical area or organ e.g. intracranial, intraspinal, intraocular, retroperitoneal, intraarticular or pericardial, or intramuscular with compartment syndrome, and/or
- (3) Bleeding causing a fall in haemoglobin level 20g/dL or more, or leading to transfusion of two or more units of whole blood or red cells.

Clinically relevant minor bleeding:

Bleeding which does not meet the definition for major bleeding, but which causes disturbance to/interruption of normal activity.

Post-thrombotic syndrome:

The presence of post-thrombotic syndrome will be assessed using the Villalta PTS scale (113) (table 1).

Long distance travel:

Air travel exceeding 6 hours duration.

STUDY FLOW (Appendix 1)

Baseline Visit

Patients will be enrolled in the study as soon as possible after the diagnosis of isolated distal DVT. Informed consent will be obtained in all patients prior to the initiation of any study procedures. All patients will be treated with therapeutic anticoagulation in the form of low molecular weight heparin (LMWH) or oral rivaroxaban for an initial two week period. Compression stockings may be used (and their use recorded) at the discretion of the investigator. Patients will be asked to record symptoms of DVT, namely pain and limitation of activity, daily in a diary. Pain will be assessed using a visual analogue scale (VAS) and functional impairment will be documented using an adapted version of the VEINES-sym scale. Symptoms will be documented until complete resolution. Patients will be instructed to return for review if at any stage they experience worsening or new symptoms.

Potential prognostic factors for proximal extension of thrombus will be collected at the baseline visit. These will include DOB, weight and height, location of clot, whether the thrombus was provoked vs non-provoked, risk factors, and family history of VTE. Patients enrolled in the study will *have a D-dimer performed at diagnosis*, as part of standard of care.

2 week visit

All patients will be reviewed by study investigators at 2 weeks, and will have repeat ultrasonography of the affected limb and a repeat d-dimer. Patients will be then managed according to ultrasound appearance and symptom resolution as follows:

a) If symptoms have completely resolved at 2 weeks and there is no change or regression of thrombus on USS, anticoagulation will be stopped.

b) If there are ongoing symptoms with unchanged appearances on USS, patients will receive anticoagulation for a further 4 weeks. Patients will continue to record symptoms of DVT daily until complete resolution. Patients will be then re-evaluated at the 6 week mark, and a repeat USS and repeat d-dimer will be performed. If symptoms have completely resolved anticoagulation will be stopped. Patients with persistent symptoms or proximal extension on USS will be managed at the discretion of the investigator.

c) If there is thrombus extension into the proximal veins on the 2 week USS, patients will be taken off study and will continue anticoagulation with the duration at the discretion of the investigator.

3 month visit

All patients completing 2 or 6 weeks of therapy will be followed for objectively proven recurrent symptomatic DVT or PE for a minimum of 3 months. Patients with ongoing or recurrent symptoms during the 3 month follow-up period will present for investigation of suspected recurrent VTE. Patients will receive written instructions regarding symptoms/signs suspicious for recurrence, with a contact number should they have any concerns. Patients will be reviewed at 3 months to confirm that there have been no episodes of recurrent VTE during this time period, and to assess for post-thrombotic syndrome using the Villalta PTS scale (Appendix 2). Functional impairment will be documented using an adapted version of the VEINES-sym scale.

6 month visit

Where possible, patients will be reviewed at 6 months for symptomatic, clinically relevant late recurrence and post-thrombotic syndrome assessment. The latter will be assessed using the Villalta PTS scale. Functional impairment will be documented using an adapted version of the VEINES-sym scale.

Patients with thrombus extension into proximal veins at any stage of the study are primary treatment failures and will be managed by their doctors independently of the study.

Symptom documentation

Pain Score – will be assessed each day prior to bed, using a 0-10 cm VAS, with 0 cm = no pain and 10cm = severe pain (Appendix 3.)

Functional Score – will be recorded using questions adapted from the VEINES-sym questionnaire (Appendix 4).

STOPPING RULES

The literature suggests our management approach will be safe and effective. Interim analysis of the primary outcome will be performed after 100, 200 and 300 patients and study will be stopped if the lower 99% confidence limit of the composite primary outcome (symptomatic proximal extension or symptomatic recurrent vein thrombosis) is greater than 2%. This rate of thrombus propagation/embolism is clinically relevant and would suggest extended anticoagulant therapy is required.

Below are the suggested event rates, point estimates and **99%CI**.

100	7/100 7%	2.1-16%
150	9/150	2.1-13%
200	11/200 5.5%	2.2-11%

MINIMISATION OF BIAS

Standardised, validated criteria will be used to diagnose and exclude distal vein thrombosis and extension/recurrence, and complications, as defined above.

DATA COLLECTION

Results will be recorded electronically using Microsoft ACCESS software.

STATISTICAL ANALYSIS

Primary Outcomes

- Symptomatic recurrence of venous thrombosis within 3 months
- *simple descriptive statistics for baseline variable with simple frequencies, proportions and means for normal distribution of data. The primary outcome will be expressed as the proportion of patients that have recurred (proportion of patients and 95% CI)*

Secondary Outcomes

- Asymptomatic proximal thrombus extension at 2 weeks:
 - *proportion of patients + 95% CI*
- Time course of symptom resolution including time to complete resolution of symptoms, and the proportion of patients with complete resolution at two weeks:
 - *descriptive statistics*
- All-cause mortality:
 - *descriptive statistics*

- Post-thrombotic syndrome at 3 and 6 months:
- *descriptive and comparing those on 2/52 Clexane vs those receiving extended anticoagulation, using a Chi square or Fishers exact test.*
- Predictors of recurrent or progressive DVT or new PE:
- *depending on rate of recurrence/progression*
- VEINES QOL outcomes:

Safety Outcomes

Bleeding (fatal, major, clinically relevant minor):

- *descriptive statistics*

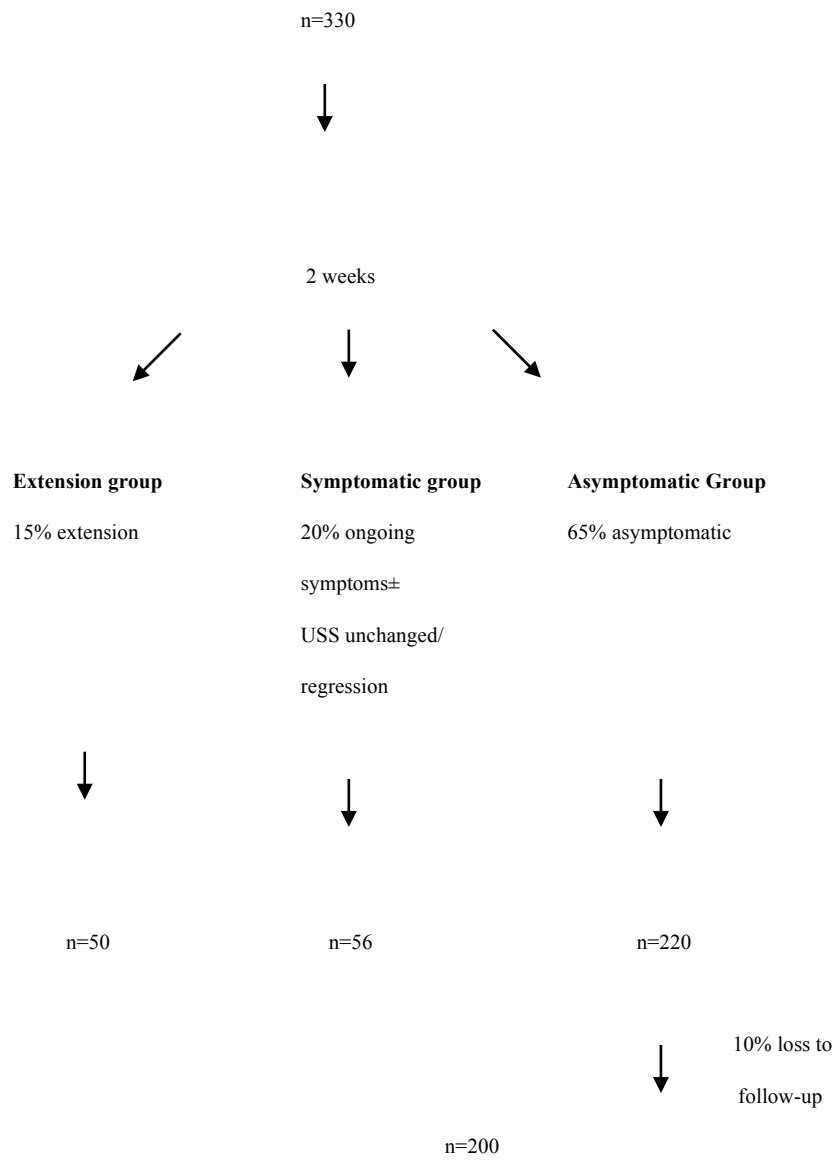
SAMPLE SIZE DETERMINATION

At least 330 patients are required to provide clinically useful confidence estimates of the primary outcomes. This is based on the assumption that:

- (a) ***Extension group*** - 15% of all the enrolled patients will experience proximal extension on USS at 2 weeks (as reported in the literature)
- (b) ***Asymptomatic group*** - 65% will be asymptomatic at 2 weeks. 10% of this arm is anticipated to be lost to follow-up or need anticoagulant therapy in the study arm.
- (c) ***Symptomatic group*** - the remaining 20% will therefore require ongoing therapy

This will give us an estimated sample size of 200 patients in the **asymptomatic group** with no symptoms and no change/regression of thrombus on USS. The sample size calculation for this arm with proportions and 95% CI are provided in appendix 5.

However the sample size of 330 may be an overestimate, therefore recruitment can cease when 200 patients have been enrolled in the experimental arm (2 weeks therapeutic anticoagulation ie. group b) or when a total of 330 patients have been recruited.



POTENTIAL FUTURE STUDIES

This is a prospective cohort study. A randomised control trial will be the optimal study to further address this clinical question, if a state of clinical equipoise is still felt to exist.

SIGNIFICANCE

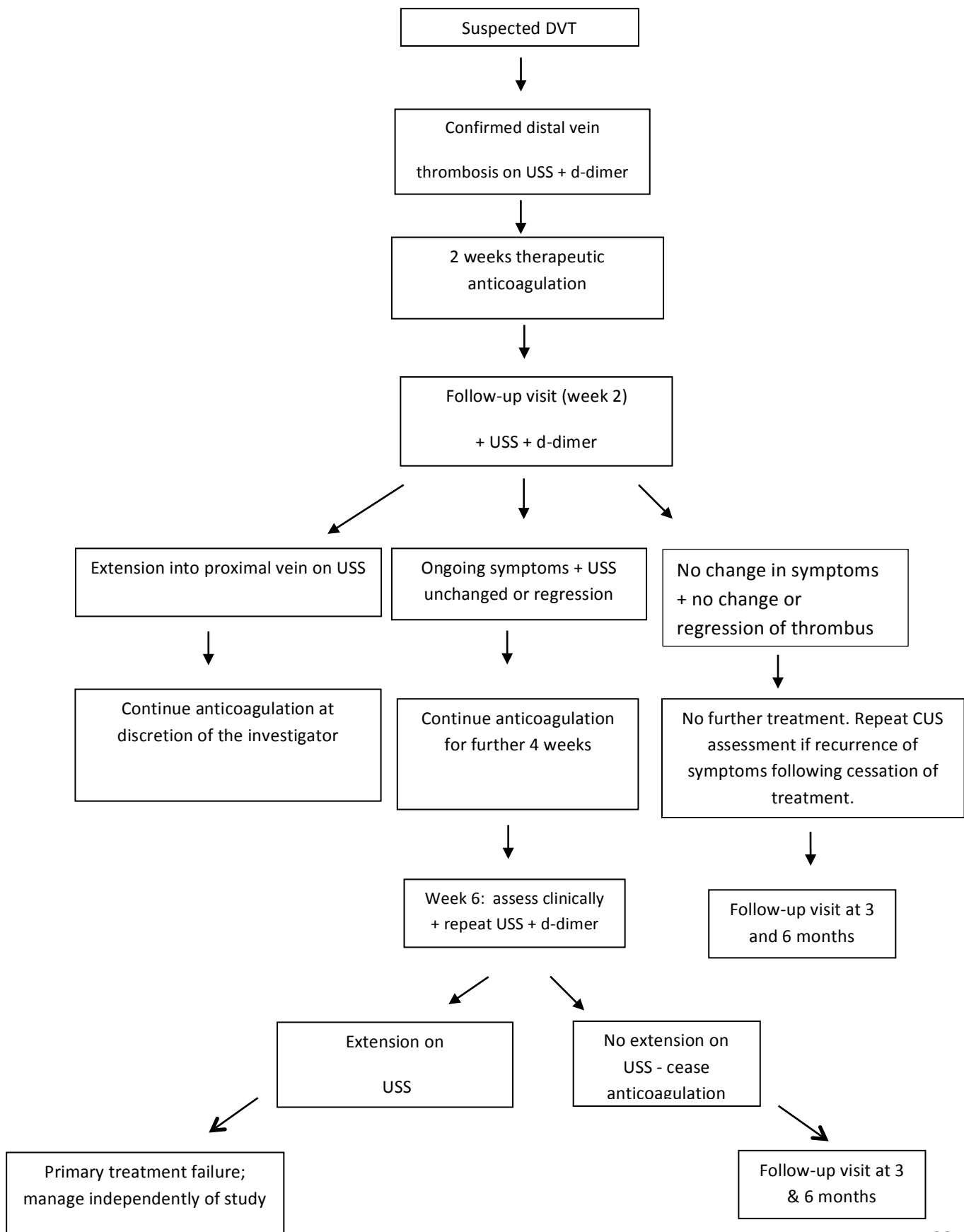
This study is important as there is still much controversy regarding the duration of treatment for distal DVT. It will determine whether limited duration of anticoagulation for distal DVT is safe and efficacious, therefore improving the management of this group of patients. If our hypothesis is correct, limited anticoagulation could be expected to be associated with fewer episodes of both major and minor bleeding. There would also be a cost saving in terms of need for reduced anticoagulant and fewer laboratory tests for monitoring whilst on therapy.

ETHICAL ISSUES

The optimal treatment for distal DVT is not known. The risks of under-treatment of DVT versus risk of fatal and major haemorrhage with over-treatment of DVT need to be balanced, and clinical trials are required to determine this. Written consent will be obtained prior to study entry. Subjects may withdraw at any time. Details from a pilot study of 28 patients treated through Southern Health have been supplied (Appendix 5), which thus far indicate that our proposed treatment is safe and effective.

APPENDIX

Appendix 1: Experimental Flow



Appendix 2a. Villalta PTS scale..(113)

Symptoms and Clinical signs	None	Mild	Moderate	Severe
Symptoms				
Pain	0 points	1 point	2 points	3 points
Cramps	0 points	1 point	2 points	3 points
Heaviness	0 points	1 point	2 points	3 points
Paresthesia	0 points	1 point	2 points	3 points
Pruritus	0 points	1 point	2 points	3 points
Clinical signs				
Pretibial edema	0 points	1 point	2 points	3 points
Skin induration	0 points	1 point	2 points	3 points
Hyperpigmentation	0 points	1 point	2 points	3 points
Redness	0 points	1 point	2 points	3 points
Venous ectasia	0 points	1 point	2 points	3 points
Pain on calf compression	0 points	1 point	2 points	3 points
Venous ulcer	Absent	Present		

Points are summed into a total score (range 0–33). PTS is defined by a total score of ≥ 5 or the presence of a venous ulcer. PTS is classified as *mild* if the Villalta score is 5–9, *moderate* if the Villalta score is 10–14, and *severe* if the Villalta score is ≥ 15 or a venous ulcer is present. To use the Villalta score as a continuous measure, it is recommended that patients who meet criteria for severe PTS based solely on the presence of an ulcer (i.e. total Villalta score is < 15) be assigned a score of 15 [2].

Appendix 2b: Visual Guide for the Assessment of Post-Thrombotic Syndrome

VISUAL GUIDE FOR THE ASSESSMENT OF POST-THROMBOTIC SYNDROME					
No or Minimal		Mild	Moderate	Severe	
Edema		 <p>No loss of bony landmarks; no pitting with pressure over ankle or shin</p>	 <p>Minimal loss of bony landmarks; shallow pitting with pressure over ankle or shin</p>	 <p>Noticeable swelling and loss of bony landmarks; moderate pitting with pressure over ankle or shin</p>	 <p>Severe swelling and loss of bony landmarks; deep pitting with pressure over ankle, shin or knee</p>
Hyperpigmentation		 <p>None</p>	 <p>Faint, speckled brownish discoloration around ankle</p>	 <p>Obvious brownish discoloration around ankle and lower shin</p>	 <p>Patches of dark, confluent, brownish discoloration around ankle and shin</p>
Venous ectasia		 <p>No venules or varicose veins</p>	 <p>A few faint reddish or purplish venules around the ankle or foot area</p>	 <p>Prominent purplish venules around the ankle and foot area</p>	 <p>Numerous confluent and prominent purple venules or varicose veins around the ankle, shin or elsewhere on leg</p>
Redness		 <p>Normal color of leg</p>	 <p>Faint redness of foot or lower leg</p>	 <p>Moderate redness of foot or lower leg</p>	 <p>Pronounced redness or purplish color of foot and lower leg</p>
Skin induration		 <p>Skin of shin and ankle not thickened and freely mobile over underlying tissue or bony structures</p>	 <p>Skin of shin or ankle slightly thickened or slightly adherent to underlying tissue or bony structures</p>	 <p>Skin of shin or ankle moderately thickened or moderately adherent to underlying tissue or bony structures</p>	 <p>Skin of shin and ankle very thickened or tightly adherent to underlying tissue or bony structures</p>
Pain during calf compression		 <p>None</p>	 <p>Present; patient says pain is mild in intensity</p>	 <p>Present; patient says pain is moderate in intensity</p>	 <p>Present; patient says pain is severe in intensity</p>
Ulcer		<div><p>Scored as present (i.e. any ulcer) or absent.</p><p>Ulcer is typically located on medial aspect of lower leg and may be open or healed</p></div> 			
		<div><p>Note</p><p>Signs may be less apparent in patients with brown or black skin.</p></div>			
Developed for the SOX Trial by Dr. Susan Kahn, Tatiana Vydykhan and Anthony Vrakotas 2018					

Developed for the SOX Trial by Dr. Susan Kahn, Tatiana Vidykhan and Anthony Vrakotas 2003.
Adapted from Villalta et al. *Haemostasis* 1994.

Appendix 3. Visual Analogue Scale (VAS) Pain Score

No pain

Worst pain ever

0 1 2 3 4 5 6 7 8 9 10

Appendix 4. Functional impact assessment(109)

<p>4. The following items are about activities that you might do in a typical day. Does your leg problem now limit you in these activities? If so, how much ?</p> <p><i>(Check one box on each line)</i></p>				
	I do not work	YES, Limited A Lot	YES, Limited A Little	NO, Not Limited At All
a. Daily activities at work	<input type="checkbox"/> 0	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3
b. Daily activities at home (e.g. housework, ironing, doing odd jobs/repairs around the house, gardening, etc...)		<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3
c. Social or leisure activities in which you are <u>standing</u> for long periods (e.g. parties, weddings, taking public transportation, shopping, etc...)		<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3
d. Social or leisure activities in which you are <u>sitting</u> for long periods (e.g. going to the cinema or the theater, travelling, etc...)		<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3

<p>5. During the <u>past 4 weeks</u>, have you had any of the following problems with your work or other regular daily activities <u>as a result of your leg problem</u>?</p> <p><i>(check one box on each line)</i></p>		
	YES	NO
a. Cut down the amount of time you spent on work or other activities	<input type="checkbox"/> 1	<input type="checkbox"/> 2
b. Accomplished less than you would like	<input type="checkbox"/> 1	<input type="checkbox"/> 2
c. Were limited in the kind of work or other activities	<input type="checkbox"/> 1	<input type="checkbox"/> 2
d. Had difficulty performing the work or other activities (for example, it took extra effort)	<input type="checkbox"/> 1	<input type="checkbox"/> 2

Appendix 5. Sample Size Calculation for Study Arm.

Sample size	Event rate	Proportion and 95% CI	99%CI
100	1/100	1.0 (0.03-5.5%)	(0.06-7.2%)
	2/100	2.0 (0.2-7%)	(0.1-8.9%)
	3/100	3.0 (0.6-8.5%)	(0.3-10.6%)
150	1/150	0.6 (0.02-3.7%)	(0.006-4.9)
	2/150	1.3 (0.16-4.7)	(0.07-6%)
	3/150	2.0 (0.5-5.7%)	(0.2-7.1%)
	4/150	2.6 (0.7-6.7)	(0.4-8.2%)
175	1/175	0.6 (0.02-3.1%)	(0.0006-4.2)
	2/175	1.1 (0.1-4.1)	(0.05-5.2)
	3/175	1.7 (0.4-4.9%)	(0.2-6.1)
	4/175	2.3 (0.6-5.7%)	(0.3-7.0%)
200	1/200	0.5 (0.02-2.8%)	(0.006-3.7%)
	2/200	1.0 (0.12-3.6)	(0.05-4.6%)
	3/200	1.5 (0.3-4.3%)	(0.1-5.4%)
	4/200	2.0 (0.5-5.0%)	0.3-6.2%)
	5/200	2.5 (0.8-5.7%)	(0.5-6.9%)

250	2/250	0.8 (0.09-2.9%)	(0.04-3.6)
	3/250	1.2 (0.2-3.5%)	(0.1-4.3)
	4/250	1.6 (0.4-4.1)	
	5/250		

Appendix 6. Pilot Study

A cohort of 28 patients with distal DVT presenting to Southern Health for treatment over the past 12 months have been treated with less than or equal to 3 weeks anticoagulation (range 1-3 weeks). All have been treated with therapeutic dose Enoxaparin or warfarin except for one patient, who was treated with prophylactic dose Clexane for unknown reasons. The results are as shown in table 2. 24/28 patients have had a 2 week USS, none of which have shown proximal extension. 16/28 patients have had their 3 month follow-up completed so far, with no evidence of proximal extension or recurrence. This is limited data but indicates that our proposed treatment is feasible and safe.

Distal DVT Outcome in Patients Receiving ≤ 3 Weeks Anticoagulation

<i>2 Week CUS</i>	<i>Symptoms at 2 Weeks</i>	<i>3 Month Follow-Up</i>
5 Resolved	15 No Symptoms	14 Asymptomatic
8 Partial Resolution	3 Symptoms*	1 Not Recorded#
13 Unchanged	10 Not Recorded	1 “Not Right” #
4 Not done		10 Pending
0 Extension		2 Lost to follow-up
		0 Recurrences

*CUS Unchanged

CUS - Resolution

Appendix 2: D-dimer Sub-Study

Two Weeks of Low Molecular Weight Heparin for Distal Vein Thrombosis (TWISTER): D-dimer sub-study

Version 1: 28/03/2012

Eileen Merriman, Huyen Tran, Simon McRae, Sanjeev Chunilal, Tim Brighton

2012

SUMMARY

Approximately 50% of symptomatic episodes of deep vein thrombosis (DVT) will be confined to the distal veins (calf DVT). The best treatment of isolated distal DVT is controversial, and the aim of the TWISTER study (Two Weeks of Low Molecular Weight Heparin for Distal Deep Vein Thrombosis) is to determine if a shorter duration of anticoagulation (minimum 2 weeks) is a safe and effective treatment for isolated distal vein thrombosis. As part of this study, blood samples will be taken at two centres (Monash Medical Centre, Victoria and North Shore Hospital, NZ) for D-dimer analysis. The primary purpose of the D-dimer analysis would be to explore whether an elevated D-dimer at the end of treatment could identify a group at increased risk for recurrence of venous thromboembolism (VTE).

STUDY HYPOTHESIS

That a persistently elevated D-dimer performed at the time of cessation of anticoagulation will predict for an increased risk of VTE recurrence, and will be associated with a longer duration of symptoms post distal deep vein thrombosis.

PRIMARY OBJECTIVE

1. To determine the incidence of VTE recurrence at 3 months in those with persistently elevated versus normal D-dimers

SECONDARY OBJECTIVE

1. To determine the time course to symptom resolution in those with persistently elevated D-dimers at 2 weeks.

BACKGROUND

It is known that patients whose first VTE event was triggered by reversible risk factors have a lower risk of recurrence and need shorter anticoagulation than patients whose event was unprovoked (idiopathic) or who carry persistent risk factors. Factors shown to predict for increased risk of recurrence include increasing patient age, active cancer, major thrombophilias, male sex, the presence of residual thrombosis on repeat scanning, and elevated D-dimers at end of treatment. (Heit Ann Haematol et al 2012; Cosmi et al TH 2011; McRae et al Lancet 2006).

The D-dimer is a fibrin degradation product and a global marker of coagulation activation. The D-dimer level is measured in the plasma by use of well-standardised assays that are widely used for the diagnosis of acute VTE. A negative D-dimer result measured at or after cessation of coagulation has been shown to predict for a low risk of VTE recurrence in patients with both provoked and unprovoked VTE (Palareti et al Circ 2003; Eichinger et al JAMA 2010; Douketis et al Ann Int Med 2010; Cosmi et al TH 2011). A meta-analysis of 7 prospective studies in patients with a first unprovoked VTE showed that the HR for recurrent VTE in all patients with a positive versus negative D-dimer result was 2.59 (CI 1.90-3.52) (Douketis et al Ann Int Med 2010).

It has been proposed that patients with a persistently elevated D-dimer at the end of treatment warrant a longer course of anticoagulation. Numerous studies have been performed on patients with proximal DVT but data on those with distal DVT is lacking. We aim to determine whether patients with a persistently elevated D-dimer at the end of two weeks of full dose Clexane for isolated symptomatic distal DVT have a higher risk of recurrence when compared with patients with a normal D-dimer at this time. In addition, we will examine whether patients with a persistently elevated D-dimer at this time point have a longer time course to symptom resolution.

STUDY DESIGN

This is a multicentre, prospective cohort study. Study patients will be derived from patients enrolled in the TWISTER study at two sites, North Shore Hospital and Monash Medical Centre.

STUDY POPULATION

Inclusion Criteria

- Patients aged 18 years or older with acute symptomatic provoked or unprovoked unilateral distal vein thrombosis (axial or muscular veins but not involving trifurcation or distal popliteal vein)
- Enrolled on TWISTER study at either Monash Medical Centre or North Shore Hospital

TREATMENT

Therapeutic anticoagulation LMWH at a weight adjusted dose, given for an initial two week period.

STUDY OUTCOMES

Primary Outcomes

- Symptomatic recurrence of venous thrombosis (DVT, non fatal and fatal pulmonary embolism) within 3 months in those with persistently elevated versus negative D-dimers after 2 weeks of therapeutic anticoagulation.

Secondary Outcomes

- Time course to resolution of symptoms in those with persistently elevated versus negative D-dimers after 2 weeks of therapeutic anticoagulation.

DEFINITIONS & DIAGNOSTIC CRITERIA

Distal vein thrombosis:

Incompressible thrombus on ultrasound, or intraluminal defect on venography, in **infrapopliteal veins**, ie involving one or more of: posterior tibial veins, peroneal veins, anterior tibial veins and muscular calf veins.

Exclusion of DVT:

Via combination of:

- (4) Low clinical probability and negative D-dimer (143, 144)
- (5) Low probability + abnormal D-dimer **or** likely clinical probability **and** negative C-CUS examination (77)

- (6) In the case of an inconclusive C-CUS, a repeat C-CUS will be performed at one week and if this is still inconclusive, then venography will be performed.

Extension of distal vein thrombosis:

Ultrasonographically-proven extension to the popliteal, femoral or iliac vein; or appearance of a proximal DVT in the contralateral leg.

Pulmonary embolism:

Diagnosed in clinically suspected patients using widely accepted clinical criteria and either high probability V-Q scan, positive CTPA, or pulmonary angiogram demonstrating emboli.

Recurrent DVT:

- (3) Reappearance of thrombus in a previously involved vein, that was demonstrated to have resolved on subsequent USS
- (4) New thrombus in a different anatomical calf vein

Major bleeding:

- (4) Fatal bleeding and/or
- (5) Symptomatic bleeding in a critical area or organ e.g. intracranial, intraspinal, intraocular, retroperitoneal, intraarticular or pericardial, or intramuscular with compartment syndrome, and/or
- (6) Bleeding causing a fall in haemoglobin level 20g/dL or more, or leading to transfusion of two or more units of whole blood or red cells.

Clinically relevant minor bleeding:

Bleeding which does not meet the definition for major bleeding, but which causes disturbance to/interruption of normal activity.

STUDY FLOW

Baseline Visit

All patients will be treated with therapeutic anticoagulation in the form of low molecular weight heparin (LMWH) for an initial two week period. Compression stockings may be used (and their use recorded) at the discretion of the investigator. Patients will be asked to record

symptoms of DVT, namely pain and limitation of activity, daily in a diary. Pain will be assessed using a visual analogue scale (VAS) and functional impairment will be documented using an adapted version of the VEINES-sym scale. Symptoms will be documented until complete resolution. Patients will be instructed to return for review if at any stage they experience worsening or new symptoms.

Patients enrolled in the study will ***have a D-dimer performed at diagnosis***, as part of standard of care. Informed consent will be obtained in all patients for the purposes of collecting a D-dimer after 2 weeks of therapeutic anticoagulation, and again at 6 weeks for patients in group B (requiring 6 weeks anticoagulation, as per TWISTER study).

2 Week Visit

All patients will be reviewed by study investigators at 2 weeks, and will have repeat ultrasonography of the affected limb ***and a repeat D-dimer***. Patients will be then managed according to ultrasound appearance and symptom resolution as follows:

- a) If symptoms have completely resolved at 2 weeks and there is no change or regression of thrombus on USS, anticoagulation will be stopped.
- b) If there are ongoing symptoms with unchanged appearances on USS, patients will receive anticoagulation for a further 4 weeks. Patients will continue to record symptoms of DVT daily until complete resolution. Patients will be then re-evaluated at the 6 week mark, and a repeat USS will be performed ***and D-dimer*** will be performed. If symptoms have completely resolved anticoagulation will be stopped. Patients with persistent symptoms or proximal extension on USS will be managed at the discretion of the investigator.
 - a. If there is thrombus extension into the proximal veins on the 2 week USS, patients will be taken off study and will continue anticoagulation with the duration at the discretion of the investigator.

3 month visit

All patients completing 2 or 6 weeks of therapy will be followed for objectively proven recurrent symptomatic DVT or PE for a minimum of 3 months. Patients with ongoing or recurrent symptoms during the 3 month follow-up period will present for investigation of suspected recurrent VTE. Patients will receive written instructions regarding symptoms/signs suspicious for recurrence, with a contact number should they have any concerns. Patients

will be reviewed at 3 months to confirm that there have been no episodes of recurrent VTE during this time period.

DATA COLLECTION

Results will be recorded electronically using Microsoft ACCESS software.

STATISTICAL ANALYSIS

Primary Outcomes

- Symptomatic recurrence of venous thrombosis within 3 months in those with persistently positive versus negative D-dimers at two weeks
- *simple descriptive statistics for baseline variable with simple frequencies, proportions and means for normal distribution of data. The primary outcome will be expressed as the proportion of patients that have recurred (proportion of patients and 95% CI); the recurrence rates between two groups will be compared using a paired t-test*

Secondary Outcomes

- Time course of symptom resolution including time to complete resolution of symptoms, and the proportion of patients with complete resolution at two weeks, will be compared in those with persistently positive versus negative D-dimers at two weeks
- *descriptive statistics; time course of symptom resolution including time to resolution of symptoms, and the proportion of patients with complete resolution at two weeks*

SIGNIFICANCE

There is still much controversy regarding the duration of treatment for distal DVT. If a persistently elevated D-dimer at the cessation of anticoagulation in this patient group can be shown to predict for VTE recurrence and/or longer duration of symptoms, then this could be useful in tailoring treatment for this patient group.

Appendix 3: Ethics Approval

3.1 New Zealand Ethics Approval



Multi-region Ethics Committee

Ministry of Health
No. 1 The Terrace
PO Box 5013
Wellington 6145
Phone (04) 816 2403
(04) 816 2646
Fax (04) 496 2343
Email: multiregion_ethicscommittee@moh.govt.nz

6 July 2011

Dr Eileen Merriman
Haematology Department
North Shore Hospital
Shakespeare Road (private Bag 93-503)
Takapuna - Auckland

Dear Dr Merriman -

Re: Ethics ref: **MEC/11/04/034** (please quote in all correspondence)
Study title: Two weeks of Low Molecular Weight Heparin for Distal Vein Thrombosis (TWISTER)
Investigators: Dr Eileen Merriman, Dr Sharon Jackson, Dr Laura Young, Dr Mark P Smith

This study was given ethical approval by the Multi-region Ethics Committee on the **6th July 2011**.

Approved Documents

The following documents were submitted on 24th March 2011 and received by the Multi-region Ethics Committee on 29th March 2011:

National Application Form for Ethical Approval of a Research Project which includes:

- Part 4 Declaration
- Form A
- Patient Information Sheet and Consent Form (Version 1, dated 25th Feb 2011)
- Locality assessment for Waitemata District Health Board
- Evidence of Maori Consultation for Waitemata District Health Board
- Protocol, version 4, dated 18th January 2011

The following documents were submitted on 17th May 2011 and received by the Multi-region Ethics Committee on 23rd May 2011:

- Letter confirming ethics approval in Australia
- Locality Assessment for Canterbury District Health Board
- Maori Consultation approval letter for Canterbury District Health Board
- Amended pages of the National Application Form

The following documents were submitted on 14th June 2011 and received by the Multi-region Ethics Committee on 21st June 2011:

- Revised generic Patient Information Sheet, Version 3, dated 14th June 2011 with the corrected typo as requested and corrected additional typo on Page 4
- The personalised Participant Information Sheet and Consent Form for Waitemata District Health Board and Canterbury District Health Board.

This approval is valid until **1st April 2013**, provided that Annual Progress Reports are submitted (see below).

Access to ACC

For the purposes of section 32 of the Accident Compensation Act 2001, the Committee is satisfied that this study is not being conducted principally for the benefit of the manufacturer or distributor of the medicine or item in respect of which the trial is being carried out. Participants injured as a result of

treatment received in this trial will therefore be eligible to be considered for compensation in respect of those injuries under the ACC scheme.

Amendments and Protocol Deviations

All significant amendments to this proposal must receive prior approval from the Committee. Significant amendments include (but are not limited to) changes to:

- the researcher responsible for the conduct of the study at a study site
- the addition of an extra study site
- the design or duration of the study
- the method of recruitment
- information sheets and informed consent procedures.

Significant deviations from the approved protocol must be reported to the Committee as soon as possible.

Annual Progress Reports and Final Reports

The first Annual Progress Report for this study is due to the Committee by 6 July 2011. The Annual Report Form that should be used is available at www.ethicscommittees.health.govt.nz. Please note that if you do not provide a progress report by this date, ethical approval may be withdrawn.

A Final Report is also required at the conclusion of the study. The Final Report Form is also available at www.ethicscommittees.health.govt.nz.

Requirements for the Reporting of Serious Adverse Events (SAEs)

SAEs occurring in this study must be individually reported to the Committee within 7-15 days only where they:

- are *unexpected* because they are not outlined in the investigator's brochure, and
- are not defined study end-points (e.g. death or hospitalisation), and
- occur in patients located in New Zealand, and
- if the study involves blinding, result in a decision to break the study code.

There is no requirement for the individual reporting to ethics committees of SAEs that do not meet all of these criteria. However, if your study is overseen by a data monitoring committee, copies of its letters of recommendation to the Principal Investigator should be forwarded to the Committee as soon as possible.

Please see www.ethicscommittees.health.govt.nz for more information on the reporting of SAEs, and to download the SAE Report Form.

Statement of compliance

The committee is constituted in accordance with its Terms of Reference. It complies with the *Operational Standard for Ethics Committees* and the principles of international good clinical practice.

The committee is approved by the Health Research Council's Ethics Committee for the purposes of section 25(1)(c) of the [Health Research Council Act 1990](#).


We wish you all the best with your study.

Yours sincerely



Laura Jayne Burlison
Administrator
Multi-region Ethics Committee
Email: Multiregion_ethicscommittee@MOH.govt.nz

3.2 Australian Ethics Approval

HUMAN RESEARCH ETHICS COMMITTEE A CERTIFICATE OF APPROVAL	
DATE	22 December 2010
PROJECT NO.	10233A
PROJECT TITLE	Two weeks of low molecular weight heparin for distal vein thrombosis (TWISTER) TWISTER
Participant Information Sheet Version No. 03 dated 23 September 2010 Consent Form Version No. 03 dated 23 September 2010	
INVESTIGATOR(S)	Dr Huyen Tran
HREC MEETING DATE	02 September 2010
APPROVAL	29 October 2010 to 29 October 2013
The Principal Investigator is required to notify the Administrative Officer, Research Directorate of:	
<ol style="list-style-type: none">1. Any change in protocol and the reason for that change together with an indication of ethical implications (if any)2. Serious or unexpected adverse effects of project on subjects and steps taken to deal with them3. Any unforeseen events that might affect continued ethical acceptability of the project4. Any expiry of the insurance coverage provided in respect of sponsored trials5. Discontinuation of the project before the expected date of completion, giving reasons6. Any change in personnel involved in the research project including any study member resigning from Southern Health &/or the study team.	
At the conclusion of the project or every twelve months if the project continues, the Principal Investigator is required to complete and forward an annual report to the Committee.	
Annual report forms will be forwarded to the researcher.	
SPECIAL CONDITIONS:	
None	
SIGNED	 Committee Representative
DATE 29 October 2010	
<i>Please quote Project No. and Title for all correspondence</i>	

Southern Health

ABN 82 142 080 338

Dandenong Hospital
Kingston Centre
Cranbourne Integrated
Care Centre

Monash Medical Centre - Clayton
Monash Medical Centre - Moorabbin
Casey Hospital
Community Health
Services across the
South East
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Appendix 4: Publications and abstract presentations during PhD candidature

2015: Two Weeks of Low Molecular Weight Heparin for Isolated Symptomatic Distal Vein Thrombosis (TWISTER Study): Interim Analysis of First 100 Patients. **Merriman E**, Chunilal S, McRae S, Brighton T, Tran H. Poster Presentation at ISTH Conference 2015, Toronto, Canada.

2015: Two Weeks of Low Molecular Weight Heparin for Isolated Symptomatic Distal Vein Thrombosis (TWISTER Study): Interim Analysis of First 100 Patients – Post-Thrombotic Syndrome Sub-Study. **Merriman E**, Chunilal S, McRae S, Brighton T, Tran H. Poster Presentation at ISTH Conference 2015, Toronto, Canada.

2010: **Merriman E**, McRae S, Brighton T, Chunilal S, Tran H. Distal (Calf Vein) DVT: A Treatment Paradigm. Poster Presentation at HAA Conference 2010, Auckland NZ; awarded best ASTH clinical poster.

Appendix 5: Funding Sources

Sanofi-Aventis Australia, 2010 \$60,000

THANZ Clinical Trials Group 2012 \$70,000

