Venous blood flow, thromboembolism and below knee cast immobilisation for trauma

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Abstract

Venous thromboembolism (VTE) has a background incidence of between 0.7 and 2.69 per 1000 per year (L. N. Roberts et al., 2013). Risk factors are either permanent or transient. Permanent risk factors include thrombophilia (80 x increase risk if homozygous for factor V Leiden), cancer (58 x increase risk if metastatic cancer or diagnosis within last 3 months), increasing age (risk doubles for each decade over age 40 years), family or personal history of deep vein thrombosis (2-3 x increase risk) and increasing body mass index (2 x increase for BMI > 35 kg/m^2 in comparison with BMI <20 kgm^2) (Y.-H. Kim & Kim, 2007) (Blom, Doggen, Osanto, & Rosendaal, 2005) (Anderson & Spencer, 2003) (Decramer, Lowyck, & Demuynck, 2008) (Holst, Jensen, & Prescott, 2010).

Transient risk factors include surgery (165 x risk in first 6 weeks after total hip or knee replacement, equating to 2% symptomatic VTE rate) (Sweetland et al., 2009). Foot and ankle procedures including ankle fracture fixation, hindfoot fusion and 1st metatarsal osteotomy are associated with 18x, 8x and 2x increase VTE risk respectively (Jameson et al., 2011). Other transient risk factors include postpartum state (21-84 x increase in first 6 weeks), use of oral contraceptive pill or hormone replacement therapy (at least 2 x risk) and lower limb cast immobilization (Jackson, Curtis, & Gaffield, 2011) (Grodstein et al., 1996). Within 90 days of lower limb cast treatment, asymptomatic DVT affects between 4 and 40% of patients, symptomatic DVT affects 1 in 250, symptomatic pulmonary embolism affects 1 in 500, with fatal pulmonary embolism affecting 1 in 15,000 (Jameson et al., 2014). It is apparent that

patients will therefore have a differing risks depending on their permanent and transient risks.

The types of VTE include asymptomatic events, for which the relevance is not fully understood (often used in studies as a surrogate for symptomatic events). Symptomatic below knee DVT (approximately 20% propagate to become above knee) (Philbrick & Becker, 1988). Symptomatic above knee DVT (affecting popliteal vein or more proximal), which are 4 times more likely to occur (Baglin et al., 2010). Pulmonary embolism can also occur. The clinical relevance of DVT is that 6% of patients will have severe post thrombotic syndrome (venous ulceration, swelling, itching) at 10 years after the event, with 66% of patients displaying some signs (Schulman et al., 2006). Uncomplicated DVT does not appear to impact on quality of life, however if DVT is complicated by post thrombotic syndrome, patients will have significantly reduced quality of life, mental and physical health. Simple non fatal PE reduces physical health and if it is complicated by pulmonary hypertension (affecting approximately 2%) it results in significantly reduced quality of life, mental and physical health (Ghanima, Wik, Tavoly, Enden, & Jelsness-Jørgensen, 2017) (Lubberts, Paulino Pereira, Kabrhel, Kuter, & DiGiovanni, 2016).

In view that VTE has significant effects on patients quality of life, it is important to try and prevent it. In order to develop strategies for preventing DVT in patients with lower limb injury treated with leg cast, it is important to investigate the relative contributions of injury, stasis and immobility to thrombogenesis. I start by performing systematic review of the literature to determine whether thromboprophylaxis reduces symptomatic venous

thromboembolism in patients with below knee cast treatment for foot and ankle trauma.

A systematic review of randomised controlled trials of thromboprophylaxis in patients with foot and ankle injuries treated with cast immobilization was performed, searching MEDLINE and EMBASE from inception to June 2015 (B. A. Hickey, Watson, et al., 2016b). Outcomes of interest were VTE (asymptomatic and symptomatic DVT and PE) and bleeding. 3 reviewers used a data extraction form and assessed the literature according to the Cochrane risk of bias tool. Statistical analysis was performed using RevMan. 7 studies of chemical thromboprophylaxis were included, all except one used venography to assess for DVT, with one study using venous ultrasound. 2 studies reported on mechanical thromboprophylaxis, neither reported symptomatic DVT events. Neither study of mechanical thromboprophylaxis found a reduction in asymptomatic DVT in the intervention group.

Funnel plot of studies of chemical thromboprophylaxis suggested no publication bias. Pooled symptomatic DVT occurred in 1.58% of patients in the control group, with 0.43% sustaining symptomatic PE. At meta analysis, symptomatic DVT was reduced in the low molecular weight heparin chemical thromboprophylaxis group (OR 0.29, CI 0.09-0.95). Chemical thromboprophylaxis did not influence PE. There was one non-fatal retroperitoneal haemorrhage (major bleed), which equated to 0.11% (1 in 886). Based on these findings, 11 symptomatic VTE events would be prevented for every 1 major bleed. These findings are comparable with the recent Cochrane review, which included 2 additional studies and a total of 2924 participants. Meta analysis found reported a reduction of VTE in the

LMWH chemical thromboprophylaxis group (OR 0.40, 95% CI 0.21-0.76) (Zee, van Lieshout, van der Heide, Janssen, & Janzing, 2017).

In order to develop strategies for prediction and prevention of VTE in patients with foot and ankle injury treated with cast immobilization, it is necessary to consider why venous thrombosis occurs in these patients. As previously discussed, patients may have permanent risk factors, which may influence hypercoagulability. The transient risk factors of injury and cast treatment may also influence risk by causing endothelial dysfunction and venous stasis (Virchow, 1856). Several important mechanisms for prevention of venous stasis have previously been found. Weight bearing is important; with Gardner et al (1990) reporting that 30ml of venous contrast was pumped out of the foot during weight bearing (Gardner & Fox, 1983). This is not always possible for a patient with foot and ankle injury treated with a cast, because they may be non-weight bearing. For patients who are non-weight bearing, it is still possible to influence venous flow. For example, Elsner et al (2007) previously found that movement of the 1st metatarsophalangeal joint increased popliteal vein flow from 13 to 39 cm/s (Elsner, Schiffer, Jubel, Koebke, & Andermahr, 2007). In patients without leg casts, intermittent pneumatic compression of the leg or thigh to prevent venous stasis was found to be effective in reducing DVT and PE in a meta analysis of over 16, 000 patients (RR 0.43, 95% CI 0.36-0.52) (Ho & Tan, 2013). It therefore seems that this is a viable mechanism. Furthermore, Whitelaw et al (2001) found that none of the IPC devices studied resulted in significantly better calf pump function when compared with simple passive or active ankle movements (Whitelaw et al., 2001). To assess the influence of toe and ankle movement on venous stasis, I

examine the effect of these movements on venous velocities measured at the popliteal vein with ultrasound. To determine whether this is a viable strategy for prevention of DVT, I then assess the impact of application of below knee cast on venous velocities.

In this proof of principle study, 20 healthy volunteers were recruited (B. A. Hickey, Morgan, Pugh, & Perera, 2014). All had measurement of calf pump function in the un-casted leg whilst seated, using ultrasound at the popliteal vein. Baseline and peak velocities were measured during active toe movement (dorsiflexion and plantarflexion) and during ankle movement (dorsiflexion and plantarflexion). A below knee cast was then applied and measurements were repeated. Mean resting baseline venous velocity was 10 cm/s, which remained unchanged when the below knee cast was applied. There was approximately 5-fold increase in venous velocities with active toe movement (mean 54 cm/s for toe dorsiflexion, mean 50 cm/s for toe plantarflexion), and 10 fold increase from baseline with ankle movements (mean 115 cm/s ankle dorsiflexion, mean 87 cm/s ankle plantarflexion). All were statistically significant. When the below knee cast was applied, there was no statistically significant decrease in the peak velocities achieved during movement excepting for ankle dorsiflexion (isometric), however this was still increased approximately 8 times compared with baseline (88 cm/s). It was therefore apparent that venous stasis did not occur when a below knee cast was applied to healthy volunteers and that active toe movement may have a role in preventing stasis in patients with injury, with subsequent reduction in DVT. To determine whether this is true I assess the effect of active toe

movement on calf pump function and asymptomatic deep vein thrombosis in patients with foot and ankle injury treated with leg cast.

In this prospective randomized controlled trial, patients between the ages of 18 and 60 years with acute foot and ankle injury treated with non-weight bearing cast were recruited (B. A. Hickey, Cleves, et al., 2016a). Patients were within 3 days of their injury and considered low risk for VTE after risk assessment. Those with additional risk factors were provided with LMWH and not recruited. Patients who consented, were randomized to either active toe movement (AToM) intervention group (advised to perform active toe dorsiflexion and plantar flexion 60 times every 6 hours minimum, but more often if possible). Patients were managed through the trauma clinic according to their injury then had assessment of calf pump function on removal of cast and assessment for lower limb DVT of both lower limbs using venous ultrasound.

Interim analysis was performed after the first 100 patients were recruited. 78 patients completed the study, mean age was 37 years, 65% were male. 59% had leg cast for ankle fracture. Analysis of calf pump function revealed no significant difference between the intervention and control groups for any of the parameters, with mean baseline popliteal velocity of 7 cm/s in both groups, popliteal venous velocity during active toe dorsiflexion 44 cm/s (AToM) v 34 cm/s (control), p=0.36 and popliteal venous velocity during active toe plantar flexion 39 cm/s (AToM) v 32 cm/s (control) p=0.35). 27% of patients were found to have asymptomatic DVT, with no significant difference between groups. The important finding was that all asymptomatic DVTs occurred in the lower limb that had been injured and treated with cast. This

basic finding had not been previously reported in the literature. It was important to determine whether this finding could be attributable to venous stasis alone, or whether general immobility or the injury itself had any significant role. To answer this, I assess the association between patient mobility and development of asymptomatic DVT.

As part of the AToM study, a triaxial accelerometer (MOVBand) was attached to the leg cast at time of recruitment to the study. This was removed in the trauma clinic at the first appointment and the first 5 days of accelerometer data was extracted. Unpaired t test was used to determine statistical significance between group means for patients who did and did not develop asymptomatic DVT. 78 patients completed the AToM study, 10 patients were excluded from accelerometer data analysis (4 trackers lost, 6 failed to record any data). There was no significant difference in accelerometer data between patients who did and did not sustain asymptomatic DVT. Average moves were 1057/day (no DVT group) vs 1005/day (DVT group), p=0.85. Average steps were 877/day (no DVT group) vs 825/day (DVT group), p=0.82. In view of this, it appeared that mobility of patients during the first week of cast treatment for injury did not predict subsequent finding of asymptomatic DVT. In view that all DVT's occurred in the lower limb that had been injured and treated in cast, it was apparent that local factors such as venous stasis or tissue injury were more important than general patient mobility, otherwise it would be anticipated that some DVT's would have occurred in the uninjured, un-casted limb. To investigate the role of the injury I examine the association between biomarkers of coagulation and tissue injury with the outcome of asymptomatic DVT.

As part of the AToM study, 3.5ml venous blood was taken at time of recruitment. Centrifuged plasma was stored at -70 degrees centigrade. After the last patient exited the study, plasma was analysed for levels of tissue factor, interleukin 6, vascular cell adhesion molecule 1 (VCAM-1) and D-dimer (B. A. Hickey et al., 2017). 77 patients were included, 1 patient did not provide blood sample. Analysis of results found no difference between levels of tissue factor, IL-6, VCAM-1 and D-dimer in groups who did and did not sustain asymptomatic DVT. Mean Tissue factor 23.9 pg/mL (no DVT group) vs 20.3 (DVT group), p=0.422. Median IL-6 3.9 pg/mL (no DVT group) vs 4.6 (DVT group), p=0.76. Median VCAM-1 553 ng/mL (no DVT group) v 496.8 ng/mL (DVT group), p=0.11. Median D-dimer 203.5 (no DVT group) v 236.0 (DVT group), p=0.49. I therefore appeared that severity of injury, measured using plasma levels of FT, IL-6, VCAM-1 and D-dimer could not predict which patients would develop asymptomatic DVT. This suggested that local factors of venous stasis might play a greater role in thrombogenesis than tissue injury.

In summary, it appears that chemical thromboprophylaxis reduces the risk of symptomatic DVT in patients with foot and ankle injury treated with leg casts. Active toe movements can prevent venous stasis in healthy volunteers, however this does not appear to influence calf pump function or occurrence of DVT in patients with injury and leg cast. The important finding from this work is that asymptomatic DVT only occurs in the lower limb that has been injured and treated with leg cast. Biomarkers of coagulation do no appear to predict DVT in this patient group. Similarly, general patient mobility measured objectively with accelerometer during the first week of cast treatment does not

appear to be associated with development of DVT in patient with foot and

ankle injury treated with leg casts.

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1   Introduction

Patients with trauma to the lower limb and cast treatment can develop venous thrombosis in the form of deep vein thrombosis or pulmonary embolism. It is important to prevent these from occurring if possible, because both have negative impact on patient health.

Symptomatic Venous thromboembolism (VTE), presenting as either Deep Vein Thrombosis (DVT) or Pulmonary Embolism (PE) occurs in between 0.7 and 2.69 per 1000 people per year in the United Kingdom (L. N. Roberts et al., 2013) (Holst et al., 2010) (Oger, 2000) (Isma, Svensson, Gottsäter, & Lindblad, 2009) (Eekhoff, Rosendaal, & Vandenbroucke, 2000) (Silverstein et al., 1998). DVT occurs approximately three times more often than PE (Oger, 2000). In the context of Trauma and Orthopaedic surgery, VTE is usually considered to be a problem associated with major joint replacement, however it also occurs in young patients with non-operatively treated injuries such as ankle fracture. Acute PE is a dangerous clinical event, which can be fatal (L. Chen & Soares, 2006). It is often the first clinical indication of thrombotic disease (Slaybaugh, Beasley, & Massa, 2003). For those who survive the acute pulmonary embolic event, approximately 1.7% will die from PE within 3 months (Goldhaber & Bounameaux, 2012). For those who survive, 2 to 4% will develop chronic pulmonary hypertension, marking the start of a chronic disease associated with disabling dyspnea (Exter, Van der Hulle, Lankeit, Huisman, & Klok, 2013; Goldhaber & Bounameaux, 2012).

Recently, it was reported that the incidence of fatal pulmonary embolism following non-operatively treated ankle fracture affects approximately 1 in 15000 patients within 90 days of injury (Jameson et al., 2014). A significant limitation of this study was the lack of out of hospital death data, it is therefore likely that the true incidence of fatal pulmonary embolism is underestimated. It also is important to recognize that fatal pulmonary embolism is only one presentation of VTE, with DVT being more common and also having significant implications on the health of patients.

Although Virchow suggested that endothelial dysfunction, hypercoagulability and venous stasis play a role in venous thrombosis over 150 years ago, it is still unclear which patients will develop venous thrombosis (Virchow, 1856). All people are at risk of VTE, the main determinants being either intrinsic e.g. genetic abnormalities in haemostasis or extrinsic e.g. vessel wall injury due to trauma and subsequent treatments including surgery and cast immobilization. The multifactorial aetiology of VTE makes it difficult to accurately predict who is most likely to sustain VTE. Risk factors combine to increase VTE risk until a threshold is reached i.e. no risk factor is identified to be a single cause great enough to cause VTE alone but in addition to other risk factors, VTE will occur. One theory as to why some patients with apparently the same transient risk factors will develop PE, whereas other will not, is that everyone's threshold is different (Lippi & Franchini, 2008). Several risk factors have been identified as having an association with the development of venous thrombosis.

Permanent risk factors

Age

Age has been shown to be an independent risk factor for the development of VTE, with an odds ratio of 1.03 in hospitalized patients with acute medical illness who are aged 75 years of older (Alikhan et al., 2004). After the age of 40 years, VTE risk approximately doubles for each subsequent decade (Anderson & Spencer, 2003). A large population based study of 2218 patients in America found that the incidence of first time VTE increased with age, independent of gender, with <5/100,000 per year in children, increasing to 500/100,000 over the age of 80 years (Silverstein et al., 1998). In view of this, a cut off age which dichotomises patients into high and low risk based on age is not helpful (Anderson & Spencer, 2003).

Previous VTE

As part of the IMPROVE study (International Medical Prevention Registry on Venous Thromboembolism), using Cox multiple regression analysis of data from 15,156 medical patients, previous VTE was found to be an independent risk factor for subsequent symptomatic VTE (Spyropoulos et al., 2011). In hospitalised patients with acute medical illness, the risk of VTE is highest in those with previous history of VTE (OR 2.06), acute infectious disease (OR 1.74), cancer (OR 1.62) and age >75 years (OR 1.03) (Alikhan et al., 2004).

Thrombophilia and family history of VTE

Approximately 20% of patients who sustain VTE have a family history of VTE, with 11.4% and 6.4% of patients subsequently being found to have factor V Leiden mutation or G20210A prothrombin gene variant (Noboa et al., 2008). These are the most commonly inherited thrombophilias and are present in between 3 and 5% of the population (Nizankowska-Mogilnicka et al., 2003) (Y.-H. Kim & Kim, 2007). The factor V Leiden mutation increases VTE risk 7 times in heterozygotes and 80 times in homozygotes (Y.-H. Kim & Kim, 2007). In patients with the factor V Leiden mutation and an associated injury, there is a 50 fold increase in risk (van Stralen, Rosendaal, & Doggen, 2008). The prothrombin promotor G20210A mutation is less potent, with homozygotes having a 20 times increased VTE risk (Y.-H. Kim & Kim, 2007). Even in the absence of these inherited thrombophilias, it was shown in a case control study of 698 patients that those with first time episode of VTE and a first degree relative history of VTE had a 2.7 times increased risk of VTE compared to those without (Noboa et al., 2008). Sonnevi et al (2013) reported similar findings in a large case-control study of 1288 women between the ages of 18 and 64 years with first time VTE. Those with a positive family history of VTE had at least doubled risk of VTE compared to controls if they used HRT/CHC or had surgery and/or cast, regardless of presence of one of the two most common thrombophilias (Sonnevi, Bergendal, Adami, Lärfars, & Kieler, 2013).

Cancer

A diagnosis of cancer is an independent risk factor for objectively confirmed VTE (Alikhan et al., 2004). In a population based case control study, with 3220 patients with cancer and 2131 patients without cancer, the diagnosis of cancer was associated with a 7 fold increase in VTE risk (Blom et al., 2005). It is important to recognize that the risk was much greater in those who were diagnosed within the preceding 3 months (53 fold increase in VTE compared with those without cancer). These patients are not routinely advised to receive chemical thromboprophylaxis by NICE (National Clinical Guideline Centre – Acute and Chronic Conditions (UK), 2010). The extent of cancer is also relevant, with the highest VTE risk being present in patients with metastatic disease (58 times increased risk) (Blom et al., 2005).

Obesity

Obesity is perceived by many to be a risk factor for VTE. To support this, the MEGA study found that BMI >30kgm^2 increases the venous thrombosis risk 2.4 fold (Pomp, le Cessie, Rosendaal, & Doggen, 2007). Holst et al (2010) confirmed this in the Copenhagen City Heart study of 18, 954 patients, at a median follow up of 19.5 years. BMI >35kgm^2 was associated with an increased risk of VTE when compared to a BMI of <20kgm^2 (HR 2.1), even after adjustment for age and cardiovascular risk factors (Holst et al., 2010). The addition of oral contraceptive pill increases the VTE risk in patients with BMI >30kgm^2 from 2.4 to 24 fold in comparison to non obese women who do not use the OCP (Pomp et al., 2007). It is important to consider this when

treating patients with lower limb injuries treated with cast, who may appear

unlikely to develop VTE. An example is a 24 year old lady who developed a

calf DVT 8 days after a non-operatively treated ankle fracture, with

subsequent non fatal PE 2 weeks later (Harvey & Runner, 2011). Apart from

her injury and cast treatment, her additional risk factors were current use of

the oral contraceptive pill and that her body mass index was 34 kgm^2. This

patient required intensive care treatment for 2 days, with a total in patient stay

of 6 days. She also required warfarin treatment for 6 months. This may have

been prevented with chemical thromboprophylaxis (Harvey & Runner, 2011).

Transient risk factors

Pregnancy and post-partum related thrombosis

VTE is responsible for 14.9% of maternal deaths in developed countries

(Friedman, Ananth, Lu, D'Alton, & Wright, 2013). In a 30 year population

based study of VTE during pregnancy or postpartum including 50 080 births,

the annual incidence of VTE during pregnancy was 95.8 per 100 000 (Heit et

al., 2005). The annual incidence of VTE in the postpartum period was 5 times

higher (511.2 per 100 000). When pregnant women are admitted to hospital

for one or more days other than for delivery or VTE, their annual risk of VTE

was found to be 1752 per 100 000 in a study of 206 785 pregnant women in

the United Kingdom. The annual incidence remained increased at 28 days

after discharge (676/100 000) (Abdul Sultan et al., 2013). To put this into

context, a systematic review of comparative studies of VTE in first 6 weeks postpartum with non-pregnant, non-postpartum women of reproductive age found rates were 21.5 - 84 times higher in the postpartum women in first 6 weeks postpartum (Jackson et al., 2011). In view of this increased risk of VTE in the postpartum period, which is higher than found in patients with lower limb trauma and cast treatment it is surprising that RCOG only recommends 6 weeks of postpartum thromboprophylaxis for patients with thrombophilia and other VTE risk factors. In comparison, NICE guidelines recommend all patients with lower limb casts should be risk assessed for increased risk of VTE and provided with thromboprophylaxis where increased risk is identified (National Clinical Guideline Centre – Acute and Chronic Conditions (UK), 2010).

Oral Contraceptive Pill

In an early study of 155 women who sustained VTE, with no risk factors for VTE apart from OCP use in the month prior to VTE, their relative risk of VTE was found to be increased (RR 3.8, 95% CI 2.4 - 6.0), when compared to 169 age matched healthy controls who did not use the OCP (Vandenbroucke et al., 1994). It seems apparent that the influence of OCP on VTE depends not only on the dose of estrogen, but also on the type of progestogen (Van Hylckama Vlieg & Rosendaal, 2014). A systematic review and meta analysis in 2013 confirmed early findings by Vandenbrouke et al (1994), concluding that all combined oral contraceptives increased the risk of venous thrombosis (RR 3.5, 95% CI 2.9 to 4.3) (Stegeman et al., 2013). The safest combination reported as being 30 micrograms of ethinylestradiol with levonorgestrel by the recent Cochrane review (de Bastos et al.,

2014). Progestin only contraceptives and implants to not appear to increase risk of venous thrombosis when used for contraceptive purposes (Mantha et al., 2012) (Tepper, Whiteman, Marchbanks, James, & Curtis, 2016).

Hormone Replacement Therapy

In a large case control study including 1082 women over age 50 years who sustained first time VTE and 1468 controls, oral contraceptive pill used to treat symptoms of the menopause was associated with an 6.3 fold increased risk of VTE (4.6 - 9.8). Other types of oral hormone replacement therapy increased the risk of VTE 4 fold (range 1.8 - 8.2) (Roach et al., 2013). A recent Cochrane review of HRT in postmenopausal women confirmed this increased risk with both oestrogen only and combined therapy (Marjoribanks, Farquhar, Roberts, Lethaby, & Lee, 2017). Surprisingly, retrospective and prospective cohort studies suggest that transdermal HRT does not appear to increase VTE risk, although there are no randomized controlled studies to confirm this (Roach et al., 2013) (Eisenberger & Westhoff, 2014).

Achilles tendon rupture

Fatal pulmonary embolism can occur in young patients with no risk factors for VTE as early as within the first week following Achilles tendon rupture (Nesheiwat & Sergi, 1996; Venkatachalam & Wright, 2012). In a series of 205 patients with partial or complete Achilles rupture, 6.3% sustained symptomatic VTE within 90 days of injury in the absence of thromboprophylaxis (Healy, Beasley, & Weatherall, 2010). Even after excluding patients who sustained below knee DVT, the combined incidence of symptomatic DVT or PE was

2.9% (n=7), all of whom were under the age of 60 years of age, with 3 patients having no additional VTE risk factors (Healy et al., 2010). A study by Wallace at al (2011) found a lower incidence of symptomatic VTE, with 1.1% and 0.2% of 945 patients with Achilles tendon rupture sustaining DVT and PE respectively within 14 weeks of non-operative functional management (Wallace, Heyes, & Michael, 2011). Considering asymptomatic DVT events, it was recently shown that 62% (n=15) of 24 patients with open Achilles repair had evidence of thrombosis on Doppler ultrasound within 6 weeks of surgery in the absence of chemical thromboprophylaxis (Domeij-Arverud, Latifi, Labruto, Nilsson, & Ackermann, 2013). It appears that patients with Achilles tendon rupture are at particularly high risk of DVT, with many of these asymptomatic DVT's resolving without causing symptoms. Considering that VTE events can occur early following injury it is critical that any form of VTE prophylaxis is provided as soon as possible following injury.

Surgery

The background risk of VTE for a middle aged female is approximately 0.017% within a 90 day period (Sweetland et al., 2009). Comparing this to patients who undergo day surgery, including varicose vein surgery, the risk ranges from 0.15% to 1.2% depending on associated risk factors, such as active cancer (Pannucci et al., 2012). For patients who undergo in patient surgery, the risk of VTE is 100 times increased compared to non-operative controls in the first 6 weeks following surgery (Sweetland et al., 2009). The

risk of symptomatic VTE or death from PE is also highest in first 6 postoperative weeks (Sweetland et al., 2009).

In the context of Orthopaedic surgery, foot and ankle operations are considered by most health care professionals to be low-risk for the development of VTE (Wukich & Waters, 2008) (Jameson et al., 2011). However, in a study of symptomatic VTE rates within 90 days of 88,241 foot and ankle procedures over a 42 month period across the English NHS it was found that VTE rates following ankle fracture fixation, hindfoot fusion or first metatarsal osteotomy, were 0.28%, 0.142% and 0.027% respectively (Jameson et al., 2011). Comparing this with the data from the million women study by Sweetland et al (2009), these rates are 17, 8 and 1.6 times the background VTE rate respectively (Sweetland et al., 2009). This paper concluded that thromboprophylaxis was not necessary in these patients (Jameson et al., 2011). It is important to recognize however that the aforementioned studies may not be directly comparable. The background incidence of VTE found in the million women study may differ from the background risk in the study by Jameson et al (2011) due to differences in the patient population (gender, age, co-morbidities). When compared to surgery in general, which increases risk 100 times in the first 6 post-operative weeks, and compared to VTE risk following hip or knee replacement (165 times increased risk) these rates may appear low, however they are still increased compared to patients who do not undergo surgery (Sweetland et al., 2009). It is also important to acknowledge that the study by Jameson et al (2011) has several limitations, including that it was not even stated whether patients

received thromboprophylaxis (Jameson et al., 2011). It also appears that VTE

rates in this study underestimate true VTE rates. One example is that only

0.061% of 1633 patients who underwent total ankle replacement (TAR)

sustained symptomatic VTE. This is considerably lower than the incidence in

the study by Barg et al (2011), in which 3.9% of 665 patients sustained

symptomatic DVT within a mean of 15.2 days following TAR, despite the use

of chemical thromboprophylaxis (Barg, Henninger, & Hintermann, 2011). Even

if the below knee DVT's are excluded from the study by Barg et al (2011), the

symptomatic DVT incidence is still 0.45%, which is more than 7 times greater

rate than reported by Jameson et al (2011) (Jameson et al., 2011).

In contrast, elective hip or knee arthroplasty is universally considered high risk

for VTE irrespective of other factors (Mont et al., 2011). Symptomatic VTE or

fatal PE is even more common in patients undergoing hip or knee arthroplasty

than those who undergo cancer surgery in first 6 postoperative weeks

(Sweetland et al., 2009). Within 90 days of surgery, between 1.69 and 1.84%

of over 100,000 patients who underwent hip or knee replacement sustained

symptomatic VTE (Jameson, Bottle, Malviya, Muller, & Reed, 2010). The

increased risk in comparison with foot and ankle surgery is multifactorial but is

likely to be related to the greater surgical insult to bone and soft tissue.

Although patients who undergo TKR may have complete venous stasis in the

lower limb secondary to tourniquet, a study by Hanslow et al (2006) did not

find an association between tourniquet use and objectively confirmed

symptomatic VTE in 602 patients who underwent foot and ankle surgical

procedures, despite use in 90% of cases for a mean duration of 62 minutes (Hanslow, Grujic, Slater, & Chen, 2006).

Lower limb trauma and cast treatment

Lower limb trauma and fracture is considered to be a major transient risk factor for VTE (White, 2012) (Bělohlávek, Dytrych, & Linhart, 2013). For example, in a large case-control study of 1470 women with first time VTE and 1590 controls, the highest risk for VTE was a combination of lower limb cast and surgery, which resulted in a 50 times increased risk of VTE in both pre and post-menopausal women (Bergendal et al., 2012). However, it is difficult to isolate the trauma from other factors that influence VTE, including treatment with cast, surgery and associated immobility. In a large population based, case-control study of 2471 patients and 3534 controls with minor lower limb injuries, not requiring surgery or plaster cast were associated with 3 fold increase in VTE. The excess risk disappeared at 10 weeks post injury (van Stralen et al., 2008). In reality, almost 80% of patients with a lower limb cast or splint have an additional risk factor, including age over 40 years, personal or family history of VTE, thrombophilia, immobilization (>4 days bed rest), pregnancy or post partum (within 6 weeks), cancer, heart failure, respiratory failure or inflammatory disease, hormonal therapy or obesity (BMI >30 kgm2) (Decramer et al., 2008).

Many studies suggest that lower limb cast and trauma account for a relatively small number of cases of deep vein thrombosis. For example, of 882 patients who sustained symptomatic DVT, only 3.9% of patients reported use of a lower limb cast in the preceding month (Isma et al., 2009). Similarly, in a series of 577 patients who underwent duplex USS for suspected DVT, Geersing et al (2010) reported that only 4% had leg trauma in the preceding 4 weeks (Geersing et al., 2010). Galanaud et al (2009) reported a greater association in a series of 933 patients with symptomatic distal DVT, in which 9% reported a history of recent plaster cast of the leg (Galanaud et al., 2009). Similarly, Aldington et al (2008) found in a cross sectional study of 61 patients under the age of 65 years of age who sustained VTE that 12% reported an injury in the preceding 4 weeks (Aldington et al., 2008). However, there was no control group for comparison.

Many assumptions have been made regarding the pathogenesis of deep vein thrombosis in patients treated with lower limb cast, however many basic questions remain unanswered. For example, it is unknown whether DVT occurs solely in the limb that has been injured and casted. Furthermore, the cast has been assumed to cause stasis, but this also has never been proven. The cast has also been assumed to cause immobility of the wearer, but again, this has never been studied. Perhaps it is the tissue injury that predisposes to venous thrombosis?

2   Narrative Review

2.1   Thromboprophylaxis in patients with below knee cast immobilization for trauma

DVT has a consistent relationship with PE (National Clinical Guideline Centre – Acute and Chronic Conditions (UK), 2010), with 20% of calf DVT's propagating to the thigh before they embolise (Philbrick & Becker, 1988). One of the challenges is that symptoms and signs for DVT are unreliable, with approximately 50% of patients with suspected DVT having normal venous phlebography (Cranley, Canos, & Sull, 1976). Distal DVT is more often associated with transient risk factors whereas proximal is associated more with permanent risk factors such as cancer (Galanaud et al., 2009). However, after excluding deaths by cancer, the 3 month mortality rate following proximal and distal DVT is equivalent (Galanaud et al., 2009). Patients with first time symptomatic DVT have a recurrence rate of 23.2% at 5 years follow up. However, the risk of recurrence in patient with symptomatic DVT confined to the calf is 4 times lower compared with patients with symptomatic proximal DVT or PE (Baglin et al., 2010). Nevertheless, despite chemical treatment for a VTE event provoked by a non-surgical factor such as lower limb trauma with cast immobilisation, 5.8% will develop a recurrence within the following year (Iorio et al., 2010). Even if this risk of morbidity and mortality associated with

acute venous embolism was acceptable to the patient, one of the greatest burdens to society is post-thrombotic syndrome and the cost of treating long term complications of VTE. In England alone, this is estimated to cost in excess of £640 million per year (L. N. Roberts & Arya, 2011).

Another significant outcome of DVT is the development of post-thrombotic syndrome. This is a chronic condition characterized by lower limb skin changes and ulceration, which results from damage to the deep veins of the lower limbs following deep vein thrombosis. During the process of recanalization, venous valves are destroyed which causes venous hypertension secondary to reflux (O'Donovan, O'Keeffe, Grace, & Lyons, 2005). Incomplete thrombus resolution results in residual venous obstruction which further impairs venous return (Kahn, 2006). Following first time DVT, 20% of all segments develop reflux on duplex scanning at 3 months, despite anticoagulation and compression stockings (M. C. Janssen, Wollersheim, Haenen, van Asten, & Thien, 1998). However, Janssen et al (1998) also found a statistically significant reduction in thrombus mass in all of 60 patients on duplex ultrasound at 3 months following symptomatic first time DVT, who had been treated with coumarin derivatives and compression stockings, with total clot resolution in 40% (M. C. Janssen et al., 1998). Clinical symptoms and signs of post-thrombotic syndrome occur in between 20 to 50% of patients who sustain symptomatic DVT (Kahn & Ginsberg, 2004). Even with elastic compression stocking treatment, 25% of 1668 patients with first time DVT were found to develop post-thrombotic syndrome within the following year (Tick, Kramer, Rosendaal, Faber, & Doggen, 2008). In a prospective

study of 111 patients by Tick et al (2010), post-thrombotic syndrome was clinically evident in 46% of patients at 3 months on CEAP scoring, with no further increase at 2 years (Tick et al., 2010). In a series of 254 patients with objectively proven DVT, Kahn et al (2008) reported similar findings, with 43% of patients showing signs of post-thrombotic syndrome at 2 years after VTE event, 3% of which were severe (Kahn et al., 2008). However, at follow up of 10 years following symptomatic DVT, only 27% of 82 patients had no signs of post-thrombotic syndrome (Haenen et al., 1999) suggesting that with increased time following the index DVT event, chronic venous hypertension will eventually result in post-thrombotic changes. Milne et al (1993) found all of 107 patients at median follow up of 8 years after venographically proven DVT had signs of post-thrombotic syndrome, of which 35% of these cases were severe with lipodermatosclerosis or skin ulceration (Milne, Stonebridge, Bradbury, & Ruckley, 1994). In view of the costs to society and the fact that there are few effective treatments for post-thrombotic syndrome, it is critical that VTE is respected and prevented (Prandoni & Kahn, 2009) (Kahn & Ginsberg, 2004).

Venous thromboembolism affects approximately 1 in 1000 people per year (Silverstein et al., 1998). Lower limb trauma and cast immobilization increases this risk, with incidences of VTE ranging from 4.3 to 40% in patients who have been treated with cast immobilization for at least 1 week (Testroote, Stigter, de Visser, & Janzing, 2011b). Chemical thromboprophylaxis can reduce the risk of developing VTE in this patient group (Testroote, Stigter, Janssen, & Janzing, 2014). In view of this, it is recommended that all patients with lower

limb trauma treated with cast immobilization should be individually assessed for risk of venous thromboembolism risk and provided with thromboprophylaxis where increased risk is identified (National Clinical Guideline Centre – Acute and Chronic Conditions (UK), 2010). The assumption is that patients without additional risk factors for VTE do not require thromboprophylaxis because their risk is lower. However, according to some VTE risk assessment tools, it is suggested that any patient with an immobilizing cast is at moderate risk of developing DVT (10-20%) and thromboprophylaxis is indicated (Caprini, 2005). Some authors recommend thromboprophylaxis for all patients with immobilisation of the lower extremity, irrespective of age and other risk factors (Testroote, Morrenhof, & Janzing, 2011a). The benefit of this practice is that all patients who would otherwise develop VTE would be provided with prophylaxis, however many patients who would not otherwise develop this complication would un-necessarily receive LMWH with associated risks and costs.

Patients with lower limb trauma and immobilization are at risk of venous thromboembolism (VTE). The most serious complication of this is death from Pulmonary Embolism (PE), which occurs in approximately 1 in 15000 patients (Jameson et al., 2014). Although fatal pulmonary embolism is the most serious thromboembolic complication, it is not the only significant outcome. Approximately 1 in 500 patients will develop a symptomatic PE within 90 days of injury (Jameson et al., 2014). Many of these patients will be functionally impaired at long term follow up (Chow et al., 2014). The other significant complication is symptomatic deep venous thrombosis, which occurs in

approximately 1 in 250 patients with non-operatively treated lower limb trauma (Selby et al., 2014). 20 to 50% of these patients will develop post-thrombotic syndrome (Kahn & Ginsberg, 2004). This condition is difficult to treat and therefore it is important to avoid. Considering that lower limb casts and splints are commonly used for a variety of soft tissue and bony traumatic conditions, the population of patients at risk of developing VTE is significant.

Many consider immobilisation of the lower limb to be an independent risk factor for deep vein thrombosis and pulmonary embolism and therefore provide chemical prophylaxis to all patients with leg casts (Menakaya et al., 2013) (Hanslow et al., 2006). Other authors have suggested prevention of VTE should be provided to patients with additional risk factors, which include age over 50 years of age, non-weight bearing and severe injury (Horner, 2011). National guidance recommends that patients with trauma and lower limb immobilization should be risk assessed for VTE. Where elevated risk is identified they should be provided with chemical thromboprophylaxis (National Clinical Guideline Centre – Acute and Chronic Conditions (UK), 2010).

2.2   Effect of below knee cast application on calf pump function in healthy volunteers

Plaster of Paris has been used to immobilise fractures for over 150 years (R. T. Austin, 1983). An assumption has been made that lower limb cast immobilization results in venous stasis, which predisposes these patients to VTE (Craik, Clark, Hendry, Sott, & Hamilton, 2015). However, there is little published literature to support this. Although Craik et al (2015) found that full weight bearing in 10 healthy volunteers with below knee casts applied resulted in significantly greater time averaged peak and mean venous velocities measured in the popliteal vein as compared to partial or non-weight bearing, they failed to compare popliteal velocities before and after cast application (Craik et al., 2015). Therefore, although weight bearing with as cast applied is important, this study did not show that application of a below knee cast causes venous stasis. In a small study of 8 healthy volunteers who had below knee casts applied, Sugwara et al (2004) found that arterial blood flow and femoral artery lumen diameter was reduced after just 7 days, however these changes were completely reversed without any specific rehabilitation within 14 days of removal of cast (Sugwara et al., 2004). In the absence of cast application, lower limb trauma has been shown to result in impaired venous outflow. Wilson et al (2002) found in their study of 126 patients with hip fracture, that venous outflow measured using strain gauge plethysmography was significantly reduced in the injured limb at 6 weeks postoperative as compared to pre operative measurements and the un injured lower limb (D. Wilson et al., 2002). This reduction in venous outflow was

found to be associated with the development of venous thromboembolism. Considering that none of these patients were treated with lower limb casts, it appears that venous stasis and subsequent thromboembolism occurs following lower limb trauma even in the absence of below knee cast (D. Wilson et al., 2002). The starkest example of stasis causing thrombogenesis is in patients who sustain cardiac arrest. In a study of 88 adult patients with out of hospital cardiac arrest, all patients had blood drawn at time of CPR for arrest and measured for thrombin-antithrombin (TAT) complexes. Mean time prior to initiating CPR was 12.1 minutes, during which time complete circulatory stasis can be assumed. Mean TAT was 159.2 micrograms per litre (range 0.79 to 1343.9). Only one of these patients had a TAT in the normal range, indicating that increased thrombogenesis is universally present among patients with non-traumatic out of hospital cardiac arrest. It is not stated what proportion of these patients went on to develop venous thromboembolism (Hostler, Callaway, Newman, & D'Cruz, 2007). Considering that complete circulatory stasis results in increased thrombin-antithrombin complexes, which indicate a hypercoagulable state, it would be reasonable to consider that tourniquet use may have similar effects. However, although a lower limb tourniquet was applied in 97% of 1000 consecutive patients who underwent foot and ankle surgery, only 4 patients sustained symptomatic DVT. In one of these cases, an intra-operative tourniquet was not used. The other 3 who did have tourniquet had it applied for 21, 60 and 90 minutes respectively. None of the other patients developed symptomatic DVT despite their tourniquet use. In view of this, it appears that even complete venous stasis below knee is unlikely to result in symptomatic DVT and is likely to be a weak factor in the

pathogenesis of venous thromboembolism (Wukich & Waters, 2008). It therefore appears unlikely that application of a below knee cast will result in significant stasis. Rather, other factors common to patients with casts, including the injury, resulting in subsequent endothelial dysfunction may be more important. This requires further study.

Muscle bulk and joint range of motion has consistently been shown to decrease in a lower limb which has been treated with cast immobilization (Halanski & Noonan, 2008). In a study of 18 patients with stable ankle fractures, MRI cross sectional imaging of calf muscles revealed a statistically significant reduction in calf muscle anatomical cross-sectional area within just 8 days of below knee cast treatment (Psatha et al., 2011). After cast treatment for 6 weeks, the gasctrocnemius and soleus muscle atrophied by 17-24% (Psatha et al., 2011). The longest recorded duration of below knee cast treatment was in a 45 year old lady who wore a below knee polymer cast for 28 months due to psychological dependence. Her calf circumference was reduced by 5.5cm compared to her uncasted limb. Surprisingly she did not develop deep vein thrombosis. In healthy volunteers, above knee cast application has similar effects on muscle bulk. Thom et al reported that after just 10 days of non-weight bearing above knee cylinder cast application to the lower limb in 8 healthy females, quadriceps muscle cross sectional area decreased significantly by almost 12 percent (Thom et al., 2001). Snijders et al (2013) reported similar findings in 12 men of mean age 24 years after 2 weeks of above knee cast application. Quadriceps cross sectional area reduced by 8%, with 7% and 13% reduction in type 1 and type 2 muscle fibre sizes respectively (Snijders et al., 2013). In the same study, lower limb muscle

strength was also found to decrease, with a reduction in single leg extension one rep max by 23% (+/-3). Following 6 weeks of natural rehabilitation, strength returned to baseline values (Snijders et al., 2013). In a larger study of 48 healthy volunteers by Hortobágyi et al (2000), above knee cylinder cast for 3 weeks resulted in a 47% reduction in eccentric, concentric and isometric quadriceps muscle strength. Interestingly, spontaneous recovery occurred over the 2 weeks following cast removal (Hortobágyi et al., 2000). Type 1 and 2 muscle fibre cross sectional area from vastus lateralis muscle biopsy also reduced by between 13 and 10% respectively (Hortobágyi et al., 2000). Similar findings were reported by Hvid et al (2011) in vastus lateralis biopsies of 9 healthy young men who worse above knee casts for 2 weeks. Cross sectional area of myosin heavy chain 2a fibres significantly reduced by 4% (p=<0.05), however myosin heavy chain 1a remained unchanged (Hvid, Ortenblad, Aagaard, Kjaer, & Suetta, 2011). A marked reduction in single muscle fiber force of contraction, of between 21 and 30% was also evident.

Even bone density is affected in patients lower limb trauma treated with casts. This occurs in both children and adults. In an age and sex matched study of adolescents between 10 and 16 years of age who were treated with cast for leg or ankle fracture, bone mineral density was reduced by between -5.8 and -31.7% (Ceroni, Martin, Delhumeau, et al., 2012a). These changes were not present at 18 months follow up (Ceroni et al., 2013). In a series of 14 adult patients with non-operatively stable ankle fractures, bone mineral density was significantly reduced at 6 weeks following injury (12% reduction) compared to un injured ankle as control (Ingle, Hay, Bottjer, & Eastell, 1999). This returned

to normal within 1 year following injury. However, there was also a statistically significant reduction in bone mineral density in the greater trochanter (3% reduction), which did not return to normal within a year following injury (Ingle et al., 1999).

Application of a lower limb cast has traditionally been considered to cause venous stasis in the casted limb (Thomas & Van Kampen, 2011). Stasis leads to hypoxia at the level of the deep venous valve cusps, which can trigger thrombogenesis (Agutter, Malone, & Silver, 2012). The two predominant factors influencing venous blood flow are 'vis a tergo', resulting from the continuous entry of blood into venules from capillary beds and secondly, the upward pressures of weight bearing on the foot with subsequent muscle contraction. In the absence of weight bearing or muscle contraction, the 'vis a tergo' results in continuous non-pulsatile flow in the deep veins, which subsequently results in vortices at the mouth of deep vein valve cusps with stagnation of blood and local hypoxia at the level of valve cusp endothelium. This thrombogenic environment results in local damage to valve cusp endothelium, with release of tissue factor, which attracts platelets and leukocytes with subsequent thrombus formation. During calf pump contraction or weight bearing, intermittent flow is restored, which empties stagnant valve pockets, bringing fresh blood with active platelets and leuokocytes, which subsequently attack the necrotic valve cusp endothelium. It is felt that these cycles of continuous and turbulent blood flow result in formation of layered thrombus (Agutter et al., 2012). With this in mind, application of a lower limb cast may interfere with the normal balance of continuous and pulsatile flow in the lower limb venous system, due to restriction of muscular contraction of the

calf pump. This may be the result of both immobilization of joints, but also by functional inactivation of the calf pump resulting from restricted weight bearing in these patients.

The first demonstration of this theory in practice was by Gardner and Fox in 1983. In their study, intravenous contrast was injected into the dorsal vein of the foot of 5 participants, who subsequently had imaging of lower limb venous system with video phlebography during weight bearing. During weight bearing, it was shown that 20 to 30ml of blood was forcefully pushed into the deep veins of the legs. The majority of this blood came from the venae comitantes of the lateral plantar artery of the foot (Gardner et al., 1990). Surprisingly, it appeared that active toe or ankle movement in the non-weight bearing limb did not influence the pooling of contrast in the plantar veins. The interpretation of this study was that weight bearing is the more important than muscular activity in influencing venous return from the leg. However, it is important to recognize that although active muscular contraction of the calf pump did not empty the veins of the foot, it is also clearly stated that active ankle dorsiflexion and plantar flexion both also empty the deep veins of the calf (Gardner et al., 1990).

Elsner et al subsequently examined the venous anatomy of the foot and found networks of veins closely related to the plantar aspect of flexor hallucis longus tendon at the levels of the inter phalangeal and metatarsophalangeal joints (Elsner et al., 2007). These veins had valves and were surrounded by a thick

connective tissue capsule, which was put under tension during passive dorsiflexion of the great toe. Passive movement of the 1st metatarsophalangeal joint in 10 cadaveric feet revealed that this venous system acted like a pump, to expel blood proximally into the foot (Elsner et al., 2007). In the clinical setting this was also found to be the case in 40 healthy volunteers who performed passive toe mobilization. In this study, Doppler ultrasound was used to measure baseline and peak venous velocities at the popliteal vein at rest and during passive and active toe movement. Baseline velocity significantly increased from 13.7 cm/s to 33.3 cm/s during passive and 38.9 cm/s with active toe movement respectively. The conclusion of this study was that toe movements could provide a means for mechanical thromboprophylaxis (Elsner et al., 2007). It is interesting to note that in a similar study by Craik et al, baseline popliteal velocity in a non-weight bearing casted leg was 8cm/s, which significantly increased to 24.3cm/s during simulated weight bearing in cast (Craik et al). However, comparing these findings with those by Elsner et al, it appears that passive and active toe movement result in greater increases in popliteal velocity as compared to weight bearing alone.

In a dynamic study of foot pump function during gait, the foot pumping mechanism was examined in 20 healthy volunteers during treadmill walking at differing speeds. This examined the combined effect of weight bearing and active toe movement. It is impractical to measure popliteal vein velocity during gait, therefore venous pressures in the dorsal vein of the foot were directly measured by a transducer system. In the upright resting position, foot

pressures were approximately 90mmHg. During walking, blood was pumped out of the lower limb and pressures decreased to 60-70mmHg. The relevance of this study to patients with lower limb cast immobilization was that restricting the range of motion at the knee or ankle resulted in reduced foot pump efficiency, with less reduction in venous pressures during walking. This study suggests that a patient with cast immobilization of the ankle, will have less efficient foot pump function. In contrast to the study by Elsner et al, the effect of great toe movements were not examined (Kügler, Strunk, & Rudofsky, 2001).

The clinical implication of isolated ankle immobilization appears to have an insignificant effect on venous thromboembolism. For example in patients with hind foot fusion, the symptomatic rates of deep vein thrombosis or non-fatal pulmonary embolism were found to be only 0.028% and 0.114% respectively in a series of 7033 patients in the English NHS (Jameson et al., 2011). This suggests that the foot pump mechanism may be able to compensate for reduced ankle motion, providing toe movement and/or weight bearing continue.

Cast application to the lower limb appears to have significant detrimental effects on the lower limb muscle bulk and strength, with changes seen also in the bones of the lower limb. However, there is little evidence that cast application to the lower limb in isolation results in venous stasis. Further study is necessary to determine the relative importance of venous stasis,

endothelial dysfunction and hypercoagulability in patients with lower limb trauma treated with casts in order to improve understanding of the development of venous thromboembolism in these patients.

2.3 The effect of active toe movement (AToM) on calf pump function and asymptomatic deep vein thrombosis in patients treated with acute foot and ankle injury treated with cast – A Prospective Randomised Controlled Trial

Thromboprophylaxis is clinically and financially beneficial compared with treatment of thromboembolic events once they have occurred (Alikhan et al., 2004). In patients with lower limb trauma treated with cast, thromboprophylaxis significantly reduces venous thromboembolism rate, with thirteen patients requiring prophylaxis with low molecular weight heparin to prevent one asymptomatic DVT (C. Roberts, 2012) (Testroote, Stigter, de Visser, & Janzing, 2011b) (Ettema, Kollen, Verheyen, & Buller, 2008). Considering that chemical thromboprophylaxis for patients with lower limb cast immobilisation represents 5.3% of the outpatient tariff for trauma, this is a significant expense (Menakaya et al., 2013). This also represents a potential risk, in view that chemical thromboprophylaxis can cause major bleeding (National Clinical Guideline Centre – Acute and Chronic Conditions (UK), 2010). Patients with lower limb trauma treated with casts are also advised to participate in activities of early mobilisation and calf pumping exercises to reduce their risk of developing DVT (Le Sage, Marianne McGee BSc, &

Jessica D Emed RN, 2014). Although it has previously been found that active toe movement increases venous velocities in the popliteal vein in healthy volunteers, even with a below knee cast applied, the clinical importance of these findings requires further investigation (B. A. Hickey et al., 2014) (Parsons, Hiskens, Price, Achten, & Costa, 2011). Most studies rule out mechanical thromboprophylaxis in patients with below knee casts for various reasons (Parsonage, 2009) (Decramer et al., 2008). However, considering that venous stasis is thought to contribute to thrombogenesis, it is possible that regular active toe movement (AToM) in the form of regular active dorsiflexion and plantarflexion could reduce the development of deep vein thrombosis in patients with injury and cast treatment (Virchow, 1856).

2.4 The association between mobility and subsequent development of Deep Vein Thrombosis in patients with lower limb trauma and cast treatment

Immobility has long been recognized to be a contributory factor in the development of deep venous thromboembolism. Almost 60 years ago, Gibbs et al (1957) reported in a post mortem study of 253 patients, that patients who were confined to bed rest of 0 to 4 days prior to death had a deep vein thrombosis (DVT) incidence of 8% (n=6) at post mortem as opposed to 28% (n=10) in those who were confined to be for 4 to 7 days prior to death (Gibbs, 1957). In view of this, patients are encouraged to mobilise early following surgery, as it is felt that early mobilization will stimulate the calf muscle pump

and limit venous stasis (E. Wilson, 2007). One of the challenges in the study of mobility and subsequent development of VTE is that the definition of immobility is variable between studies and mobility in many patient groups, such as those with lower limb casts, has not been studied (Emed, Mossison, Rosiers, & Kahn, 2010). For example, Spyropoulos et al defined immobility as confinement to a bed or chair for more than 24 hours (Spyropoulos et al., 2011). In contrast, other studies have used definitions of immobility as expected bed rest of up to 10 or more days (Harenberg et al., 1990). A recent systematic review of immobility in hospitalized medical patients acknowledged this considerable inconsistency in the definition of immobility and it was concluded that further research is needed to define and standardise this concept (Emed et al., 2010). Although the most frequent definition of immobilization in epidemiological studies of venous thromboembolism is confinement to bed or bed rest lasting more than 3 days (Pottier et al., 2009), this issue is further complicated by the recent finding that overall correlation between patient reported mobility and accelerometer-assessed physical activity can be low (Spearman's r = 0.3) (Sabia et al., 2014).

Although seated immobility at work for more than 8 hours in a 24 hr period, within the 4 weeks prior to VTE is common in patients who develop DVT/PE (34% of 61 patients), this is not conclusive evidence of a causal relationship because there was no control group (Aldington et al., 2008). It could be argued that this level of mobility reflects the sedentary jobs of modern society. The term eThrombosis was coined in 2003, when a 32 year old man developed a life threatening pulmonary embolism after prolonged immobility

sitting at a computer, regularly for periods of between 12-18h per day, with consecutive sitting of 1-6 hrs without standing. (R. Beasley, Raymond, Hill, Nowitz, & Hughes, 2003). A similar case was reported by Chang et al (2013) of a 31 year old man who developed a DVT after sitting playing computer games for 8 hours per days for four days whilst on holiday (Chang, Burbridge, & Wong, 2013). Death from fatal pulmonary embolism has also been attributed to several consecutive days of immobility following sleeping in a car following an earthquake and from physical restraint in a man with acute psychosis (Inoue, 2006) (Cecchi, Lazzaro, Catanese, Mandarelli, & Ferracuti, 2012).

Patients who are physically trapped prior to hospital admission, for example in a car and requiring extrication have been shown to have increased VTE rates. In a retrospective review of 15,159 trauma patients over a 10 year period, 1176 patients were identified from a database which recorded pre-hospital entrapment. This was significantly associated with both increased risk of DVT (OR 5.22) and PE (OR 3.16). Even with multivariate analysis this proved to be a contributing factor for VTE (OR 1.54) (Rogers et al., 2011). It appears that there is an association between reduced mobility and development of VTE, however this is difficult to quantify.

2.5 Can we use biomarkers of coagulation to predict which patients with foot and ankle injury will develop deep vein thrombosis?

Patients with foot and ankle trauma treated with leg casts are at risk of venous thrombosis (VTE). The majority of VTE risk assessment tools assess patient and treatment factors, including mobility, body mass index and surgery to risk stratify patients, but they have limited accuracy for predicting VTE in this patient group. None consider the endothelial dysfunction and hypercoagulability that occurs secondary to injury. It would be useful to be able to predict which patients will develop DVT after injury because this would enable thromboprophylaxis to be provided to those at greatest risk, which would provide greatest benefit.

Tissue injury results in activation of the coagulation cascade through initiation of the extrinsic coagulation pathway. The primary cellular activator of this process is tissue factor (TF), which is released by tissues in response to injury (Manly, Boles, & Mackman, 2011) (Caprini, Arcelus, & Reyna, 2001). Tissue injury may be the result of trauma, such as an ankle fracture. More recently it has been suggested that other mechanisms including heavy smoking with oxidative stress, cancer and immobilization with focal vessel ischaemia may share a common pathway of cell death to trigger venous thromboembolism (Golomb, Chan, Denenberg, Koperski, & Criqui, 2014). Once TF is exposed to blood, it acts as a receptor for factors VII and VIIa. This complex subsequently

activates coagulation factors IX and X, which marks the beginning of the common coagulation pathway whereby prothrombin is converted to thrombin, which stimulates the formation of a fibrin clot (Manly et al., 2011).

Under normal conditions, the endothelial surface is not thrombogenic, with substances including thrombomodulin, tissue factor pathway inhibitor and plasminogen activator acting to prevent thrombosis (Wakefield, Myers, & Henke, 2008). However, in the conditions of endothelial injury, the endothelial surface vasoconstricts to become pro-inflammatory, due to the action of endothelin 1, platelet activating factor and other procoagulant molecules (Wakefield et al., 2008) (Piazza, 2013). Activation of the endothelial surface also results in movement of the adhesion molecule, soluble p-selectin (sPsel) from the endothelial cell Wiebel-Palade body to the endothelial surface (Ley, 2003). This regulates leukocyte migration from the circulation and supports initial tethering to the endothelial cells and platelets (Antonopoulos, Sfyroeras, Kakisis, Moulakakis, & Liapis, 2014). Combined with abnormal venous flow with relative stasis and low shear at the endothelial surface, these conditions are optimal for thrombogenesis (Virchow, 1856).

Samuels and Webster (1952) first documented the effects of vascular endothelial injury on haemostasis in a dog model. In this experiment, 14 femoral veins, 25 jugular veins and 7 inferior vena cavae were dissected and removed under anaesthetic and subjected these to mechanical and chemical injury to assess the response. The initial phase of thrombosis occurred with

minimal trauma as a sequence of platelet adherence to cement substance, with coalescence of platelets to form thrombi. Finally, fibrin was deposited on the platelet thrombi surface with subsequent fibrin masses spread along inter-cellular lines and across the cell bodies (Samuels & Webster, 1952). If the injury was severe or prolonged, the process progressed to phase 2 in which the endothelial surface was disrupted, with pathologic anatomic changes to the endothelial cells. These changes were proportional to the time of application of crushing, indicating that more severe trauma results in more significant changes in the venous endothelium (Samuels & Webster, 1952).

As discussed, tissue factor release from endothelium and p selectin represent the initiation of thrombogenesis. In the clinical context, these markers of haemostasis have been used to identify hypercoagulable states secondary to injury or identify cases in which venous thrombosis has occurred. For example, p selectin has been shown at meta analysis level of 2429 patients to be significantly elevated in patients with deep vein thrombosis, independently of other factors know to be associated with increased levels e.g. solid organ tumour, HIV (odds ratio for venous thromboembolism 2.89, 95% CI 2.32-3.61, P<0.001) (Antonopoulos et al., 2014). In the context of Orthopaedic surgery, encrypted mononuclear cell tissue factor procoagulant activity significantly increases on day 1 and 2 after knee replacement, decreasing to normal levels by day 6. In this study, it was raised in all 19 patients (G. J. Johnson, Leis, & Bach, 2009).

Recently, Inflammatory cytokines such as Interleukin 6 and adhesion molecules including Vascular cell adhesion molecule 1 (VCAM-1) have been found to be associated with development of venous thrombosis (Mosevoll, Lindås, Tvedt, Bruserud, & Reikvam, 2015) (Hou et al., 2012). However, it is unclear whether these can be used to predict which patients will develop venous thrombosis.

During clot resolution, fibrin is degraded into d-dimer and fibrin degradation products (Rectenwald et al., 2005). Thrombin-antithrombin (TAT) complexes are also found in patients with thrombus formation (Hostler et al., 2007). In a study of d-dimer levels pre and post total hip replacement in 63 patients, mean d-dimer levels increased approximately 10 times baseline levels at day 7 following surgery (preoperative level 0.88 +/- 0.71 micrograms per mil to 9.26 +/- 4.87). D-dimer was still significantly raised compared to pre surgery levels at 21 days following surgery. Although none of these patients had lower limb venous imaging to screen for deep vein thrombosis, this study suggests that hip replacement surgery is a prothrombotic event (Fujisawa, Naito, Asayama, Kambe, & Koga, 2003). This has been well established in previous studies (Mont et al., 2011) (Abraham, Ternisien, Hubert, Pidhorz, & Saumet, 1999).

Considering the literature discussed, it may be possible to use biomarkers of tissue injury and coagulation to identify patients with prothrombotic states following lower limb trauma. This may enable risk stratification of those who would benefit most from thromboprophylaxis.

2.6   Aims and objectives of this work

The aim of this work is to firstly to critically interrogate the literature for the effects of chemical and mechanical thromboprophylaxis in patients with foot and ankle trauma treated with leg cast immobilisation. The secondary aims are to determine the laterality of venous thrombosis in relation to the casted and injured leg, and determine the roles of venous stasis, immobility and tissue injury in the development of DVT in these patients.

Only by understanding the relative contributions of each will it be possible to develop strategies to identify those at greatest risk of developing thrombosis and accurate methods of prevention.

Objectives

In order to identify best practice in the prevention of VTE in patients with acute foot and ankle injury treated with leg cast, it is pertinent to review the literature for or against use of chemical or mechanical thromboprophylaxis in these patients. In Chapter 3, the evidence is critically analysed by systematic review. Results are pooled and presented as a meta-analysis.

To quantify the role of below knee cast immobilization on venous stasis, I will assess the effect of below knee cast immobilization on baseline popliteal venous velocity in healthy volunteers. Further, to develop potential thromboprophylactic strategy I will assess the effect of active toe and ankle

movements on popliteal venous velocity before and after application of a below knee polymer cast.

To confirm that active toe movements are an effective strategy for prevention of venous stasis and venous thrombosis in patients with foot and ankle injury, I will assess the effects of AToM on calf pump function and asymptomatic deep vein thrombosis in patients with below knee cast immobilization of the leg.

To determine the relative contribution of mobility in the development of venous thrombosis I will objectively measure mobility in patients with lower limb trauma treated with non-weight bearing below knee cast and determine the association between level of mobility and subsequent development of DVT.

In order to develop potential strategies to predict which patients with foot and ankle injury treated with leg cast will subsequently develop venous thrombosis, I will measure tissue factor, interleukin 6, VCAM-1 and d-dimer at time of presentation to the Emergency department (within 3 days of injury) and examine the association with subsequent finding of asymptomatic venous thrombosis.

3 Systematic review and meta-analysis of thromboprophylaxis in patients with below knee cast immobilization for trauma

The OVID interface was used to search MEDLINE and EMBASE databases up to 1st June 2015. The following search strategy, previously used by Roberts et al. (2012) was used: (exp venous thrombosis OR exp thromboembolism OR exp pulmonary embolism OR DVT.mp OR deep vein thrombosis.mp OR PE.mp OR pulmonary embolism.mp OR venous thromb$.mp) AND (exp casts surgical OR plaster cast$.mp OR exp immobilization OR immobilization.mp) (C. Roberts, 2012). The search was limited to randomized controlled trials, with no language exclusions. One author performed the study selection based on the following defined inclusion and exclusion criteria. Inclusion criteria: Studies including adult patients of any venous thromboembolism risk stratification (including operative and non-operative treatment) with foot or ankle trauma treated with below knee cast or immobilizing splint. Study interventions were chemical or mechanical thromboprophylaxis started within 72 h of injury, with a control group, which had no thromboprophylaxis. The outcomes of efficacy were symptomatic venous thromboembolism (pulmonary embolism and deep vein thrombosis) objectively proven with imaging.

The outcomes of safety were:

1. Major bleeding i.e. bleeding resulting in death, risk to life or blood transfusion.

2. Clinically important non-major bleed i.e. bleeding that required withdrawal from the study.

3. Minor bleed i.e. any other type of bleed which was not major or clinically important.

Only full papers were reviewed. For trials that reported results in more than 1 publication, data from the most complete publication was extracted and used the other publications to clarify the data. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement for reporting of systematic reviews and meta-analyses of randomized clinical trials was followed. Two reviewers performed data extraction independently using standardized data extraction sheets (Figure 3.1). Discrepancies between the reviewers were reviewed by a third reviewer. Odds ratio and absolute risk reduction for symptomatic DVT were used to calculate number needed to prevent with thromboprophylaxis. Mantel Haenszel method was used to assess dichotomous outcomes. Statistical heterogeneity was determined using I^2 statistics. Fixed effects model was used when heterogeneity was <30%, using Review Manager (RevMan 5.0).

Fig. 3.1 Data items extracted from the included papers

Study Title:

Reference:

Study design/type of study:

Source of funding:

Included injuries with number of each:

Included participants (risk factors for thromboembolism):

Exclusion criteria:

Treatment (cast only, splint only, surgery +/- splint or cast):

Treatment group intervention:

Time between injury and recruitment into study:

Control group (e.g. placebo):

Outcome measure (symptomatic or asymptomatic venous thromboembolism)

Method of objective confirmation of thromboembolism:

	Study drug/intervention		Control group		HR/RR (95% CI)	p-value
	n	%	n	%		
Patients						
Symptomatic DVT – state location (vein involved, above or below knee)						
Asymptomatic DVT – state location (vein involved, above or below knee)						
Symptomatic PE						
Asymptomatic PE						
Major bleed						
Adverse events						
Death						

The risk of bias for each article was determined by two authors, who independently reviewed the full articles. Data was extracted from articles and a judgment with supporting information was made according the Cochrane Risk of Bias tool (Figure 3.2). In cases where authors disagreed, the evidence for the judgment was discussed and a consensus opinion was reached. A score from 1 to 3 was given for each of the 7 parameters. Where an item was deemed low risk of bias, a score of 1 was given for the item. A score of 2 was given if the risk of bias was deemed unclear. A score of 3 was given if the item was deemed high risk. The lowest risk of bias score for the 7 items was 7. The highest score was 21. Studies were ranked in decreasing order of risk of bias. Where assessors of outcome were not blinded to intervention group, the study was rated as high risk of bias.

Fig. 3.2 Risk of bias tool

Bias domain	Source of bias	Support for judgment	Review authors' judgment (assess as low, unclear or high risk of bias)
Selection bias	Random sequence generation	Describe the method used to generate the allocation sequence in sufficient detail to allow an assessment of whether it should produce comparable groups	Selection bias (biased allocation to interventions) due to inadequate generation of a randomised sequence
Selection bias	Allocation concealment	Describe the method used to conceal the allocation sequence in sufficient detail to determine whether intervention allocations could have been foreseen before or during enrolment	Selection bias (biased allocation to interventions) due to inadequate concealment of allocations before assignment
Performance bias	Blinding of participants and personnel*	Describe all measures used, if any, to blind trial participants and researchers from knowledge of which intervention a participant received. Provide any information relating to whether the intended blinding was effective	Performance bias due to knowledge of the allocated interventions by participants and personnel during the study
Detection bias	Blinding of outcome assessment*	Describe all measures used, if any, to blind outcome assessment from knowledge of which intervention a participant received. Provide any information relating to whether the intended blinding was effective	Detection bias due to knowledge of the allocated interventions by outcome assessment
Attrition bias	Incomplete outcome data*	Describe the completeness of outcome data for each main outcome, including attrition and exclusions from the analysis. State whether attrition and exclusions were reported, the numbers in each intervention group (compared with total randomised participants), reasons for attrition or exclusions where reported, and any re-inclusions in analyses for the review	Attrition bias due to amount, nature, or handling of incomplete outcome data
Reporting bias	Selective reporting	State how selective outcome reporting was examined and what was found	Reporting bias due to selective outcome reporting
Other bias	Anything else, ideally pre-specified	State any important concerns about bias not covered in the other domains in the tool	Bias due to problems not covered elsewhere

*Assessments should be made for each main outcome or class of outcome

Patients and Interventions

Nine prospective randomized controlled trials were included in this review (Table 3.1). Study details according to data extraction form are displayed in Table 3.2. A Funnel plot revealed no publication biases (Figure 3.3) (Egger, Davey Smith, Schneider, & Minder, 1997).

Table 3.1 Studies included

| Author	Year	Title	Journal
Selby R, Geerts WH, Kreder HJ, Crowther MA, Kaus L, Sealey F	2015	A double-blind, randomized controlled trial of the prevention of clinically important venous thromboembolism after isolated lower leg fractures	J Orthop Trauma. 2015 May;29(5):224-30
Sultan, M. J., Zhing, T., Morris, J., Kurdy, N., & McCollum, C. N.	2014	Compression stockings in the management of fractures of the ankle: a randomised controlled trial	The Bone & Joint Journal, 96-B(8), 1062–1069.
Domeij-Arverud, E., Latifi, A., Labruto, F., Nilsson, G., & Ackermann, P. W.	2013	Can foot compression under a plaster cast prevent deep-vein thrombosis during lower limb immobilisation?	The Bone & Joint Journal, 95-B(9), 1227–1231.
Lapidus, L. J., Ponzer, S., Elvin, A., Levander, C., Lärfars, G., Rosfors, S., & De Bri, E.	2007	Prolonged thromboprophylaxis with Dalteparin during immobilization after ankle fracture surgery: a randomized placebo-controlled, double-blind study	Acta Orthopaedica, 78(4), 528–535.
Lapidus L.J., Rosfors S., Ponzer S., Levander C., Elvin A, Lärfars G, de Bri E.	2007	Prolonged Thromboprophylaxis With Dalteparin After Surgical Treatment of Achilles Tendon Rupture: A Randomized, Placebo-Controlled Study	J Orthop Trauma. 2007 Jan;21(1):52-7.
Jørgensen, P. S., Warming, T., Hansen, K., Paltved, C., Vibeke Berg, H., Jensen, R., et al.	2002	Low molecular weight heparin (Innohep) as thromboprophylaxis in outpatients with a plaster cast: a venografic controlled study.	Thrombosis Research, 105(6), 477–480.
Lassen, M. R., Borris, L. C., & Nakov, R. L.	2002	Use of the low-molecular-weight heparin reviparin to prevent deep-vein thrombosis after leg injury requiring immobilization	The New England Journal of Medicine, 347(10), 726–730.
Kock, H. J., Schmit-Neuerburg, K. P., Hanke, J., Rudofsky, G., & Hirche, H.	1995	Thromboprophylaxis with low-molecular-weight heparin in outpatients with plaster-cast immobilisation of the leg	The Lancet, 346(8973), 459–461.
Spannagel, U., & Kujath, P.	1993	Low molecular weight heparin for the prevention of thromboembolism	Seminars in Thrombosis and

| | in outpatients immobilized by plaster cast | Hemostasis, 19 Suppl 1, 131–141. |

Table 3.2 Details of included studies

Study	Year	Injuries included and total number of participants	Participants included (VTE risk factors)	Exclusion criteria	Treatment	Treatment group intervention	Control	Time between injury and recruitment	Outcome measure	Method of objective confirmation of VTE
Selby, R. et al	2015	Operated lower limb trauma (tibia, fibula, ankle +/- associated foot or patella fractures)	Age 18-87 years Surgery BMI not stated OCP not stated Smoking status not stated	Major trauma Other anticoagulant use Allergy to LMWH Pregnancy Active cancer Previous VTE Hypercoagulable state Active bleeding or bleeding disorder Intracranial bleed in previous 4 weeks Vascular injury needing repair	All surgical and cast or splint	Dalteparin 5000 and Xa for 14 +/- 2 days	Matching placebo 14 +/- 2 days	<72 hours	Symptomatic VTE within 3 months or asymptomatic proximal DVT on lower limb venous ultrasound at 14 days	Lower limb venous ultrasound
Sultan, M.J. et al	2014	Operated and non operated ankle fractures (n=90)	Age 16-90 years BMI up to 40 Some patients had surgery	Previous ankle fracture or ankle surgery Open fracture Pilon complex fracture Injury or condition that impaired mobility Peripheral arterial disease or abnormal Doppler waveform Malignancy Chronic debilitating illness Treating Orthopaedic surgeon choice	Aircast boot +/- surgery Patients who had surgery, also had clexane 20mg Non-weight bearing for all	Ankle Injury Stocking – 25mmHg pressure at ankle, 17mmHg mid calf and 10mmHg upper calf – equivalent to class 2 stockings	Tubigrip under aircast	Within 72hrs of injury	A secondary outcome measure was asymptomatic DVT on duplex at 4 weeks	An experienced vascular technologist undertook duplex ultrasound imaging of the deep veins in the calf and thigh at four weeks.
Domeji Averyd, E. et al	2013	Acute unilateral Achilles rupture (n=24)	Age 18-75 years BMI up to 39.1 Operated within 72 hours	Inability to give informed consent Ongoing anticoagulation treatment (including high dose Aspirin) Planned follow up at different hospital Inability to follow instructions Kidney failure Heart failure with pitting oedema Thrombophlebitis Previous VTE – during preceding 3 months Surgery in previous month Malignancy Haemophilia Pregnancy Unwillingness to participate	Surgery and postoperative cast. Then at 2 weeks post surgery, all patients went into DonJoy walking boot for 4 weeks.	Foot intermittent pneumatic compression beneath the cast	Nothing	Within 72 hours	Symptomatic and Asymptomatic DVT	All patients were screened for DVT in the operated leg using unilateral color duplex sonography (CDS) at two and six weeks post-operatively
Lapidus, L.J., Ponzer, S. et al	2007	Operatively treated ankle fractures (n=272)	18-75 years All operated	Inability or refusal to sign consent Inability to comply (dementia, alcoholism) Current anticoagulants Allergy to contrast Planned follow up at another hospital Renal disorders including transplant VTE within 3 months Surgery within 1 month Malignancy Current bleeding disorder Pregnancy Aspirin use >325mg or other platelet inhibitors Polytrauma	Surgery and cast	1,000ml Dextran 60 on admission for all patients, Dalteparin subcut 5,000 IU once daily for 1 week for all patients, then randomized to continue or placebo until plaster removed at 5 weeks post surgery	Placebo in identical syringe to intervention	Within 72 hours of injury	Asymptomatic at time of cast removal	All patients had phlebography on operated limb only, on day of cast or splint removal. Colour duplex was done is phlebography failed. – All duplex done by 1 of 4 vascular technologists. Also did venography if clinical VTE during study period. CTPA or V/Q if PE clinically suspected

Author	Year	Injury	Inclusion	Exclusion	Treatment setting	Intervention	Control	Timing	Outcome	Imaging
Lapidus, L.J., Rosfors, S. et al	2007	Achilles tendon ruptures (n=105)	18-75 years Surgical treatment	Refusal or inability to consent Ongoing anticoagulants Contrast allergy Intended follow up in alternative hospital Inability to comply with study Renal disorder Recent VTE within 3 months Surgery in preceding month Malignancy Bleeding disorder Pregnancy High dose aspirin or platelet inhibitors Other injuries	Surgery with a postoperative cast or orthosis for 6 weeks	Dalteparin 25,000 units anti Xa/ml, 5000 Unit dose once daily injection	Placebo injection with 0.9% saline in an identical syringe, 6 weeks supply	Within 72 hours of injury	Asymptomatic and symptomatic VTE	Unilateral colour duplex USS followed by plethysmography for confirmation if duplex positive
Jorgensen, P.S. et al	2002	Fracture and tendon injuries (n=300)	Planned lower limb cast for at least 3 weeks Oral contraceptive pill Previous DVT Smokers Varicose veins Surgery	Pregnancy Allergy to heparin or contrast media Renal or liver impairment Uncontrolled hypertension Bleeding disorders Cerebral insults due to bleeding GI bleeding Inability to perform self-injection	All patients had below knee casts. 86pts in Innohep group had surgery, 89 pts in control had surgery. Injury demographics and surgery was not significantly different between groups.	3,500 IU anti Xa tinzaparin (Innohep) s/c once daily for total casting period – mean duration was 5.5 weeks	Nothing	Not stated	Asymptomatic DVT	Unilateral ascending venography
Lassen, M.R. et al	2002	Fractures (tibia, patella, malleoli, foot), Achilles tendon rupture (n+438)	Age up to 56 years BMI up to 28 Previous VTE Varicose veins OCP use HRT use Surgery Casts Smokers	Body weight <35kg Current VTE Systolic BP >200mmHg or diastolic >110 Cerebral aneurysm CVA within 3 weeks Active GI ulcer Haemorrhagic diathesis Bacterial endocarditis Platelets <100,000 per cubic mm Previous heparin use Immobilisation .4 days prior to enrolment Heparin or contrast allergy Contraindication to venography	Surgery or cast immobilisation	1750 anti-Xa units of reviparin (Clivarine, Knoll) subcut to be injected once daily whilst immobilised	The patients received identical prefilled syringes with placebo to take once daily for time of immobilization	Within 4 days of injury	Symptomatic or asymptomatic VTE	ascending venography of the injured leg within one week after removal of the plaster cast or brace. Venography was performed earlier if there was a clinical suspicion of thrombosis
Kock, H.J. et al	1995	Fractures or sprains	Age up to 65 years Obesity (Broca index >1.2) Varicose veins Smokers OCP Cast	Surgical treatment Previous DVT Pregnancy Clotting disorders or anticoagulant medication Bleeding sources Chronic venous insufficiency Contra-indication to heparin	Non-operative treatment with cast	LMWH (Mono-Embolex) daily s/c injection 32mg for duration of cast	Nothing	Not stated – but all had imaging to exclude DVT prior to randomization	Asymptomatic DVT	Ultrasound and phlebography
Spannagel, U. et al	1993	Fractures, soft tissue injuries	Age up to 76 years Cast Smoking Overweight (110% Broca) Previous thrombosis Varicose veins Malignant disease OCP Pregnancy Heart failure	Hypersensitivity to heparin Thrombopathy Treatment with oral anticoagulation or platelet inhibitors Acute cerebral or GI bleeding Acute pancreatitis Inflammatory heart disease Arterial hypertension (diastolic more than 120mmHg) Renal failure (serum creatinine 3mg/dL or greater)	Non-operative soft tissue and bony injury with plaster cast for at least 7 days If patients went on to have operation they were scanned prior to this and exited the study	LMWH 36mg injection once daily for period of cast	Nothing	Not stated	All were scanned. Asymptomatic DVT. Some had symptoms at time of scanning (33.3% of those with positive scans, but criteria for 'symptomatic' not defined)	Compression ultrasound on cast removal Phlebography on cases of positive compression ultrasound

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Fig. 3.3 Funnel plot of Total DVT (Symptomatic and Asymptomatic)
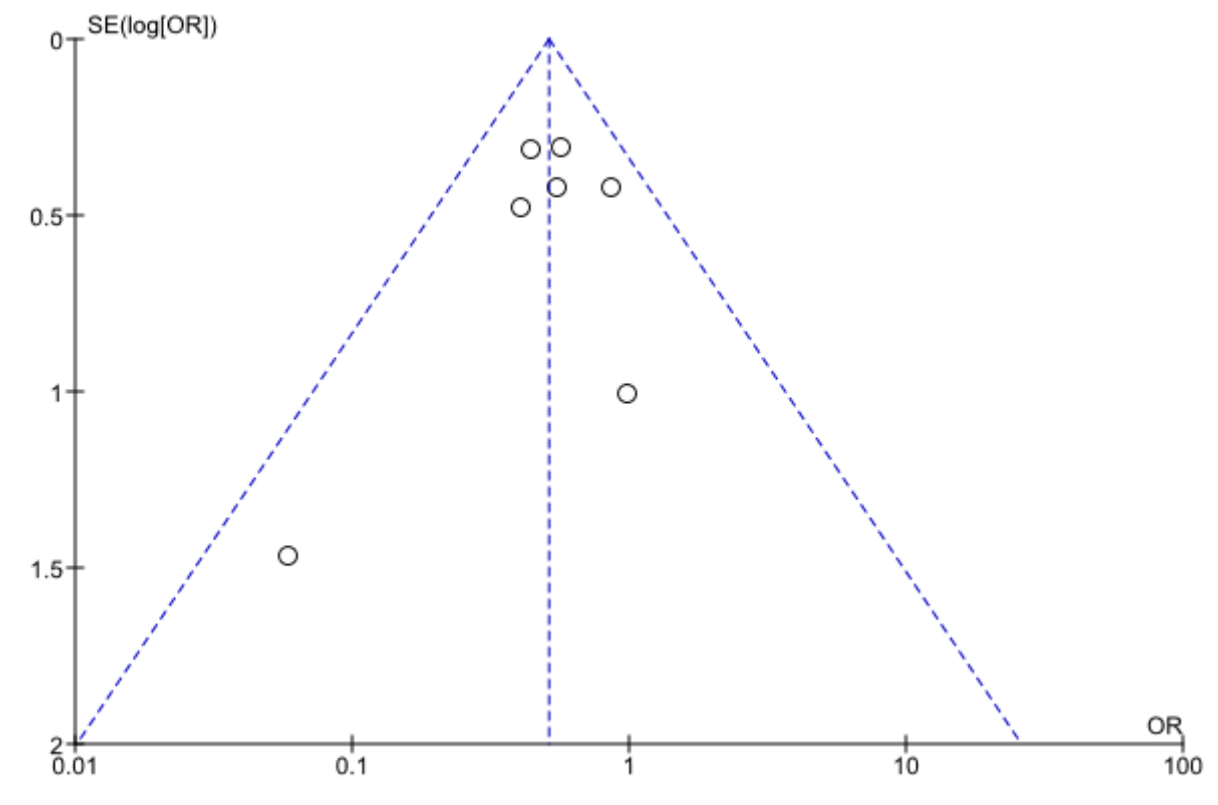
Seven of these studies focused on chemical thromboprophylaxis and two studied mechanical thromboprophylaxis. Only two studies considered patients treated non-operatively (Kock, Schmit-Neuerburg, Hanke, Rudofsky, & Hirche, 1995; Spannagel & Kujath, 1993), with all others including patients who underwent surgery. Two have focused on patients with ankle fractures (Lapidus et al., 2007; Sultan, Zhing, Morris, Kurdy, & McCollum, 2014), two have focused on Achilles tendon ruptures (Domeij-Arverud et al., 2013), (Lapidus et al., 2007), and the remaining 5 studies include patients with a variety of soft tissue and bony injuries (Kock et al., 1995; Lassen, Borris, & Nakov, 2002; Spannagel & Kujath, 1993; Wille-Jørgensen, Jorgensen, & Crawford, 2005) (Selby et al., 2015). Included studies did not provide details

of numbers of patients with individual risk factors for venous thromboembolism.

In view of current knowledge of risk factors for venous thromboembolism (VTE in patients with lower limb immobilization, and in accordance with guidelines of the Emergency College of Medicine, the most important permanent risk factors appear to be: current hormone replacement therapy/oral contraceptive pill, personal or first degree relative history of VTE, active smoker, any recent hospital admission or major surgery, pregnancy or immediate postpartum, any serious co-morbidity including cardiac failure, chronic obstructive pulmonary disease, chronic renal failure or inflammatory bowel disease, extensive varicosities, active cancer, obesity (BMI >30), known thrombophilia, age >60 years. Considering these risk factors, all of the studies included at least some patients who would be considered 'high risk' for VTE, with current recommendation to provide low molecular weight heparin. The earlier four papers even included patients with malignancy, which would be ethically impossible by today's standards. (Kock et al., 1995; Lassen et al., 2002; Spannagel & Kujath, 1993; Wille-Jørgensen et al., 2005). In 5 studies, patients were recruited within 72 hours of injury (Sultan et al., 2014) (Domeij-Arverud et al., 2013) (Lapidus et al., 2007) (Selby et al., 2015). Lassen et al recruited patients within 4 days of injury (Lassen et al., 2002). In the study by Kock et al, the time to recruitment was not stated, however all patients underwent imaging to exclude DVT prior to entering the study (Kock et al., 1995). In two studies, the time between injury and recruitment is not stated (Wille-Jørgensen et al., 2005) (Spannagel & Kujath, 1993). It is

therefore possible that some patients in these studies may have developed asymptomatic DVT prior to entering the study.

Mechanical thromboprophylaxis studies, included an Ankle Injury compression stocking in one study (25mmHg pressure at the ankle, 17mmHg mid calf and 10mmHg upper calf) (Sultan et al., 2014). The second mechanical thromboprophylaxis study examined the effect of a pneumatic foot compression device beneath the cast (Domeij-Arverud et al., 2013). It is clearly difficult to blind patients to the latter, in which the control group received no placebo.

In the 7 studies of chemical thromboprophylaxis, all focused on low molecular weight heparin as the intervention: Subcutaneous Dalteparin 5000 international units once daily (Lapidus et al., 2007) (Selby et al., 2015), Subcutaneous Tinzaparin (Innohep) 3500 international units once daily (Jørgensen et al., 2002), 1750 anti-Xa units of reviparin (Clivarine, Knoll) subcutaneous once daily (Lassen et al., 2002), LMWH (Mono-Embolex) daily s/c injection 32mg (Kock et al., 1995), LMWH 36mg injection once daily (Spannagel & Kujath, 1993). Some of these studies included overweight patients, and without dose adjustment for body weight it is possible that doses may have been sub prophylactic in some patients (Spannagel & Kujath, 1993), (Kock et al., 1995).

All chemical thromboprophylaxis studies used venography to confirm asymptomatic DVT, except for the most recent study by Selby et al (2015) (Selby et al., 2015). It is important to recognize that technological advances and increased operator experience in the use of non-invasive duplex ultrasonography has made this commonplace. Venography has generally been replaced by utrasound which is more economical, less invasive and safer (Rectenwald et al., 2005) (L. N. Roberts & Arya, 2011) (Goldhaber & Bounameaux, 2012). In the hands of an experienced operator, ultrasonography has a sensitivity of 100%, specificity of 98% and accuracy of 98% for patients with lower limb DVT when compared with venography (Garino, Lotke, Kitziger, & Steinberg, 1996). In the three recent studies, conducted in 2013 (Domeij-Arverud et al., 2013) and 2014 (Sultan et al., 2014), duplex ultrasound was used to image the lower limb venous system (Selby et al., 2015).

Asymptomatic Venous Thromboembolic Outcomes

All studies presented data for asymptomatic DVT. A summary of events is displayed in Table 3.3. None of the included studies assessed for asymptomatic pulmonary emboli. Events that occurred are denoted numerically with percentages. The incidence of asymptomatic DVT in the control group ranged from 4.3% (Kock et al., 1995) and 42% (Domeij-Arverud et al., 2013). Two studies considered the effect of mechanical thromboprophylaxis on asymptomatic DVT rate (Domeij-Arverud et al., 2013; Sultan et al., 2014). In the latter paper this was a secondary outcome

measure and with 90 patients recruited it was underpowered to detect a statistically significant difference. The paper by Domeij-Averyd et al considered the effects of an intermittent pneumatic foot compression pump under cast in patients with Achilles tendon rupture. The asymptomatic DVT rate was very high in both the intervention group (67% n=8/12) and the control group (42% n=5/12), which did not reach statistical significance. In view of this high DVT rate the study was stopped early. There were no reported symptomatic DVT's reported in these studies and no Pulmonary Emboli occurred (symptomatic or asymptomatic). In view of this, there is no present evidence that mechanical thromboprophylaxis is effective in patients with lower limb trauma and cast immobilization. Further adequately powered, prospective randomized controlled studies are necessary to answer this question.

Table 3.3 Summary of Asymptomatic venous thromboembolic events

Note that no studies reported asymptomatic pulmonary embolism

Study	Year	Intervention group	Control group	p-value
Selby, R. et al	2015	1/128 (0.78%) all proximal	1/130 (0.77%) all proximal	0.99
Sultan, M.J. et al	2014	5/42 (12%)	10/44 (23%	0.26
Domeji Averyd, E. et al	2013	8/12 (67%) All distal	5/12 (42%) All distal	0.08
Lapidus, L.J., Ponzer, S. et al	2007	21/101 (21%)	27/96 (28%)	0.25 *note that a secondary analysis of patients with casts (excluding splints) did find a statistically significant reduction
Lapidus, L.J., Rosfors, S. et al	2007	16/47 (34%) 1 proximal DVT	16/36 (36%) 3 proximal DVT	0.8
Jorgensen, P.S. et al	2002	10/99 (10%)	18/106 (17%) All below knee except one above knee in this group	0.16
Lassen, M.R. et al	2002	3 proximal/189 (2%) 14 distal/183 (8%)	10 proximal/191 (5%) 25 distal/188 (13%)	0.09 0.09
Kock, H.J. et al	1995	0/176 (0%)	7/163 (4.3%) 3 proximal, 4 distal	<0.006
Spannagel, U. et al	1993	6/126 (4.8)	21/127 (16.5)	<0.01

The seven prospective randomized controlled trials studying the effects of chemical thromboprophylaxis in patients with trauma and lower limb immobilization were conducted between 1993 and 2015. The first of these was the study of 253 patients with lower limb fractures or soft tissue injuries treated with casts by Spannagel et al (1993), in which LMWH resulted in a statistically significant reduction in asymptomatic DVT (4.8% LMWH group v 16.5% control p <0.01). However, there were no details of the location of the DVT's therefore this study was not included in the meta-analysis. Furthermore, 33% of 27 patients found to have DVT on screening were reported to have some clinical symptoms, but it is not discussed whether these were in the control or intervention group. Only 3 DVT events were above knee (Spannagel & Kujath, 1993). Although Kock et al (1995) found pooled total of above and below knee asymptomatic DVT events were statistically significantly reduced in the intervention group (p=0.006) in their study of 339 patients with lower limb fractures or sprains (176 provided with LMWH), this was not the case when analyzing above and below knee DVT events individually (Kock et al., 1995). 3 studies (Table 3.4) provided details of asymptomatic below knee DVT outcomes. In all of these, the confidence interval of the odds ratio crossed 1, therefore there was no statistically significant reduction in DVT in patients who received LMWH. However, on pooling the results at meta analysis, the odds ratio was 0.54 (95% CI 0.32 – 0.90), indicating that asymptomatic below knee DVT is reduced in these patients. Meta-analysis of asymptomatic above knee DVT events included 4 studies (Table 3.5), none of these found statistically significant DVT reductions in individual studies or on pooling of results. Meta-analysis of

Asymptomatic total DVT (Table 3.6) found a reduction in total asymptomatic

total DVT (OR 0.53, 95% CI 0.36 – 0.77). Based on this, 19 patients would

require thromboprophylaxis with LMWH to prevent one asymptomatic DVT.

Table 3.4 Asymptomatic distal DVT
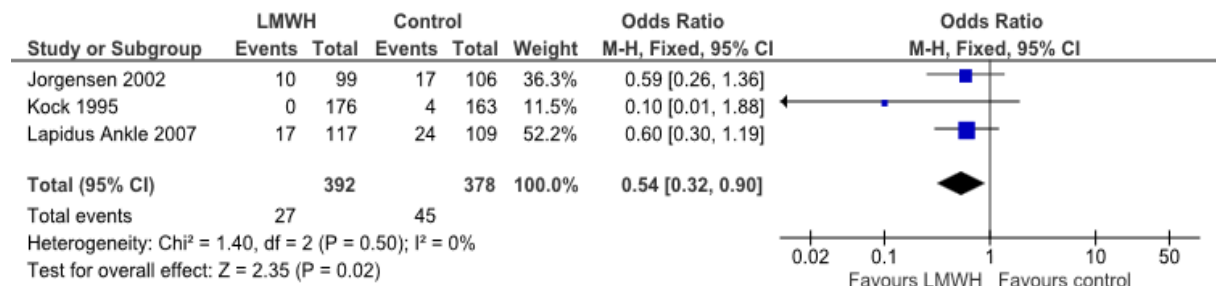
Table 3.5 Asymptomatic proximal DVT
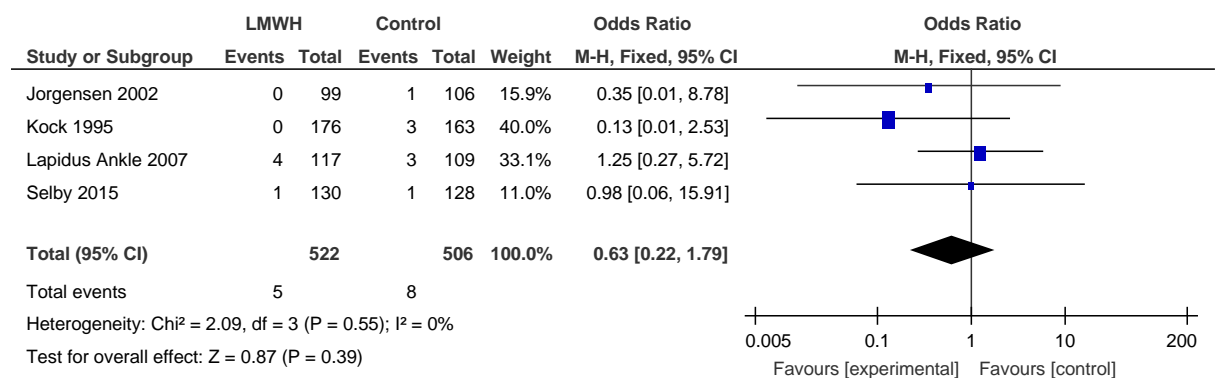
Table 3.6 Asymptomatic total DVT
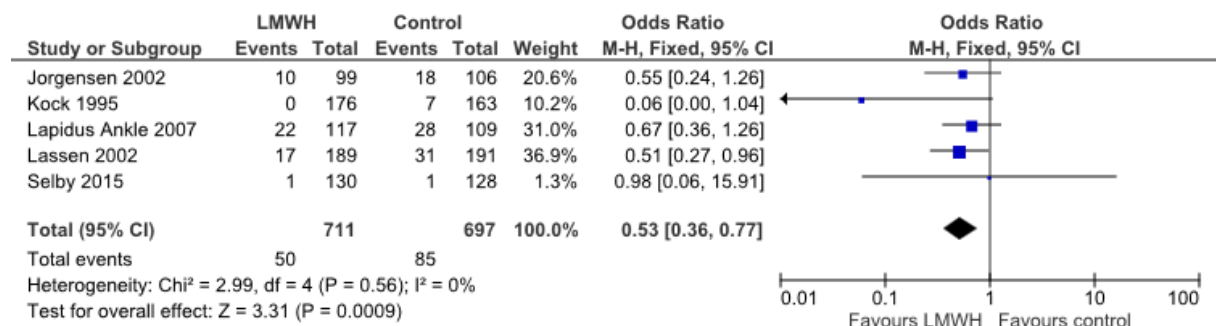
Number needed to prevent asymptomatic DVT = 1/((711-50/711) – (697-85)/697)) = 19

Symptomatic Venous Thromboembolic Outcomes

Five of the nine studies presented data on symptomatic DVT events (Jørgensen et al., 2002; Lapidus et al., 2007; Lassen et al., 2002; Spannagel & Kujath, 1993) (Selby et al., 2015). 2 of these studies reported no symptomatic events in control of intervention groups and were excluded from meta-analysis (Table 3.7) (Jørgensen et al., 2002) (Spannagel & Kujath, 1993). Considering the 5 studies presenting symptomatic DVT data, the overall incidence was 11/697 (1.58%) in the control group (Table 3.8). Considering the 3 studies included in the meta-analysis (Table 3.7) none of these studies found a statistically significant symptomatic DVT reduction individually, however at meta-analysis of pooled results there was a statistically significant reduction in symptomatic DVT in the patients who received LMWH (OR 0.29, 95% CI 0.09 – 0.95) (Lapidus et al., 2007) (Lassen et al., 2002) (Selby et al., 2015).

Table 3.7 Symptomatic DVT meta analysis

Study or Subgroup	LMWH Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Lapidus Ankle 2007	2	117	6	109	52.8%	0.30 [0.06, 1.51]
Lassen 2002	0	189	4	191	38.6%	0.11 [0.01, 2.06]
Selby 2015	1	130	1	128	8.6%	0.98 [0.06, 15.91]
Total (95% CI)		436		428	100.0%	0.29 [0.09, 0.95]
Total events	3		11			

Heterogeneity: Chi² = 1.17, df = 2 (P = 0.56); I² = 0%

Test for overall effect: Z = 2.04 (P = 0.04)
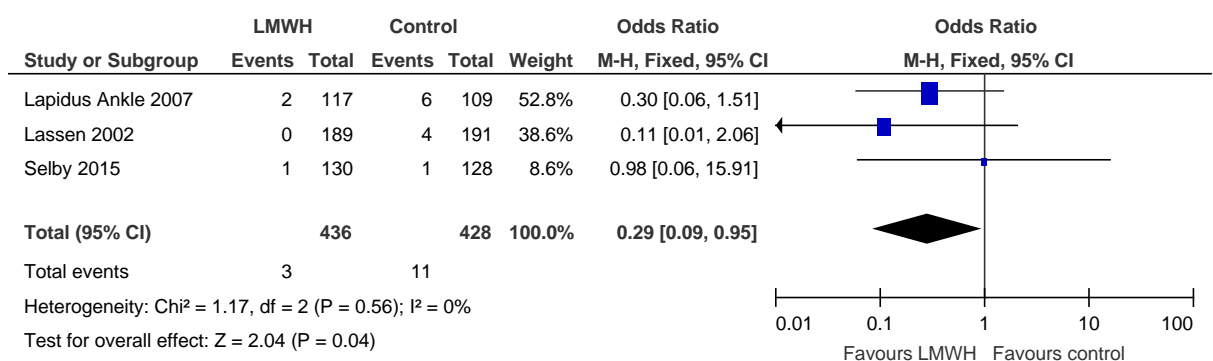
Table 3.8 Symptomatic DVT

Study	Intervention (n)	Symptomatic DVT (total)	Control	Symptomatic DVT (total)
Kock 1995	176	0	163	0
Lassen 2002	189	0	191	4
Jorgensen 2002	99	0	106	0
Lapidus Ankle 2007	117	2	109	6
Selby 2015	130	1	128	1
Total	711	3	697	11

Six studies presented data for symptomatic pulmonary embolism (Lapidus et al., 2007) Lapidus and Rosfors 2007, (Jørgensen et al., 2002; Lassen et al., 2002; Spannagel & Kujath, 1993) (Selby et al., 2015) (Table 3.9). The highest symptomatic pulmonary embolism occurrence was 1%, found in the study by Lassen et al (Lassen et al., 2002). The overall symptomatic pulmonary embolism rate considering studies presenting this data was 3/692 (0.43%). None of these pulmonary emboli were fatal. Meta-analysis was not possible with only 3 events. LMWH did not result in statistically significant reductions in symptomatic pulmonary emboli in any of these studies. Symptomatic VTE events were not reported in either of the two studies which examined the effect of mechanical thromboprophylaxis (Domeij-Arverud et al., 2013; Sultan et al., 2014).

Table 3.9 Symptomatic Pulmonary Embolism

Study	Intervention (n)	Symptomatic PE	Control	Symptomatic PE
Kock 1995	176	0	163	0
Spannagel 1993	126	0	127	0
Lassen 2002	217	0	221	2
Jorgensen 2002	99	0	106	0
Lapidus Ankle 2007	117	0	109	0
Lapidus Achilles 2007	49	0	47	0
Selby 2015	130	0	128	1
Total	914	0	901	3

Considering the overall number of symptomatic VTE events reported by included studies (Table 3.10), 82 patients would require thromboprophylaxis with LMWH to prevent on symptomatic VTE (including Pulmonary Embolism and Deep Vein Thrombosis).

Table 3.10 Symptomatic Venous Thromboembolism (PE or DVT)

Study	Intervention (n)	Symptomatic VTE	Control	Symptomatic VTE
Kock 1995	176	0	163	0
Spannagel 1993	126	0	127	0
Lassen 2002	217	0	221	6
Jorgensen 2002	99	0	106	0
Lapidus Ankle 2007	117	2	109	6
Lapidus Achilles 2007	49	0	47	0
Selby 2015	130	1	128	2
Total	914	3	901	14

Number needed to prevent one symptomatic VTE = $1/((914-3)/914) - ((901-14)/901) = 81.6$

Bleeding events

The 7 studies of chemical thromboprophylaxis reported major bleeding, with one non fatal retro-peritoneal hemorrhage event occurring in the study by Lassen et al (2002) (Lassen et al., 2002) (Table 3.11). Considering the total number of patients who received LMWH in these studies, the overall incidence of major bleeding was 1 in 886 (0.11%), number needed to harm = 886. Considering that the number needed to prevent symptomatic VTE was found to be 82, 10 symptomatic VTE events would be prevented for every major bleed. Clinically relevant non-major and minor bleeds were more likely to occur in the LMWH groups (Table 3.12, Table 3.13) however at meta-analysis the 95% confidence interval crossed 1 in both cases and therefore

the difference in bleeding event rates for these outcomes was not statistically

significant.

Table 3.11 Major bleeding events

Study	Intervention	Major bleed	Control	Major bleed	
Kock 1995	176	0	163		0
Spannagel 1993	126	0	127		0
Lassen 2002	189	1	191		0
Jorgensen 2002	99	0	106		0
Lapidus Ankle 2007	117	0	109		0
Lapidus Achilles 2007	49	0	47		0
Selby 2015	130	0	128		0

Table 3.12 Clinically relevant non-major bleed
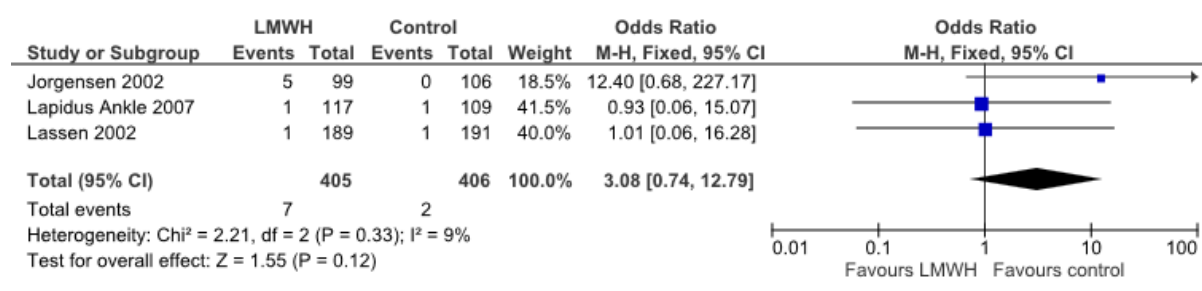
Table 3.13 Minor bleed
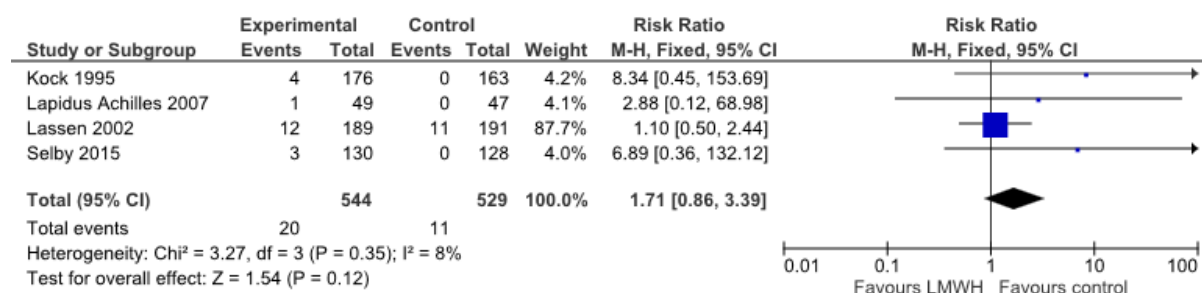
Risk of bias assessment

The risk of bias for each article was determined by two authors, who independently reviewed the full articles (BH and UW). Data was extracted from articles and a judgment with supporting information was made according the Cochrane Risk of Bias tool (Table 3.14) (Higgins et al., 2011). In cases where authors disagreed, the evidence for the judgment was discussed and a consensus opinion was reached (RA). A score from 1 to 3 was given for each of the 7 parameters. Where an item was deemed low risk of bias, a score of 1 was given for the item. A score of 2 was given if the risk of bias was deemed unclear. A score of 3 was given if the item was deemed high risk. The lowest risk of bias score for the 7 items was 7. The highest score was 21. Studies were ranked in decreasing order of risk of bias. Where assessors of outcome were not blinded to intervention group, the study was rated as high risk of bias.

None of the studies were free of risk of bias. The study deemed to be at least risk of bias was by Lassen et al. This study was generally low risk of bias, however there were no details provided in the paper of the method of group allocation concealment (Lassen et al., 2002). A recent study of mechanical thrombopropylaxis using intermittent pneumatic compression under a cast was also deemed low risk of bias (Domeij-Arverud et al., 2013). This study was stopped early due a very high rate of asymptomatic deep vein thrombosis in the intervention group (8/12 in intervention group v 5/12 in control p=0.08). The study by Lapidus and Ponser (Lapidus et al., 2007) scored a 3 'high risk

of bias' in the category of 'Other' because all patients (those in intervention and those in control) received LMWH for 1 week prior to being randomized. It is possible that this may have reduced the effect size of the LMWH provided to the intervention group after randomization. This may account for the failure of this study to find statistically significant reduction in asymptomatic DVT between intervention and control groups. However, despite this, there was a statistically significant reduction in asymptomatic DVT in the LMWH group for a secondary analysis of patients treated with cast immobilization (excluding those with splints). The studies by Kock and Spannagel (Kock et al., 1995; Spannagel & Kujath, 1993) were considered to be at highest risk of bias overall. Also, these studies did not state whether assessors of outcome of DVT were blinded to participant intervention group. The results of these studies should be viewed with caution. The study of ankle injury stockings in patients with ankle fracture by Sultan et al (Sultan et al., 2014) was generally low risk of bias however the study is inadequately powered to find a statistically significant reduction in DVT as an outcome (n=90), which was a secondary outcome measure.

Table 3.14 Risk of bias assessment

Study	Year	Selection bias – Random sequence generation	Selection bias – Allocation concealment	Performance bias – Blinding of participants and personnel	Detection bias – Blinding of outcome assessment	Attrition bias – Incomplete outcome data	Reporting bias – Selective reporting	Other bias	Total risk of bias score
Selby, R. et al	2015	Randomisation using a computer randomization code Low risk of bias (1)	Medications were pre-packaged by study sponsor and distributed by them Low risk of bias (1)	Patients and all research personnel were blinded to the study medication. However, does not state that study/placebo were identical Unclear risk (2)	Bilateral Doppler ultrasound by certified vascular technologist with confirmation by staff radiologist, both blinded Low risk (1)	Compliance assessed by syringe count. Clear about who was lost and why Low risk of bias (1)	Made it clear that they only looked at clinically important venous thromboembolism events Low risk of bias (1)	Nil. Low risk of bias (1)	8
Domeji Averyd, E. et al	2013	Computer-generated random numbers in permuted blocks of four to undergo either standard plaster cast treatment alone or plaster cast Low risk of bias (1)	Computer generated Low risk of bias (1)	Participants could not be blinded High risk of bias (3)	Two experienced ultrasonographers (FL, AL), who were blinded to the treatment regimens, performed all the screening for DVTs Low risk of bias (1)	26 were randomized, only 2 dropped out because they could not tolerate IPC Low risk of bias (1)	All VTE outcomes were reported Low risk of bias (1)	No power calculation but incidence was much higher than anticipated Low risk of bias (1)	9
Lassen, M.R. et al	2012	Randomization was performed by computer in blocks of four Low risk of bias (1)	No details of allocation concealment Unclear risk (2)	Control group received identical placebo injection. All the data were collected by a Danish contract research organization and transferred to the statistical department of the sponsor Low risk of bias (1)	Review of venography centrally by 3 radiologists blinded to group Low risk of bias (1)	4 patients dropped out due to adverse events but not stated what these events were Unclear risk of bias (2)	Reported that one patient had venogram more than 1 week after cast removal and excluded this because it did not meet inclusion, which was good. Reported on planned outcome measure. Low risk of bias (1)	Malleolar fractures significantly less common and Achilles tendon rupture more common in LMWH group Unclear risk of bias (2)	10
Lapidus, L.J., Ponzer, S. et al	2007	Randomisation method not stated Unclear risk (2)	Allocation concealment not stated Unclear risk (2)	Control group received identical placebo injection pen Low risk of bias (1)	An independent radiologist who was blinded to the randomization Low risk of bias (1)	All patients accounted for Low risk of bias. 75/272 randomised were lost to follow up. Reasons were stated but this is high (28%). (1)	All patients accounted for and intention to treat analysis done for primary outcome i.e. venographic DVT Low risk of bias (1)	All patients received LMWH for a week before randomization, which could have equalized the results. This was felt to be necessary ethically High risk of bias in a sense that LMWH effect size could have been reduced between groups after randomization (3)	11
Lapidus, L.J., Rosfors, S. et al	2007	Consecutive recruitment computer randomization (1)	Not stated Unclear risk (2)	All participants got an identical injection (control was placebo) Low risk of bias (1)	Radiologist blinded to randomized group Low risk of bias (1)	All accounted for Low risk of bias (1)	Secondary analysis was done based on USS endpoint whereas it was planned that USS+phlebo Unclear risk of bias (2)	This study is likely to be underpowered High risk (3)	11

Study	Year								Total
Jorgensen, P.S. et al	2002	Random numbers in sealed envelopes – details of randomization sequence not given Low risk of bias – randomization appeared to work (1)	Sealed envelopes. Not stated when patients were allocated Unclear risk of bias (2)	Control group did not receive anything therefore may have known which group they were in. Patients were therefore unlikely to be blinded. Unclear risk. Even if patients knew which group they were in, I don't think this would influence outcome (2)	Two experienced radiologists, independent of treatment group assessed venograms. Assessor blinded. Low risk of bias (1)	Although all randomized participants were accounted for, the loss to follow up was very high (32% i.e. 95/300 lost). The study is therefore underpowered. High risk of bias due to inadequate participants completing study, making it underpowered to detect a significant difference (3)	No flow diagram. Difficult to follow how and why patients were lost through study. Unclear risk (2)	With such a high rate of patient withdrawal from study due to injections, how did they check compliance with injections? Unclear risk of bias (2)		13
Sultan, M.J. et al	2014	Computer based randomization Low risk of bias (1)	Not described Unclear risk of bias, however, randomization worked (2)	Assessor blind Low risk of bias (1)	The vascular technician undertaking duplex imaging was also blinded Low risk of bias (1)	4 patients did not attend for Duplex. Not stated what happened to them. Could have died of fatal PE Unclear risk of bias (2)	DVT reporting was not clear. Table of DVT rates between operated and non-operated not displayed. Surgically treated patients also received clexane (in a subtherapeutic dose) Unclear risk of bias (2)	Study underpowered to assess secondary outcome measure of VTE rates (n=90 only) High risk of bias for VTE outcome, study underpowered (3)		14
Spannagel, U. et al	1993	Not stated Unclear risk of bias (2)	Not stated Unclear risk of bias (2)	Not stated if sonographers were blinded to group Control did not receive a placebo injection Unclear risk of bias (2)	Not stated if sonographers were blinded to group Unclear risk of bias (3)	306 randomised. 53 excluded (14 in control got LMWH by someone else, 12 in LMWH group stopped it on own accord, 3 casts removed before 7 days, 6 underwent surgery before 7 days in cast, 18 casted patients did not return for any follow up!). Therefore 253 included. Low risk of bias (all accounted for. 17% lost, therefore still high quality (1)	USS was performed to detect DVT, then phlebography to confirm. Phlebographic rates slightly lower. Authors chose to present USS rates. Unclear risk (2)	They have done stats on multiple outcome measures, Not stated if they used Bonferroni correction Unclear risk (2)		14 Not stated if assessors were blinded to treatment group
Kock, H.J. et al	1995	Randomised with lists stratified for varicose veins and obesity (>20% above ideal Broca weight) Unclear risk of bias (2)	Not stated Unclear risk of bias (2)	Control did not get a placebo injection because it was felt to be unethical. Therefore patients were unlikely blinded Unclear risk of bias (2)	Not stated if assessors of DVT imaging were blinded Unclear risk of bias (3)	Its unclear how many patients were randomized and subsequently were lost to follow up Unclear risk of bias (2)	Primary outcome was reported Low risk of bias (1)	Significantly more patients in control group on OCP and significantly longer duration of cast in control group also High risk of bias (3)		15 Not stated if assessors were blinded to treatment group

Orthopaedic surgical patients are generally regarded as high risk of venous thromboembolic complications. Patients undergoing surgery for hip fracture, total hip or knee replacement have deep vein thrombosis incidences of up to 60% (National Clinical Guideline Centre – Acute and Chronic Conditions (UK), 2010). Prevention of VTE is more desirable than treating VTE events from both a clinical and financial perspective (Alikhan et al., 2004), and there is no doubt that mechanical and chemical thromboprophylaxis are effective in patients undergoing major Orthopaedic surgery. For example, in the context of total knee replacement, the addition of mechanical to chemical thromboprophylaxis (pneumatic compression) significantly reduces the incidence of DVT from 18.7% to 3.7% with combined prophylaxis. The effects are similar for patients undergoing total hip replacement (reduction in DVT from 9.71% to 0.94%) (Kakkos, Warwick, Nicolaides, Stansby, & Tsolakis, 2012). In patients who undergo hip fracture surgery, heparin or mechanical thromboprophylaxis with foot or calf pumping devices are also effective in reducing the incidence of DVT (Handoll et al., 2000). In view of this, it has become accepted that patients undergoing major Orthopaedic surgery should be provided with thromboprophylaxis unless contra-indicated.

Another group of patients which account for a large workload for the Orthopaedic surgeon are patients with lower limb trauma. Many of these patients are treated as outpatients, non-surgically with casts or splints. Some studies indicate that these patients are also at increased risk of venous thromboembolism due to a combination of patient, injury and treatment factors (Spannagel & Kujath, 1993). In the United Kingdom, it is recommended that

all patients treated with cast or splint immobilization should be assessed for risk of venous thromboembolism and provided with thromboprophylaxis where increased risk is identified (NICE, Gemnet). Some authors recommend thromboprophylaxis for all patients with immobilisation of the lower extremity, irrespective of age and other risk factors (Testroote, Morrenhof, & Janzing, 2011a) (Spannagel & Kujath, 1993). In some U.K. hospitals, all patients treated with lower limb immobilization are provided with chemical thromboprophylaxis. It has been reported that this practice is cost effective when considering the potential savings in litigation (Menakaya et al., 2013). This may be true, however it is vital to review the evidence of the effects of thromboprophylaxis in patients with lower limb cast or splint immobilization to enable clinicians to make informed decisions for or against its use.

Considering studies which reported symptomatic venous thromboembolism, deep vein thrombosis occurred in 1.58% of patients randomized to no prophylaxis group (11/697) (Table1.3) (Kock et al., 1995) (Lassen et al., 2002) (Jørgensen et al., 2002) (Lapidus et al., 2007) (Selby et al., 2015) Table 3.7, Table 3.8). Symptomatic pulmonary embolism occurred in 0.33% of patients (3/901) (Kock et al., 1995) (Spannagel & Kujath, 1993) (Lassen et al., 2002) (Lapidus et al., 2007) (Selby et al., 2015) (Table 3.9).

The most serious complication of this is death from Pulmonary Embolism (PE), which has recently been shown to occur in approximately 1 in 15000 patients (Jameson et al., 2014). None of the included studies in this review are therefore adequately powered to assess a reduction in this outcome. It is

therefore not surprising that there is uncertainty amongst Orthopaedic surgeons on the effectiveness of chemical thromboprophylaxis to prevent fatal PE (Mirkazemi, Bereznicki, & Peterson, 2012).

The majority of studies included in this review, focus on the effects of chemical thromboprophylaxis (n=7). Two have studied mechanical thromboprophylaxis. All chemical thromboprophylaxis studies focused on low molecular weight heparin. None considered alternatives such as aspirin or newer oral anticoagulant direct thrombin inhibitors.

Effect of Chemical Thromboprophylaxis

None of the included studies found a significant reduction in symptomatic DVT at individual study level, however at meta analysis (Table 3.7), there was a statistically significant reduction (OR 0.29, 95% CI 0.09 – 0.95) (Lapidus et al., 2007) (Lassen et al., 2002) (Selby et al., 2015). The number needed to prevent symptomatic VTE was 82, considering all studies reporting this outcome for each group (Table 3.10). To put this into context, the numbers needed to prevent recurrent hip fracture in post menopausal women with hip fracture is 100 (Wells et al., 2008). For asymptomatic DVT, clinically important DVT has recently been restricted to events which are above knee, or symptomatic events within 90 days of injury (Selby et al., 2015). With focus on studies which reported outcomes of asymptomatic above knee DVT (Table 3.3), none of these studies found significant reductions in asymptomatic

above knee DVT, pooled meta-analysis also failed to find an effect of LMWH (Table 3.5). It is important to discuss the earliest two studies into the effect of LMWH in patients with lower limb trauma and cast treatment, which both found statistically significant reductions in asymptomatic composite results of above and below knee DVT (Kock et al., 1995; Spannagel & Kujath, 1993). In the larger study by Kock et al (1995), 339 patients with lower limb fractures or sprains treated with non-operatively with below knee cast or above knee cylinder cast, LMWH (Mono-Embolex) daily s/c injection 32mg resulted in a statistically significant reduction in asymptomatic deep vein thrombosis, with no events occurring in the LMWH group and seven in the control (p=0.006). Out of the seven asymptomatic DVT's which occurred in the control group, only 3 were above knee. It is also of note that this paper is 20 years old and current management of lower limb traumatic injuries has changed significantly. For example, the majority of patients in this study (74%) were treated with cast for soft tissue injuries and sprains. Considering the 7 patients in the control group who sustained asymptomatic deep vein thrombosis, 2 of these patients sustained fractures of the 5th metatarsal of hallux, both of which would nowadays be treated with a heel weight bearing shoe rather than a cast. 2 patients with knee contusions or meniscal injuries would likely to have been provided with a temporary brace rather than an above knee cylinder cast. The other three patients had casts for ankle sprains, contusion or distortion and it is likely that they would have also been provided with a walking boot or functional brace rather than cast immobilization in a modern fracture clinic. A final important limitation is that it is not stated in this paper

whether the assessors of outcome of venous thromboembolism were blinded to patient intervention group.

The second paper, which found a statistically significant effect of LMWH on overall asymptomatic DVT outcomes, was by Spannagel et al (1993). This paper found the greatest reduction in asymptomatic deep vein thrombosis of all studies, from (4.8% LMWH group v 16.5% control p <0.01). It is unclear in this paper how many of these asymptomatic deep vein thrombosis were below or above knee. Patients were all treated with cast immobilization. Similarly to the study by Kock et al (1995), 70% of these patients had soft tissue injuries rather than fractures. There are no details provided of the reasons for cast immobilization. This study suffers from the same limitation as the former study by Kock et al, because it is not stated whether the assessment out outcome of deep vein thrombosis was blinded. These studies are at high risk of bias. This has been previously noted in other reviews of this literature (Ettema 2008).

Even if a composite reduction in asymptomatic DVT has clinical relevance in regards to longer term outcomes such as post thrombotic syndrome, it is important that patients are provided with chemical thromboprophylaxis as soon as possible following their injury. If there is a delay in commencing thromboprophylalxis, which may be due to patients presenting several days after their injury, they may have already developed asymptomatic deep vein thrombosis. There are no studies which quantify this effect, however the

majority of RCT's included in this review recruited patients within 3 days of injury. In a study of thromboprophylaxis in patients with acute stroke, both leg doppler USS performed at 3 days following admission found that 8% had already developed an asymptomatic DVT (Bembenek, Karlinski, Kobayashi, & Czlonkowska, 2011).

Another important consideration is that patients must comply with LMWH injections in order to achieve reductions in venous thromboembolism. Some studies suggest that 12% of patients stop taking LMWH due to discomfort (Jørgensen et al., 2002). In a prospective study of 214 patients of mean age 34 years, using enoxaparin injections in France, 20.5% were deemed inappropriate for training or refused, with a further 12% failing to use LMWH after being trained to perform injections (Le Gall et al., 2006). This will significantly affect the efficacy of prophylaxis in clinical practice.

Effect of Mechanical Thromboprophylaxis

There is no evidence that mechanical thromboprophylaxis results in a statistically significant reduction in either asymptomatic or symptomatic venous thromboembolic events in patients with lower limb trauma treated with cast immobilization (Domeij-Arverud et al., 2013; Sultan et al., 2014). Both of these studies lacked statistical power to detect a statistically significant effect on deep asymptomatic deep vein thrombosis, which was a secondary outcome measure for the paper by Sultan et al. It was acknowledged by the

authors that a larger study is required to examine the effects of the ankle injury stocking on deep vein thrombosis incidence.

In theory, intermittent pneumatic compression, as used in the study by Domeij Averud (2013) could have been effective in reducing venous thromboembolism. IPC foot pumps work by compression of the plantar venous plexus (Broderick et al., 2010). It may also increase fibrinolytic activity (Ho & Tan, 2013) (Salzman et al., 1987). In empirical studies, A-V foot pumps do result in statistically significant increases in calf pump function (measured as increases in popliteal venous velocities from baseline) (Killewich, Sandager, Nguyen, Lilly, & Flinn, 1995). This is also true for neuromuscular electrical stimulation (Kaplan, Czyrny, Fung, Unsworth, & Hirsh, 2002) (Corley, Birlea, Breen, & Olaighin, 2009) (Warwick et al GEKO), weight bearing (Craic et al 2014) and active or passive foot and ankle movements (Whitelaw et al., 2001) (Hickey et al 2014). The limitation of these studies of calf pump function is that the majority were performed under test conditions in healthy volunteers. Most of these studies conclude that further study is required to determine the effects of these interventions on venous thromboembolism, however very few persist to investigate this.

Early weight bearing and range of motion has been studied in patients who underwent ankle fracture fixation. Although there was a non statistical reduction in DVT in the early range of motion group, this benefit was outweighed by wound complications, which were statistically more likely in

those treated with splint and early motion as compared to the group treated in cast. (Lehtonen et al., 2003).

There is good clinical evidence that intermittent pneumatic compression is effective at reducing venous thromboembolism in hospitalized patients (Pascarella, 2014). At meta-analysis level of 16,164 surgical and non-surgical patients from 70 trials, IPC was significantly more effective than control in reducing DVT (7.3% v 16.7%, absolute risk reduction 9.4% $p < 0.01$, relative risk 0.43), as well as pulmonary embolism (1.2% v 2.8%), RR 0.48). It was as effective as chemical thromboprophylaxis, and the addition of the two was most effective (RR 0.54) compared to IPC alone. 39% of these studies were on Orthopaedic surgical patients, however none were patients with lower limb trauma and cast immobilization (Ho & Tan, 2013). It is of note that in clinical practice, pneumatic compression devices are frequently applied with errors (Elpern, Killeen, Patel, & Senecal, 2013).

There are several potential reasons why Domeij Arverud et al (2013) failed to show a significant effect of IPC on DVT rates in patients with casts, including patient discomfort leading to withdrawal from the study in 2 out of 14 patients within 1 week, the mean duration of IPC being only 5.4 hours per day. Furthermore, 58% of patients in the intervention group reported IPC malfunction within 2 weeks of use (the majority due to low pressure readings). It is therefore un surprising that IPC failed to work effectively in this study (Domeij-Arverud et al., 2013).

The study by Sultan et al used an ankle injury stocking under an immobilizing boot in patients with ankle fractures. The main aim was to reduce ankle swelling and improve function, which was convincingly shown to be the case (Sultan et al., 2014). DVT was a secondary outcome, for which the study is underpowered. This requires further study. Using an ankle injury stocking in clinical practice may be difficult, as it was stated in this study that many patients in the ankle injury stocking study required entonox inhalation analgesia for application. Although there were no serious complications reported in this study, it is difficult to monitor for skin complications if this was used under a cast rather than a boot (Warwick et al., 2013).

The main strength of this review is that only included level 1 evidence studies. The benefit of this is that randomization equalizes both known and unknown confounding variables between groups (Bederman, Chundamala, & Wright, 2010). Symptomatic and above knee asymptomatic DVT were differentiated from below knee asymptomatic DVT, which may account for differences in conclusions as compared with previous reviews of LMWH in patients with lower limb immobilization (Ettema et al 2008, Testroote et al).

4   Methods

4.1   Effect of below knee cast application on calf pump function in healthy volunteers

Twenty healthy adult volunteers between the ages of 21 and 58 years of age (mean 31 years) were enrolled in this prospective study. Eleven were female. The Institutional review board and local research and ethics committee approved this proof of principle study (REC reference number 11/WA/0089). All participants signed a consent form prior to participation. Participants with previous medical history of peripheral vascular disease, diabetes, varicose veins, venous thromboembolism or previous foot and ankle surgery were excluded because these factors could have an adverse effect on calf pump function.

All participants had baseline and peak popliteal velocity measurements measured at the Popliteal vein by one Medical Physicist using a Doppler ultrasound probe. This technique has been validated and used recently by Izumi et al (2010) and Griffin et al (2010) to determine the effects calf compression and electrical stimulation on calf pump function. Baseline and peak velocity measurement has been in many studies of calf pump function, particularly to examine the effects of intermittent pneumatic foot or calf compression devices (R. J. Morris & Woodcock, 2010). Measurements of

peak venous velocities at the Popliteal vein have previously been found to accurately reflect flow volumes in the Femoral vein (Delis, Slimani, Hafez, & Nicolaides, 2000) (Ricci, Fisk, Knight, & Case, 1997). Measurements were performed at the Popliteal vein due to ease of access.

A standardized protocol was followed for each participant. Baseline popliteal vein velocity was measured in the right leg using Doppler ultrasound (cm/s) with the participant in the seated position, with hips, knees and ankles in the 90 degrees position. The participant was then shown a toe or ankle movement and was asked to perform the movement to their best effort, during which peak systolic velocity was measured at the popliteal vein. It has previously been shown that it takes approximately 7.5 seconds for the leg veins to refill (Griffin, Nicolaides, Bond, Geroulakos, & Kalodiki, 2010). Therefore, after each exercise, the toe and ankle were returned to the neutral position for at least 1 minute to allow the baseline velocity to return to normal before performing the next movement. Peak velocities were measured for the following movements: active ankle dorsi flexion, active ankle plantar flexion, active great toe dorsi flexion, active great toe plantar flexion. Ankle movements have previously been shown to result in high levels of calf muscle activity, measured using electromyography (O'Donovan et al., 2005). Toe movements were included because these can easily be performed with a below knee plaster cast in situ.

Once all measurements were recorded for each movement, a below knee 3-layer fiberglass polymer cast (3M Scotch-cast) was applied over wool and stockinet, supervised by a plaster technician. Popliteal vein velocity measurements at baseline and during movement were then repeated for each of the four movements with the cast in situ. Although ankle movements of dorsiflexion and ankle plantar flexion were not possible with cast in situ, participants could still perform isometric contraction of muscle groups. Group means were calculated for baseline and each movement pre and post cast application to allow comparison between groups.

Statistical analysis

A sample size calculation performed by Professor R Newcombe, Medical Statistics at Cardiff University. It was determined that 20 participants were required to detect a statistically significant change in pre and post cast peak systolic velocity at 80% power. Statistical analysis was performed using the group mean baseline and peak systolic velocity pre and post cast application using the paired t-test at the 5% alpha level. Two-sided p values of less than 0.05 were considered statistically significant. All statistical analysis was performed using SPSS for Windows (SPSS, Chicago, Illinois).

4.2 The effect of active toe movement (AToM) on calf pump function and asymptomatic deep vein thrombosis in patients treated with acute foot and ankle injury treated with cast – A Prospective Randomised Controlled Trial

Patients

In this prospective randomized controlled study, 100 adult patients with acute bony or soft tissue foot or ankle trauma treated with below knee cast at the University Hospital of Wales between February 2014 and February 2015 were recruited. The local Ethics committee and Research and Development department approved this study. The study was registered on UK Clinical Research Network Portfolio Database (UKCRN ID: 17648. http://public.ukcrn.org.uk). Funding for consumables to conduct this study was provided by AO UK. In accordance with NICE guidance and local hospital protocols, all patients were assessed for risk of venous thromboembolism. If patients had any permanent risk factors for VTE they were not eligible for the study and were provided with Low molecular weigh heparin (Enoxaparin) thromboprophylaxis 40mg subcutaneous injection once daily and were not eligible for study participation. Patients currently taking Warfarin or Heparin for any other reason were also excluded.

Exclusion criteria

Risk factors for VTE (exclusion criteria) were: Age >60 years, current
hormone therapy (Oral contraceptive pill, Hormone replacement therapy,
Tamoxifen), personal or first degree relative VTE history, any recent hospital
admission or major surgery (within 3 months), pregnancy or immediate
postpartum (6 weeks), any serious medical co-morbidity (including congestive
cardiac failure, chronic obstructive pulmonary disease, chronic kidney
disease, inflammatory bowel disease), extensive varicosities, active cancer,
obesity (BMI over 30 kg/m^2), known thrombophilia, achilles tendon rupture,
equinus cast. All patients assessed as 'low risk' based on this risk
assessment were potentially eligible for the study and were referred to the
Chief Investigator (BH) for review in the next available fracture clinic. On
review in clinic, VTE risk assessment was repeated to confirm patients were
'low risk' and eligible for the study. Patients who were anticipated to be
treated in a backslab or cast for less than 1 week were not recruited. Patients
with injuries requiring surgery were also not recruited because these patients
are provided with Low Molecular Weight Heparin. Patients presenting more
than 72 hours after initial injury were excluded.

Study design and intervention

The Chief Investigator (BH) recruited all patients. After consenting for
participation in this study, patients were allocated to either the intervention
(AToM) or control group according to a pre-defined computerized
randomization sequence with variable block sizes. Patients were not informed

of their randomization allocation group and all assessments of calf pump function and ultrasound for deep vein thrombosis were performed by medical physicists who were blinded to the randomization allocation. Participants who were allocated to the intervention group were provided with an active toe movement protocol to follow, with a compliance diary to complete. Patients allocated to the intervention group were instructed to perform maximal active toe flexion and extension movements as often as possible, with prescriptive advice to perform these movements at a minimum of 60 movements every 6 hours. All patients were shown how to do this and observed doing the movements to confirm they understood. Patients were managed in fracture clinic depending on their injury until discharge.

Outcome measures

When the patient no longer required their below knee cast or boot immobilization as part of their treatment they attended the Medical Physics department for assessment of calf pump function and bilateral above and below knee venous Duplex scanning to screen for asymptomatic deep vein thrombosis. Historically, venography was used to screen for DVT's, however current clinical practice is to use Duplex ultrasonography. This has previously been used in other similar studies (Selby et al., 2015). Any patient who was discharged from clinic prior to having Duplex scanning was sent an appointment letter to attend for Duplex scanning. Senior Medical Physicists who work within the National Health Service and perform these scan on patients routinely performed all scans. If a deep vein thrombosis was present

on Ultrasound scanning, the anatomical location and laterality in relation to the injured limb was recorded. The patient exited the study at this stage. Patients with positive Doppler scans for DVT were provided with treatment if the DVT was located in the above knee veins (Popliteal or more proximal). Patients with DVT in the below knee veins had a repeat Duplex scan a week later to assess for resolution or propagation of the DVT.

In patients who did not sustain deep vein thrombosis, calf pump function was assessed using Doppler ultrasonography according to a standardized protocol. With the patient seated with hips and knees at 90 degrees flexion, baseline popliteal velocity was measured in the popliteal vein after cast removal, using Doppler ultrasound at rest. Each patient then performed 3 consecutive active toe dorsiflexion exercises starting with the toe in neutral position, with a rest of 30 seconds between to allow for venous refilling. Peak popliteal velocity was recorded during active toe movement and the mean was calculated. Peak velocity was then recorded during active toe plantar flexion. This method has previously been used to assess calf pump function in healthy volunteers with and without lower limb cast applied (B. A. Hickey et al., 2014).

Statistical analysis and early stopping

A power calculation was based on the prediction that asymptomatic DVT would occur in 21.9% of patients with an anticipated intervention effect size of

63% reduction (Roderick et al., 2005). To allow for 5% withdrawal rate after randomization it was planned to randomize 150 patients to each group to achieve 80% power. To ensure randomization worked, baseline characteristics were analysed using t tests for continuous variables and chi square tests for proportions. Shapiro Wilk test was used to test for normality of calf pump function data and analysis of non-parametric results was performed with Mann Whitney U test. A generalization of the Mann Whitney U statistic was also performed to determine the effect size of AToM on calf pump function (Newcombe, 2006). All statistical analysis was performed using SPSS for Windows (SPSS, Chicago, Illinois). Analysis of DVT outcomes between groups was performed using Chi square test.

After 100 patients had been recruited, analysis of the calf pump function between groups was performed to determine whether the intervention had any significant effect on baseline or peak popliteal velocities. The outcome of this was that although the intervention appeared to influence popliteal velocities, there was no statistically significant difference when compared to the control group. In view that AToM did not significantly influence calf pump function in patients, it was decided by the Chief Investigator (BH) with agreement of the steering committee to request a formal interim analysis of the effect of the intervention on asymptomatic deep vein thrombosis. At this stage in the study, the overall event rate was 33.3% (n=13) in intervention and 20.5% (n=8) in control. Interim analysis found the confidence interval of the proportions ranged from 6.9% asymptomatic DVT reduction in the intervention to 31% increased incidence of asymptomatic DVT in the intervention group

(Newcombe, 1998). In view that the anticipated effect size was a 63% asymptomatic DVT reduction in the AToM intervention group, it appeared that the intervention was less effective than expected and the steering committee decided to stop recruitment (Briel, Bassler, Wang, Guyatt, & Montori, 2012).

4.3 The association between mobility and subsequent development of Deep Vein Thrombosis in patients with lower limb trauma and cast treatment

In this prospective study, 100 patients between the ages of 18 and 60 years of age, with acute lower limb trauma and below knee cast immobilisation were recruited from the Emergency Department at the University Hospital of Wales. This study had ethical approval and was approved by the research and ethics committee for Wales. All patients signed a consent form prior to participation. The study was funded by AO UK. All patients were assessed for risk of venous thromboembolism according to NICE and local guidelines provided with LMWH where indicated. Patients were excluded if they met any of the following criteria: Age >60 years, current hormone therapy (Oral contraceptive pill, Hormone replacement therapy, Tamoxifen), personal or first degree relative VTE history, any recent hospital admission or major surgery (within 3 months), pregnancy or immediate postpartum (6 weeks), any serious medical

co-morbidity (including congestive cardiac failure, chronic obstructive pulmonary disease, chronic kidney disease, inflammatory bowel disease), extensive varicosities, active cancer, obesity (BMI over 30 kg/m^2), known thrombophilia, achilles tendon rupture, equinus cast. All patients assessed as 'low risk' based on this risk assessment were potentially eligible for the study and were referred to the Chief Investigator (BH) for review. Patients who were anticipated to be treated in a backslab or cast for less than 1 week were not recruited. Patients with injuries requiring surgery were also not recruited because these patients are provided with Low Molecular Weight Heparin. Patients presenting more than 72 hours after initial injury were excluded.

All enrolled patients had a tri-axial accelerometer (Movband 2, Dynamic Health Strategies) applied to the cast by one author (BH) at time of cast application. Accelerometers were placed into a watertight bag and applied to a standardised position on the anterolateral aspect of their casted leg (approximately 10cm distal to the level of the tibial tubercle, lateral to the tibial crest). Accelerometers were fixed into the backslab cast between the wool and the crepe layers so that it was not possible to remove this without disrupting the backslab. Participants were advised to leave the accelerometer in the cast and only remove it if it caused irritation.

On review in fracture clinic 1 week later, the accelerometer was removed and the first 5 days of mobility data was extracted (120 consecutive hours) from the accelerometer using USB interface with MOVband Dashboard Version

2.3. Mean daily 'steps', 'moves' and 'miles' were calculated for each participant. Any accelerometers that were removed in the interim were determined 'non wear' and excluded from analysis. The first 5 continuous days of accelerometer data were analysed because it was felt this would represent the period of least mobility. Sharma et al found that this was the case in women following child birth (Sharma, Atkin, Mackillop, & Paterson-Brown, 2012). 120 hrs of consecutive accelerometer data collection was used in order to standardize data collection and reduce errors. It has previously been recommended that accelerometers should be worn for greater than 12h/day to ensure accurate estimates of daily physical activity, with shorter wear times resulting in increased error of measurement (Herrmann, Barreira, Kang, & Ainsworth, 2014). Although it is possible to compare physical activity between groups, selecting a single random day of activity, the mean of the number of days recorded was used in this study (Wolff-Hughes, McClain, Dodd, Berrigan, & Troiano, 2016). Patients were managed according to fracture clinic protocols for their injury until cast removal, at which point the patient had bilateral lower limb venous ultrasound to screen for deep vein thrombosis. Unpaired t-test was used to determine statistical significant difference in average moves and average steps per day for DVT and no DVT groups.

Accelerometer details

The MOVband 2 (Dynamic Health Strategies) is a triaxial accelerometer, which is 2 inches by 7/8 inches, weighing 0.4oz. This works using a proprietary algorithm to calculate moves, steps and miles. It can detect any type of movement as long as it is on a body part. The accelerometer generates activity when it experiences gravity <1 ms^{-2}, with an Epoch length of 30 seconds. It will only record activity when the accelerometer moves in at least 2 of 6 degrees of freedom, meaning it does not record car travel because this is one continuous axis. Under test conditions, it has been found to have significant association with orbital shaker frequencies of 1.3, 1.9 and 2.5 Hz for 5 minutes (Spearman rho 0.98) (Mendoza, Hickey, Young, & Freedson, 2015). Under free living conditions in young healthy adults, it has also been found to have strong positive correlations with the Actigraph GT1M research grade accelerometer (r=0.974 +/-0.06) (Williamson, Rebold, Carnes, Glickman, & Barkley, 2014) and the Actigraph GT1M research grade accelerometer in children (r=0.77, p<0.001) (Fennell, Kobak, Glickman, & Barkley, 2016). The majority of studies into physical activity use the Actigraph brand (Montoye, Moore, Bowles, Korycinski, & Pfeiffer, 2016). During outdoor walking at self selected speeds, the MOVband has been shown to have error rates of <15% when compared with observed steps (Taylor et al., 2014). This was acceptable for the purpose of this study.

4.4 Can we use biomarkers of coagulation to predict which patients with foot and ankle injury will develop deep vein thrombosis?

As part of the Active Toe Movement study (AToM), adult patients presenting to the Emergency Department at University Hospital of Wales with lower limb trauma requiring treatment with a below knee non-weight bearing cast for at least 1 week were assessed for eligibility (B. A. Hickey, Cleves, et al., 2016a). All patients were risk assessed for venous thromboembolism according to hospital protocol and considered 'low risk'. All patients were recruited within 72 hours of injury, none were provided with chemical thromboprophylaxis. At time of enrolment to the study, 3.5ml of venous blood was taken using standard Vaccutainer technique into a 3.2% sodium citrate coagulation tube. This was centrifuged at 1500 rpm for 15 minutes and the supernatant plasma was frozen at -70 degrees centigrade within 1 hour of blood being withdrawn. Participants were managed in the fracture clinic with lower limb cast immobilization according to their injury. On discharge from clinic, patients underwent bilateral lower limb ultrasound scan to assess for above and below DVT. All assessments were performed by medical physicists who perform these venous ultrasound studies as part of their role in the National Health Service, all with a minimum of 5 years experience. After the last patient was discharged from clinic, blood samples were thawed and analysed. Plasma was tested for quantitative levels of Human Coagulation Factor III/Tissue factor, Interleukin 6 (IL-6), VCAM-1 and D-dimer. We used the Quanikine

ELISA Immunoassay kits (R&D Systems) for each test according to the manufacturers instruction. All kits were stored between 2 and 8 degrees centrgrade and used before their expiry dates. All tests were performed with the assistance of 2 experienced laboratory technicians, who were blinded to the DVT status of the patient.

Tissue Factor testing protocol:

Human Coagulation Factor III/Tissue Factor ELISA Immunoassay (Quantikine, R&D Systems, Catalog Number DCF 300) was used for this test. All samples underwent a 2 fold dilution with 150 microlitres of calibrator diluent RD5-20. 500mL wash buffer was created by addition of 20mL wash buffer concentrate to distilled water. Color reagents A and B were mixed in equal volumes. Human coagulation factor III standards were prepared by pipetting 100 microlitres of 5000 pg/mL standard into an Eppendorf tube, with 900 microlitres of calibrator diluent RD5-20 added to the same tube (Tube A) and 500 microlitres was added to an additional 6 tubes (B,C,D,E,F,G). 500 microlitres of the mixture from Tube A was added to Tube B, then 500 microlitres was added from Tube B to Tube C. This process was repeated to create 7 standards: 500pg/mL, 250pg/mL, 125pg/mL, 62.5pg/mL, 31.3pg,mL, 15.6pg/mL and 7.8pg/mL. A standard with 0 pg/mL was also created from calibrator diluent alone.

After the samples, standards and reagents were prepared, 100 microlitres of RD1-89 Assay Diluent was added to each well of the microplate. 100 microlitres of each sample was added to individual wells. 100 microlitres of

each standard was also added to separate wells. The 96 well microplate has a pre-coated surface of monoclonal antibody which binds to Tissue Factor on contact. The microplate was then incubated at room temperature on a horizontal microplate shaker set at 500 rpm. After incubation, wells were aspirated and washed using wash buffer for a total of four washes. The plate was inverted and blotted dry. Subsequently, 200 microlitres of Human Coagulation III conjugate was added to each well, wells were covered and incubated for a further 2 hours on the horizontal microplate shaker. After 2 hours the wells were aspirated and washed. 200 microlitres of substrate solution was added to each well prior to a final incubation in dark for 30 minutes on the benchtop. After 30 minutes incubation, 50 microlitres of stop solution was added to each well. The optical density of each well was then read using a microplate reader.

Interleukin 6 testing protocol:

Quantikine ELISA Human IL-6 Immunoassay kit (R&D Systems) was used to quantify Interleukin 6. Samples were analysed according to Catalog Number D6050). Wash buffer was made using the same method used for Tissue Factor. Substrate solution colour reagents A and B were mixed in equal volumes and protected from light. Human IL-6 Standard was reconstituted with Calibrator Diluent RD6F to produce a stock solution of 300pg/mL. Standards of concentrations 100pg/mL, 50 pg/mL, 25 pg/mL, 12.5 pg/mL, 6.25 pg/mL and 3.13 pg/mL were created according to manufacturer instructions using similar methods for Tissue factor. 100 microlitres of assay

diluent RD1W was added to each well, followed by 100 microlitres of each sample (undiluted) to individual wells. 100 microlitres of each standard was also added to separate wells. The microplate was subsequently incubated at room temperature for 2 hours, after which wells were aspirated, washed four times with wash buffer and blotted dry. 200 microlitres of Human IL-6 conjugate was then added to each well and these were incubated for a further 2 hours at room temperature. Following this, wells were aspirated, washed and blotted dry as previously described. A final incubation at 20 minutes in the absence of light was performed after addition of 200 microlitres of substrate solution to each well. Finally, 50 microlitres of stop solution was added to each well and the microplate was read using an optical microplate reader.

VCAM-1 testing protocol:

Human VCAM-1/CD106 Immunoassay was performed according to R&D Systems Quantikine ELISA Catalog Number DVC00. Calibrator diluent in a 1:5 dilution was prepared first by addition of 20mL Calibrator diluent RD5P concentrate to 80ml distilled water. Samples were subsequently diluted 20-fold by addition of 380 microlitres of calibrator diluent RD5P to 20 microlitres of sample. Wash buffer was prepared as previously described for IL-6 method. Human VCAM-1 standard was reconstituted to create a standard of 400ng/mL, which was subsequently diluted with Calibrator diluent to create standards with VCAM-1 concentrations of 200ng/mL, 100ng/mL, 50ng/mL, 25ng/mL, 12.5ng/mL and 6.25ng/mL. The calibrator diluent was used as the 0ng/mL zero standard. 100 microlitres of Human VCAM-1 conjugate was

added to each well, followed by 100 microlitres of each diluted sample. 100 microlitres of each standard were added to separate wells. Wells were then incubated for 1.5 hours at room temperature before being aspirated, washed four times and blotted dry. 100 microlitres of substrate solution was then added to each well and this was incubated at room temperature in darkness for 20 minutes. Finally, 50 microlitres of stop solution was added to each well and microplates were read using the microplate reader to measure optical densities.

Optical density results

Using the optical density results for each test, a standard curve was created using optical density (y axis) against the. Sample results were then calculated using this curve. Tissue Factor/Coagulation Factor III results were multiplied by 2 to account for the initial 2-fold dilution. Interleukin 6 results were tested undiluted, therefore did not require correction. To account for the initial 20-fold dilution, VCAM-1 results were multiplied by a factor of 20.

D-dimer measurement protocol

The HemosIL D-dimer HS was used (product code 0020007700). This is an automated latex enhanced immunoassay for the quantitative determination of D-dimer in human citrated plasma. The D-dimer HS latex reagent is a suspension of polystyrene latex particles of uniform size coated with a fragment of a monoclonal antibody highly specific for the D-dimer domain

included in fibrin soluble derivatives. When a plasma containing D-dimer is mixed with the latex reagent and the reaction buffer, the coated latex particles agglutinate. The degree of agglutination is directly proportional to the concentration of D-dimer in the sample and is determined by measuring the decrease of the transmitted light caused by the aggregates (turbidimetric immunoassay). All samples were tested in batch using a fully automated, bench-top, random access analyser (ACL TOP 700) after calibration and internal quality control using IL specific quality control plasmas at low and high control levels.

Statistical analysis

Test for normality were performed using Kolmogorov Smirnov test. Unpaired t-tests were used to test for statistical significance between Group 1 (DVT) and Group 2 (No DVT) where data was parametric. Mann Whitney U test was used for non-parametric data.

5   Results

5.1   Effect of below knee cast application on calf pump function in healthy volunteers

20 healthy staff were recruited. None of the hospital staff approached to participate had reason for exclusion (no previous medical history of peripheral vascular disease, diabetes, varicose veins, venous thromboembolism or previous foot and ankle surgery). Average participant age was 31 years (range 21 to 58). 11 were female.

Baseline popliteal velocity

160 measurements of resting baseline popliteal flow were taken. The baseline was calculated as a mean due to the physiological Baseline Variability that is shown in the Doppler image in Figure 5.1.

Fig. 5.1 Doppler ultrasound image showing baseline variability and peak

systolic velocity during toe movement
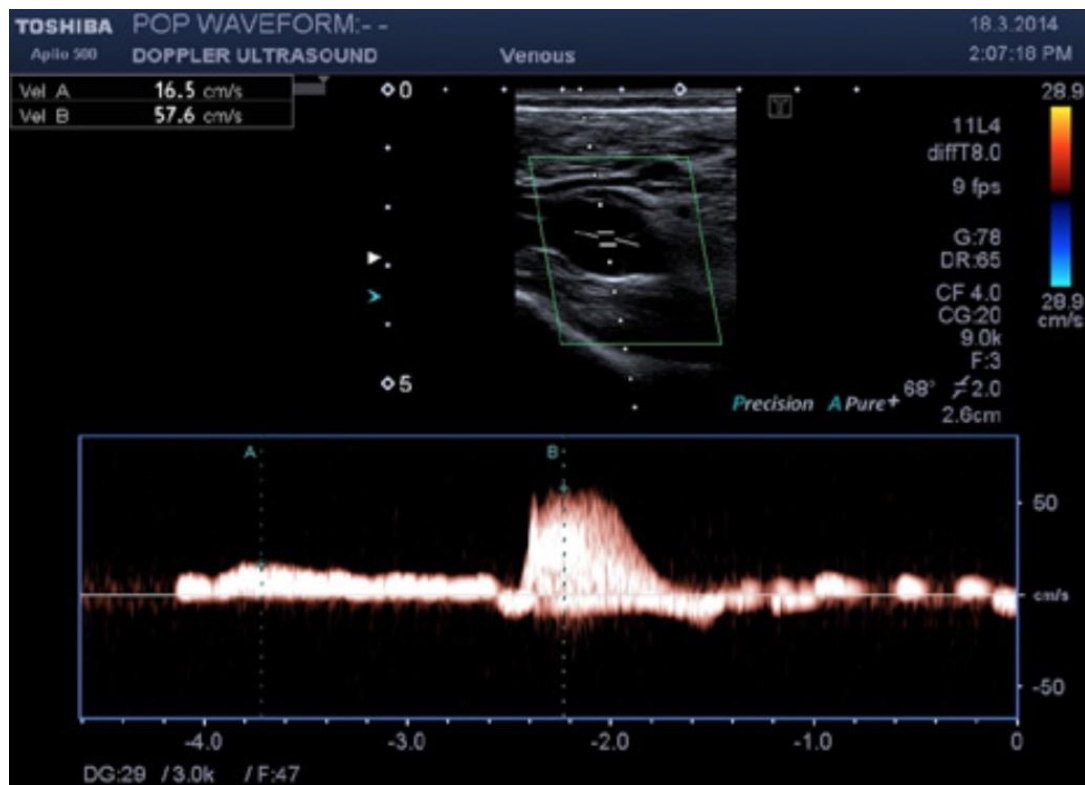
Prior to cast application, mean resting baseline popliteal vein velocity was

9.16 cm/s (4.0-24.6 cm/s). After a below knee polymer cast was applied, the

baseline velocity was 9.94 cm/s (9.5 – 26.4). The difference in baseline

popliteal velocity pre and post cast application was not statistically significant

(p=0.29).

Effect of active ankle or toe movement on popliteal velocities

All movements tested resulted in a statistically significant increase in peak systolic velocity as compared to baseline measurement (p=0.0001) without a cast. Active toe dorsi-flexion increased the velocity in the popliteal vein from the baseline to 53.6 cm/s pre cast mean peak (12.8-152.8). Active toe plantar-flexion resulted in an increase in velocity from baseline a pre cast mean peak of 49.7 cm/s (15.5-127.7). Ankle dorsiflexion increased velocity from baseline to 115.4 cm/s peak (31.5-189) pre cast. Ankle plantarflexion increased velocity from baseline to 86.6 cm/s mean peak (39.9-158.9) pre cast (Table 5.1).

Table 5.1 The effect of muscle contraction on Popliteal vein flow in the non-casted leg

Exercise	Mean Resting baseline venous velocity (cm/s)		Mean peak systolic velocity achieved with movement (cm/s)		p value
Seated toe dorsi flexion	10.42	(4.2-32.1)	53.6	(12.8-152.8)	0.0001*
Seated toe plantar flexion	10.42	(4.2-32.1)	49.7	(15.5-127.7)	0.0001*
Seated ankle dorsi flexion	10.42	(4.2-32.1)	115.4	(31.5-189)	0.0001*
Seated ankle plantar flexion	10.42	(4.2-32.1)	86.6	(39.9-158.9)	0.0001*

* statistically significant result

After a below knee cast was applied, active toe dorsi-flexion increased the velocity in the popliteal vein from the baseline to a mean 59.1 cm/s post cast mean peak (10.5-184.1) (pre-post cast peak mean difference p=0.572) (Table 5.2). Active toe plantar-flexion resulted in an increase in velocity from baseline to a post cast mean peak of 57.3 cm/s (20.9-108.3) (pre-post cast peak mean difference p=0.299). Ankle plantar-flexion increased velocity from baseline to 112.9 cm/s mean peak (34.1-265.5) post cast (pre-post mean difference p=0.23). Ankle dorsi-flexion increased velocity from baseline to 88.2 cm/s mean peak (23.2-234.2) post cast (pre-post mean difference p=0.045). Although the post cast peak velocity was reduced compared to pre cast peak for ankle dorsi-flexion, the post cast mean peak velocity still increased more than 10 times from baseline levels.

Table 5.2 Comparison of Mean Peak Popliteal vein flow without and then with a below-knee cast

Exercise	Pre cast mean peak systolic velocity (cm/s)		Post cast mean peak systolic velocity (cm/s)		p value
Seated toe dorsi flexion	53.6	(12.8-152.8)	59.1	(10.5-184.1)	0.572
Seated toe plantar flexion	49.7	(15.5-127.7)	57.3	(20.9-108.3)	0.299
Seated ankle dorsi flexion	115.4	(31.5-189)	88.2	(23.2-234.2)	0.045*
Seated ankle plantar flexion	86.6	(39.9-158.9)	112.9	(34.1-265.5)	0.23

* statistically significant result

5.2.1 The effect of active toe movement (AToM) on calf pump function and asymptomatic deep vein thrombosis in patients treated with acute foot and ankle injury treated with cast – A Prospective Randomised Controlled Trial

During the recruitment, 342 patients between the ages of 18 and 60 years had injuries treated with below knee casts, 242 were excluded prior to randomization. 100 patients entered the study and were randomized to intervention (AToM) or control groups. 78 patients completed the study (Fig. 5.2). There were no significant differences between the groups (Table 5.3).

91% of injuries were bony (n=71), which included 57 ankle fractures (72%). Other bony injuries included 5th metatarsal fracture (n=3), anterior process of calcaneus fracture (n=1), cuboid fractures (n=2), avulsions of dorsal talonavicular ligament (n=4), lateral process of talus fracture (n=2), navicular fracture (n=2). 7 patients sustained soft tissue injuries, which included 6 grade 3 ankle sprains and one Lisfranc ligament injury.

22 (56%) of the patients randomized to the AToM group returned a fully completed toe movement activity diary with number of active toe movements recorded for each day during cast or boot treatment. 13 (59%) of these patients recorded that they achieved the minimum number of active toe movements (240) per day (range 16-600). 4 (31%) of these patients sustained asymptomatic DVT. There were no reported adverse events.

Fig. 5.2 Flow diagram of study participants

| Enrolment | Assessed for eligibility (n=342) |

Excluded (n=242)

Prescribed Low Molecular Weight Heparin (n=63)
Underwent surgery +/- LMWH (n=115)
Taking Warfarin (n=1)
Out of area follow up planned (n=4)
Injury more than 72hrs prior to presentation (n=12)
Declined (n=47)

Randomized (n=100)

Allocation

Allocated to Active Toe Movement intervention group (n=51)

Allocated to control group (n=49)

Follow up

Lost to follow up
Did not attend for Ultrasound (n=11)
Withdrawn (n=1) – readmitted with new injury requiring LMWH

Lost to follow up
Did not attend for Ultrasound (n=10)

Analysis

Analysed (n=39)

Excluded from analysis (n=0)

Analysed (n=39)

Excluded from analysis (n=0)

Table 5.3 Characteristics of Patients Randomised

Characteristics	AToM (n=39)	Control (n=39)
Age (mean)	36.6 (18–60)	36.8 (18–60)
Male sex, n (%)	23 (59)	28 (72)
Bony injury, n (%)	36 (92)	36 (92)
Injuries, n		
Ankle fracture – Weber A	7	8
Ankle fracture – Weber B	15	16
Ankle sprain	3	3
Anterior process of calcaneus fracture	1	0
Cuboid fracture	1	1
Distal fibula avulsion fracture	1	0
Dorsal talonavicular ligament avulsion	3	1
Fifth metatarsal fracture	1	2
Lateral process of talus fracture	1	1
Lisfranc injury	0	1
Medial malleolus fracture	4	5
Navicular fracture	1	1
Posterior malleolus fracture	1	0
Duration of cast or boot, mean±SD (days)	42.7 (13.8)	39.6 (17.0)

Calf pump function data was not normally distributed, and was analysed using Mann-Whitney test (Newcombe, 2006). The U/nm statistic of 0.572 (95% CI 0.420 – 0.710) for both active toe dorsiflexion and active toe plantar flexion indicated that patients in the intervention group generally had greater peak velocities during these movements, however the effects were small (Table 5.4). The mean baseline popliteal vein diameter was 7.9mm in both groups. Although baseline popliteal velocity, peak velocity during active toe dorsiflexion and peak velocity during active toe plantar flexion were greater in the AToM group patients, none of these differences were statistically significant (Table 5.5).

Table 5.4 Calf pump function results

Calf pump function parameter	AToM	Control	p-value
Baseline popliteal vein diameter (mm)	7.9 (1.736)	7.95 (SD 2.084)	0.933
Baseline popliteal vein velocity (cm/s)	7.33 (3.663)	6.8 (2.193)	0.579
Peak popliteal velocity during active toe dorsi flexion (cm/s)	44.04 (30.172)	34.20 (18.878)	0.361
Peak popliteal velocity during active toe plantar flexion (cm/s)	39.04 (23.699)	31.70 (16.398)	0.348

Table 5.5 – Mann Whitney U statistic analysis of calf pump function

parameters (U/mn)

	U/mn	95% confidence limits	
		lower	upper
Baseline popliteal vein diameter - injured/cast leg	0.495	0.349	0.642
Baseline popliteal vein velocity - injured/cast leg	0.533	0.384	0.676
Peak velocity during active toe dorsi flexion (average) - injured/cast leg	0.572	0.420	0.710
Peak velocity during active toe plantar flexion (average) - injured/casted	0.572	0.420	0.710

5.2.2 Incidence and anatomical location of VTE in patients with foot and

ankle trauma and casts

Asymptomatic DVT

21 (27%) patients were found to have deep vein thrombosis on ultrasound

examination (Table 5.6). All of these occurred in the lower limb that had been

injured and treated in cast. The DVT rate was 13/39 (33.3%) in intervention

group, 8/39 (20.5%) in control group. These differences were not statistically

significant (p=0.202). 3 DVT's were above knee and were treated for oral

anticoagulants for 3 months. In all patients except 2, the peroneal vein was thrombosed. In 12 patients the DVT was an isolated peroneal DVT. 4 patients had combined peroneal and posterior tibial vein DVT, 1 patient had isolated posterior tibial vein DVT, 2 had popliteal vein DVT which appeared to arise from the peroneal vein, 1 patient had isolated popliteal vein DVT. The mean age of patients who sustained DVT was 36 years (range 21 to 53) with a mean BMI of 25kgm^2 (range 19.7 to 30.4). 13 were male (65%). There was no statistically significant difference between age or BMI between patients who sustained DVT and those who did not.

Table 5.6 Details of Deep Vein Thrombosis events

	AToM (N=39)	Control (N=39)
Asymptomatic DVT in Popliteal vein or more proximal	1	2
Asymptomatic below knee DVT details:		
Peroneal vein	8	5
Posterior tibial vein	1	0
Peroneal and posterior tibial veins	3	1
Total n (%)	13 (33.3)	8 (20.5)

5.3 The association between mobility and subsequent development of Deep Vein Thrombosis in patients with lower limb trauma and cast treatment

100 patients were recruited. 22 were lost to follow up. 10 patients were excluded because either accelerometers were lost (n=4) or failed to capture any data (n=6). 68 patients completed the study with full accelerometer data for 5 days (120 consecutive hours) and completed lower limb venous ultrasound scan to examine for DVT. The mean patient age was 38 years (range 18 to 60), 63% (n=43) were male. The majority of patients received below knee cast for ankle fracture (Table 5.7).

Table 5.7 Characteristics of patients included

Characteristics	n=68
Age, mean	38
Range	18-60
Male sex, n (%)	43 (63%)
Injuries, n	
Ankle fracture –Weber A	12
Ankle fracture – Weber B	27
Ankle sprain	6
Anterior process of calcaneus fracture	1
Cuboid fracture	1
Dorsal talonavicular ligament avulsion	3
Fifth metatarsal fracture	3
Lateral process of talus fracture	2
Lisfranc injury	1
Medial malleolus fracture	9
Navicular fracture	2
Posterior malleolus fracture	1

For the 68 patients who completed the study, mean daily accelerometer recorded moves were 1043 (SD 1019), mean daily steps were 863 (SD 848) and mean miles recorded were 0.48 per day (SD 0.48). 9 patients (13%) recorded 0 moves, steps or miles for at least one day. Out of the 9 patients who recorded 0 for at least 1 day, 3 of these recorded 0 for 1 day. 3 of these patients recorded 0 for 3 or more consecutive days during the first 5 days treated in cast.

26% (n=18) of the 68 patients were found to have asymptomatic DVT on bilateral lower limb venous ultrasound after a mean cast duration of 42 days (SD 14). One was above knee (Popliteal vein), the remaining 17 cases of DVT were below knee. None occurred in the patients who were immobile (3 consecutive days of 0 recorded accelerometer moves or steps). Move and step data was normally distributed. There was no statistically significant difference in moves or steps between Group 1 (No DVT) and Group 2 (DVT) Table 5.8.

Table 5.8 Accelerometer results for patients who did and did not sustain DVT

	No DVT (Group 1)	DVT (Group 2)	
Number of patients	50	18	
Males	32	11	
Age	38 (18-60)	36 (20-53)	
BMI	25 (19-32)	26 (20-32)	
Average Moves per day	1057 (SD 1095)	1005 (SD 793)	p=0.8538
Average Steps per day	877 (SD 909)	825 (SD 669)	p=0.8251

5.4 Can we use biomarkers of coagulation to predict which patients with foot and ankle injury will develop deep vein thrombosis?

100 patients were recruited as part of the AToM study. 22 were excluded because they did not attend for lower limb venous ultrasound to assess for DVT. 1 patient did not provide a blood sample. 77 patients were included. The majority were male (n=50). Patient demographics and injury types are displayed in Table 5.9. 27% (n=21) of the 77 patients were found to have asymptomatic DVT on bilateral lower limb venous ultrasound scanning, all of which occurred in the lower limb that had been injured and treated in cast. 2 of the DVT's were above knee (Popliteal vein or more proximal), the rest were below knee.

Table 5.9 Demographics and injury types

	No DVT (Group 1)	DVT (Group 2)
Number of patients	56	21
Males	36	14
Age	37 (18-60)	36 (20-53)
BMI	25 (19-31)	25 (20-32)
Injuries		
Ankle fracture –Weber A	13	1
Ankle fracture - Weber A and 5th Metatarsal fracture	0	1
Ankle fracture - Weber A and undisplaced Talus fracture	1	0
Ankle fracture – Weber B	18	9
Ankle fracture - Weber B and Cuboid fracture	1	0
Ankle fracture - Weber B and 5th Metatarsal fracture	1	0
Ankle fracture - Weber C	1	0
Ankle sprain	4	2
Anterior process of calcaneus fracture	1	0
Cuboid fracture	2	0
Dorsal talonavicular ligament avulsion	2	1
Fifth metatarsal fracture	3	0
Lateral process of talus fracture	1	1
Lisfranc injury	0	1
Medial malleolus fracture	5	4
Navicular fracture	1	1
Posterior malleolus fracture	1	0
Talar neck and Dorsal Talonavicular ligament avulsion	1	0

Tissue factor levels were normally distributed, therefore unpaired students t-test was used to assess for statistically significant differences between Group 1 (No DVT) and Group 2 (DVT) (Mean 23.92 pg/mL v 20.33 pg/mL, p=0.422). 18 patients (23%) had TF levels >35pg/mL, 3 of these subsequently developed DVT. TF levels ranged from 0 to 68pg/mL. The other blood results were not normally distributed, therefore Mann Whitney U tests were used. There was no significant difference in Interleukin 6 levels between Group 1 (median 3.91 pg/mL) and Group 2 (median 4.59 pg/mL), p=0.764), range 0 to 84.68 pg/mL). Median values for VCAM-1 were also similar between groups

(552.98 v 496.84 ng/mL, p=0.111). VCAM-1 levels ranged from 412.63 to

823.15 ng/mL. Although there was a trend for median D-Dimer to be higher in

those who subsequently sustained DVT, this was also not significant

(p=0.490). D-Dimer levels ranged from 31 to 1184 ng/mL. Results are

displayed in Table 5.10. Subsequently, the three Receiver Operator Curves

demonstrated that based on this sample of subjects, tissue factor, interleukin

6 and VCAM1 are poor predictors of development of DVT (area under curve

<0.5 in all cases, Fig. 5.3, 5.4, 5.5).

Table 5.10 Blood results

	No DVT (Group 1)	DVT (Group 2)	
Tissue Factor (pg/ml)	23.92 (SD 17.52)	20.33 (SD 16.83)	p=0.422
Interleukin 6 (pg/ml)	3.91 (SD 13.07)	4.59 (SD 7.03)	u=561.5, z=0.29, p=0.764
VCAM 1 (ng/ml)	552.98 (SD 92.59)	496.84 (SD 114.02)	u=448.5, z=1.58, p=0.111
D-Dimer (ng/ml)	203.5 (SD 225.27)	236.0 (SD 262.95)	u=527.5, z=-0.68, p=0.490

Fig. 5.3 ROC curve using IL-6 as a predictor of DVT - Area under the curve = 0.446
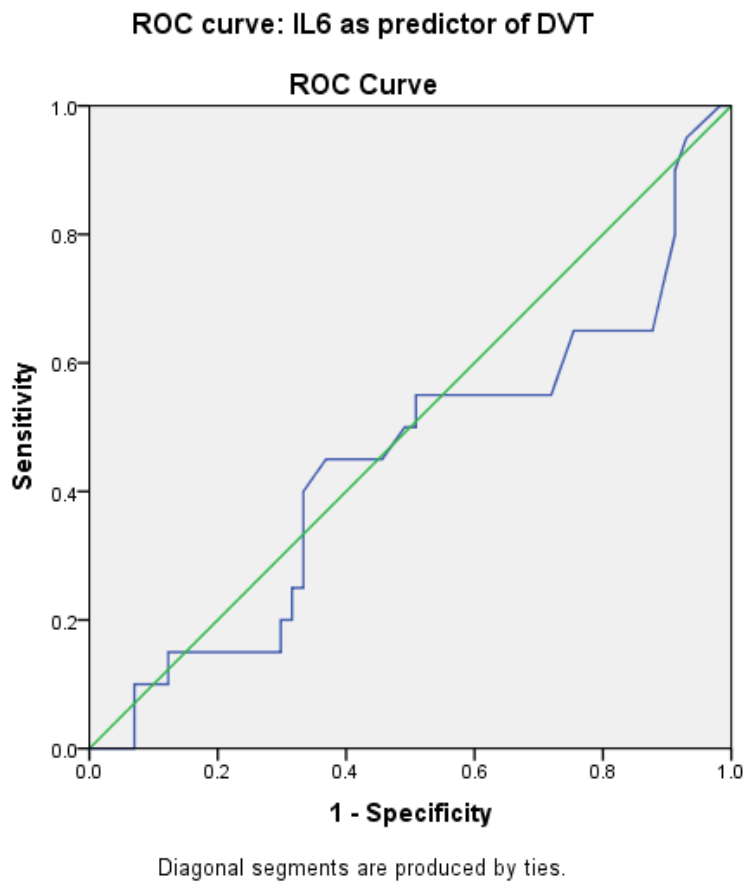
ROC curve: IL6 as predictor of DVT

Diagonal segments are produced by ties.

Fig. 5.4 ROC curve using VCAM-1 as a predictor of DVT - Area under the curve = 0.397

VCAM-1 as predictor of DVT

ROC Curve

Diagonal segments are produced by ties.
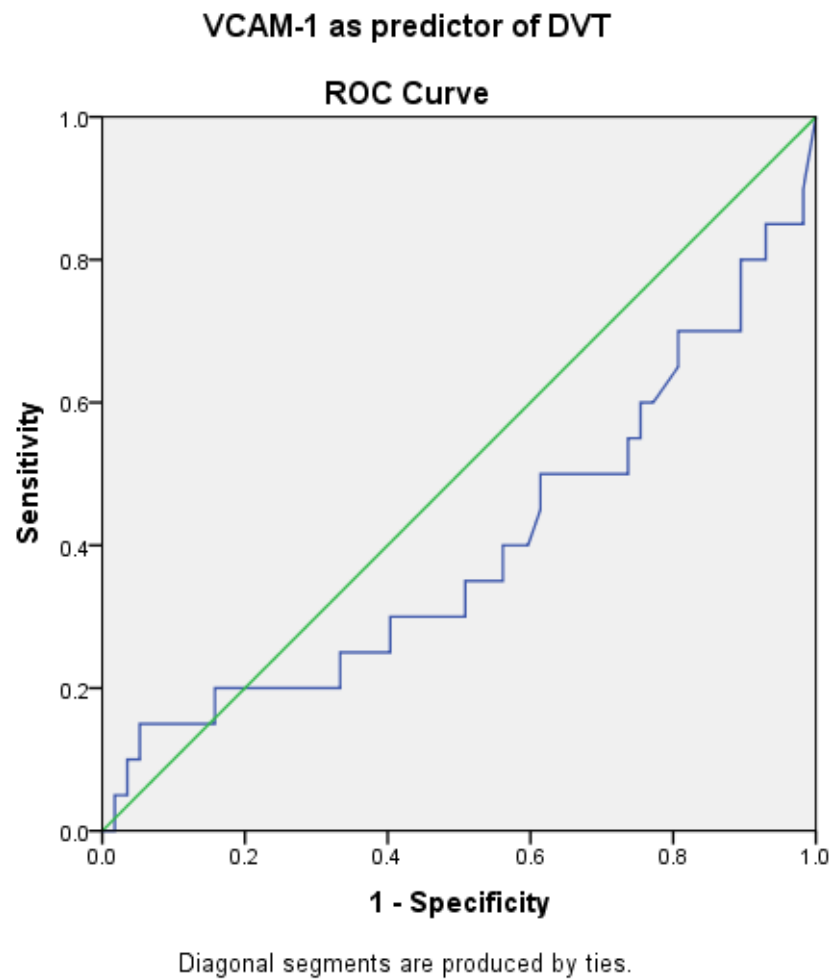
Fig. 5.5 ROC curve using Tissue Factor as a predictor of DVT - Area under the curve = 0.478

ROC curve: Tissue Factor as predictor of DVT

Diagonal segments are produced by ties.
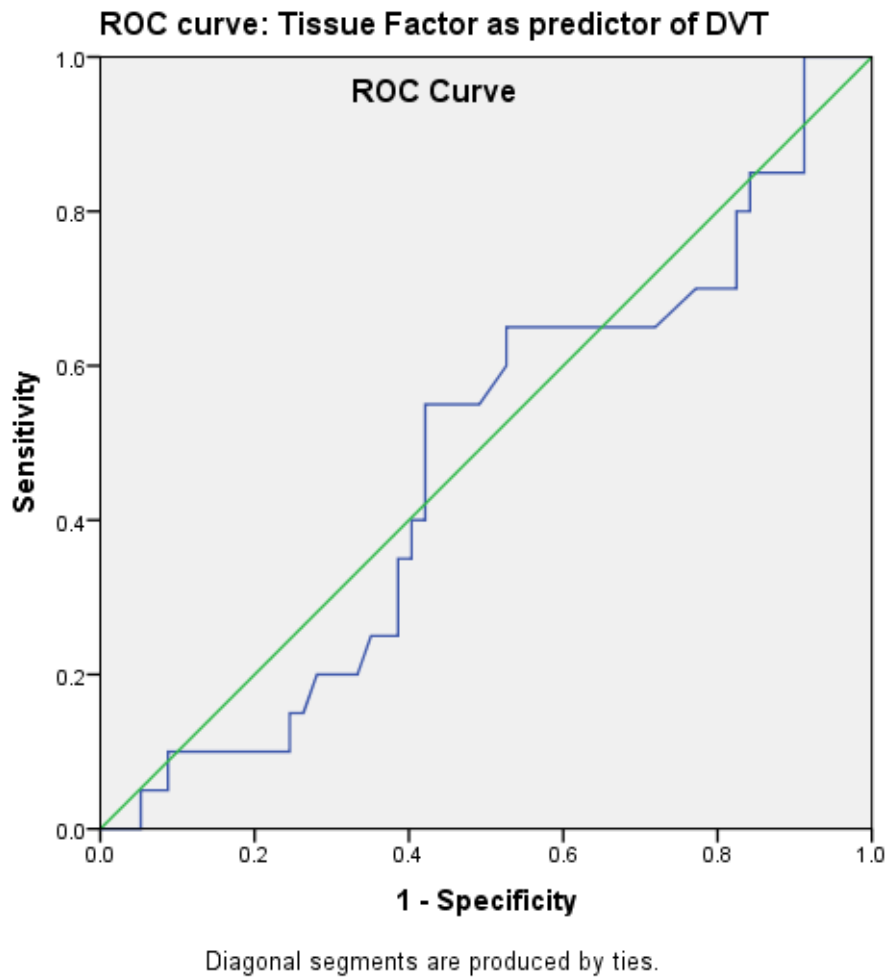
6 Discussion

6.1 Effect of below knee cast application on calf pump function in healthy volunteers

This is the first study into the effects of a below knee cast on calf muscle pump function. Surprisingly, even with a below knee cast in situ, toe and ankle dorsi-flexion and plantar-flexion movements significantly increase peak systolic velocity measured at the popliteal vein to between 57.3 cm/s and 112.9 cm/s, which is between six to ten times mean baseline levels. These findings are similar to those reported by Izumi et al (2010) who found that ankle movements in the un-casted limb resulted in popliteal velocities of 75cm/s (40-152) (Izumi, Ikeuchi, Mitani, Taniguchi, & Tani, 2010).

Comparing pre cast and post cast peak velocities, the cast did not significantly reduce velocities created by active toe movements or ankle plantar flexion. Although peak velocity resulting from ankle dorsi-flexion was reduced by the presence of a below knee cast compared to peak velocity prior to cast application (p=0.045), active ankle movement with cast in situ still resulted in a statistically significant increase in peak velocity from 10.3cm/s at baseline to 88.2 cm/s during movement (p=0.0001). This was approximately 75% of the maximum achievable without the cast.

Although it is clear that systems which influence venous haemodynamics do reduce rates of venous thromboembolism, the minimum threshold for these systems is unknown (Andrews, Sommerville, Austin, Wilson, & Browse, 1993). It is also unknown which haemodynamic parameter is most important in the prevention of DVT (Ricci et al., 1997). Furthermore, there is no clinical evidence that mechanical thromboprophylactic methods such as intermittent pneumatic compression devices, which produce higher venous velocities in the lower limb result in lower rates of deep vein thrombosis (R. J. Morris & Woodcock, 2004). However, active toe or ankle movements can influence calf pump function to a similar extent to other methods, such as intermittent pneumatic compression devices of the foot or calf. It is therefore possible that active toe movements in patients with lower limb cast immobilization could reduce rates of deep vein thrombosis.

In one of the first experimental studies using the AV foot pump at different pressures, cycle rates and duration of compression settings, it was found that there was a statistically significant increase in maximum popliteal and femoral vein velocities when the pressure was increased from 50mmHg to 200mmHg. In the popliteal vein, peak velocity at 50mmHg foot compression pressure was 31.4cm/s in men and 27.6 in women. At 200mmHg it was 53.5cm/s in men and 47.9cm/s in women. Differences between men and women were not statistically significant (Andrews et al., 1993). A similar study by Johnson et al, examined the effect of the AV foot pump on popliteal venous velocities in 14 healthy volunteers of mean age 40 years. Foot pumps were set at 200mmHg compression with a 20 second cycle. Baseline popliteal velocities of 10.3cm/s

(9.4-10.8) increased to 36.8cm/s during foot compression (B. F. Johnson, Manzo, Bergelin, & Strandness, 1997). In this study, the baseline popliteal velocity was similar (mean 9.94 cm/s). The lowest mean popliteal velocity result in this study, with below knee cast in situ, was during active toe plantar-flexion (57.3cm/s). This is higher than all popliteal velocities recorded in the studies of AV foot pumps, even at the highest foot pump setting of 200mmHg. One of the potential problems with foot pump compression devices is that the volume of blood in the plantar venous plexus of the foot is only 30ml, therefore high pressures are necessary to empty these veins (R. J. Morris & Woodcock, 2004). This can reduce patient compliance (Warwick et al., 1998).

In view that calf is a greater reservoir of venous blood than the foot, compression devices, which compress the calf or combine calf and foot compression, have been developed. Many studies have shown that calf or combined compression devices enhance venous haemodynamics to a greater extent than foot compression devices alone (Delis et al., 2000). Ricci et al compared the effect of 4 different foot compression devices at different settings against an intermittent calf compression device in 27 healthy volunteers (Ricci et al., 1997). Each participant had popliteal vein baseline (referred to as resting systolic velocity) and peak velocities (referred to as maximum venous velocity) measured during foot or calf compression using each device. All scans were done with the patient in reverse Trendelenburg position. Mean baseline velocity at the popliteal vein was 12.5cm/s (+/- 3.7 cm/s), which statistically significantly increased with all devices to a mean peak velocity of between 35.2 (+/- 16.3) and 55.3 +/- 19.5 depending on the

device. The intermittent calf compression device (encompassing the entire calf, with inflation time of 12 seconds, pressure of 40mmHg, with one cycle per minute) and the Kinetic Conceptions foot pump device, which had the highest compression setting (inflation time 2 seconds, pressure 160mmHg, 3 cycles per minute) resulted in the greatest enhancement of peak velocities. A study using similar methodology, compared the effects of high pressure rapid inflation combined foot and calf pump devices (160mmHg compression with 2 second inflation time) against standard pressure calf compression devices (50mmHg pressure, 12 second inflation time). The high pressure devices significantly increased popliteal velocities compared to standard pressures, from baseline mean of 11.3 cm/s by a mean of 37.3 cm/s (+/- 4.1) (M. D. Malone, Cisek, Comerota, Holland, & Eid, 1999). Again, the peak velocities with compression devices were still less than velocities achieved with simple active toe movements.

The greatest increase in popliteal velocities using intermittent pneumatic compression was shown by Delis et al, in a study of 45 volunteers (20 healthy and 25 with intermittent claudication) using combined calf and foot compression with pressures of 180mmHg with 3 second inflation time. At these high settings, popliteal venous velocities reached 130cm/s. There was no statistically significant difference in increases found when measured at popliteal vein compared with measurements at the femoral vein. Increases in velocities found in claudicants were comparable to results in healthy volunteers (Delis et al., 2000). This increase in popliteal venous velocity is greater than all measurements achieved in this study. However, at such high pressures, the safety of using a device, especially under a below knee cast in

which it is not possible to monitor skin integrity is of concern. It is also unlikely that patients with injuries treated with casts would be able to tolerate such pressures due to discomfort.

Alternative methods for enhancing venous return from the lower limb include neuromuscular stimulators of the peroneal nerve (transcutaneous electrical nerve stimulation) and electrical muscular stimulation of the calf muscle directly. One of the first studies to examine the effect of muscular stimulation was by Kaplan et al (2002). A neuromuscular stimulator was positioned over the gastrocnemius medial and lateral heads, followed by electrodes being placed on the sole of the foot. 49 healthy volunteers participated. Popliteal velocities were measured using Doppler ultrasound and compared with the unstimulated leg as a control, however the position of the leg during measurements was not stated. In this study, it was found that gastrocnemius stimulation resulted in a statistically significant increase in popliteal velocity of 10.2 cm/s when compared to the control leg. Foot stimulation resulted in an increase velocity of 8.66 cm/s compared to the control leg, which was also statistically significant (Kaplan et al., 2002). A conclusion of this study was that the significance of findings in reduction of DVT require prospective study, however this has not been examined. Although no participants found the device uncomfortable, this degree of venous flow enhancement is small compared to findings of the present study. A more recent study examined the effects of Veinoplus calf muscle electrical stimulator in 24 healthy volunteers at different frequencies (Griffin et al., 2010). Baseline velocity at the popliteal vein was 10cm/s, which is similar to findings in this study (10.3cm/s). The

highest peak systolic velocities in this study were achieved with stimulation frequencies of between 2 and 8 per minute, which resulted in peak systolic velocities of 96-105 cm/s, which is greater than the peak velocities achieved by the majority of calf or foot compression devices.

The only study of neuromuscular stimulation in patients with casts is by Warwick et al (2013) using the GEKO device. This is a peroneal nerve stimulator, which functions to stimulate muscles of the anterior and lateral compartment of the leg to contract. Interestingly, it is marketed as a calf pump stimulator but does not function by stimulating muscles of the deep or superficial compartment of the leg, which are traditionally considered as the calf pump. In a proof of concept study of 10 healthy volunteers, the GEKO was found to increase venous flow in the femoral vein by between 18 and 131% baseline depending on the position of the leg. The GEKO statistically significantly increased popliteal vein velocities with the cast in situ in the supine position and standing positions (both non-weight bearing and weight bearing). When the leg was elevated, baseline popliteal velocities increased therefore the addition of the GEKO stimulation did not significantly increase popliteal velocity i.e. veins of calf were less filled than in other positions. Depending on the position of the leg, the GEKO increased peak venous velocity in the popliteal vein from baseline of between 10.0cm/s and 19.5cm/s to between 22.3cm/s and 29.3cm/s during activation. This increase is less than all studies of intermittent pneumatic compression and also less than possible with simple toe movement.

Two studies have compared different methods of lower limb venous haemodynamic enhancement (Izumi et al., 2010) (Broderick et al., 2010). The former compared the effects of a TENS machine positioned over the fibula head in 10 healthy volunteers, to stimulate the common peroneal nerve against electromuscular stimulation (EMS) and intermittent calf compression set at a pressure of 40mmHg (Flowtron AC 500). The effects of manual calf squeeze and active ankle dorsiflexion movement were also studied. Popliteal venous velocities were examined with Doppler ultrasound with the patient in the prone position. The mean peak velocities achieved, in order of decreasing were: TENS 102cm/s, EMS 97 cm/s, Calf squeeze 90 cm/s, Active ankle dorsi flexion 75cm/s, Intermittent pneumatic calf compression 65cm/s. In comparison to findings in this study, patients in the present study achieved a mean peak velocity of 112.9 cm/s even with a below knee cast in situ, which is greater than all results in this study. I similar study by Broderick et al studied compared the effects of Intermittent pneumatic foot compression and neuromuscular stimulation (Duostim tibial nerve stimulator) against weight bearing for 20 seconds and standing toe flexion exercises. Popliteal venous velocity, diameter and time of flow were measured at the popliteal vein and used to calculate an ejected volume in 10 healthy volunteers. The most significant finding of this study was that both weight bearing and weight bearing toe curl exercises resulted in the greatest ejected volumes (33ml and 31ml respectively, with no statistically significant difference between results). In the supine position, simulated weight bearing did not result in significantly increased ejected volumes compared with toe curl exercises (17 v 15ml). However, standing weight bearing did result in greater ejected volumes (33ml)

when compared non-weight bearing toe curl exercises in the supine position (17ml). In the context of a patient with a below knee cast, this study may indicate that weight bearing would result in a greater ejected volume from the lower limb than non-weight bearing toe exercises (Broderick et al., 2010).

Some of the differences between findings in this study and findings of others could be influenced by differences in the leg position during testing in different studies. The position of the lower limb affects the efficiency of the calf pump. An example of this is the study of the AV impulse foot pump by Fleming et al (2000). In their study, femoral venous velocities were measured in 10 healthy volunteers at settings of 200mmHg compression at a rate of 3 compressions per minute, with the lower limb in different positions. With the lower limb in the horizontal position, foot compression increased velocity by 27.2%. In the 25 degree Trendelenberg position it increased by only 15.4% (not statistically significant difference). When the lower limb was positioned in the 25 degree reverse Trendelenberg position, femoral venous velocities increased significantly by 102.8% from baseline. Therefore, it appears that the foot down position may pre fill the lower limb veins to enable the foot and calf pump to work more effectively (Fleming, Fitzgerald, Devitt, Rice, & Murray, 2000).

There are several limitations of this study. Firstly, measurements were performed on healthy participants and excluded participants with peripheral vascular disease, diabetes, varicose veins, deep venous thrombosis or previous foot and ankle surgery. Secondly, participants were asked to perform

movements to their best efforts. This is likely to have resulted in a best-case result, which may not represent clinical practice. However, the increases between baseline and peak popliteal velocity reached extreme statistical significance (p=<0.0001) in all cases and therefore it is possible that these movements would also result in significant increases in patients who may have impaired calf pump function due to co-morbidity. The focus of this study was the effect of active ankle or toe movements on calf pump function, measured as peak velocity at the popliteal vein. Some authors have studied additional haemodynamic measures of calf pump function, including return time (the time for the peak velocity to return to baseline) (Ricci et al., 1997). These may be important parameters, however there is no evidence that alterations of these additional measures reduces venous thromboembolism. There are other methods for measurement of calf pump function, including muscle strength testing and measurement of residual and ejected volumes using plethysmography. In this study, calf pump function was measured using Doppler, based on baseline and peak velocities, because this has been used extensively in previous studies of intermittent pneumatic compression devices as discussed.

The findings of this study may have clinical relevance for several reasons. Until now an assumption has been made, that lower limb cast immobilisation resulted in loss of the calf pump and that this venous stasis could not be influenced. However, the results of this study show that despite having reduced movement of the foot and ankle active movements stimulate calf pump function. It is good practice to encourage patients to maintain knee and

toe movements when they are in a cast. These results demonstrate how important these movements are in maintaining physiological Popliteal vein flow and that this may be significant in reducing their risk of VTE. In much the same as airline passengers are now taking control of managing their flight risk, patients should be advised to perform these movements to reduce venous stasis. The recommended movements are ones that can occur naturally in patients with below knee casts as they mobilise around during the course of their day and they do not need to be dramatic in order to achieve good flow. Further study is necessary to determine the effect of active toe movement on calf pump function in patients. If this is effective, it could influence the incidence of deep vein thrombosis.

6.2.1 The effect of active toe movement (AToM) on calf pump function and asymptomatic deep vein thrombosis in patients treated with acute foot and ankle injury treated with cast – A Prospective Randomised Controlled Trial

This is the first study to examine the effects of active toe movement on DVT in patients with lower limb trauma treated with cast. Although it has previously shown that active toe movement (AToM) can influence calf pump function in healthy volunteers with below knee casts applied, it does not appear that this influences the incidence of DVT (B. A. Hickey et al., 2014). The concept that lower limb muscular contraction can influence thrombosis is not new. In a

prospective randomized study performed almost 20 years ago, 38 patients who underwent total hip replacement were randomized to a standardized foot and ankle movement protocol and venous plethysmography was performed 4 days postoperatively (McNally, Cooke, & Mollan, 1997). Venous outflow was increased by 22% in the intervention group and it was recommended that regular foot and ankle exercises should be part of a prophylactic regimen against venous thrombosis (McNally et al., 1997). Although these exercises may have influenced calf pump function, there was no further study conducted to investigate the effects on deep vein thrombosis. Similarly, Kaplan et al (2002) found that electrical stimulation of the foot or calf muscles significantly increased popliteal and femoral vein velocities, but failed to investigate the effects on DVT (Kaplan et al., 2002). More recently, Fuchs et al (2005) conducted a prospective randomized controlled trial of 227 trauma patients with bony or ligamentous trauma to the spine, pelvis including acetabulum, femur, tibia or ankle (Fuchs, Heyse, Rudofsky, Gosheger, & Chylarecki, 2005). All patients received unfractionated heparin, however the intervention group also received mechanical thromboprophylaxis with an ankle continuous passive motion machine (Arthroflow) for 30-minute sessions, three times per day (30 movements per minute, range of motion 20 degree dorsiflexion to 40 degrees plantar flexion). Asymptomatic DVT diagnosed on ultrasound or venogram was significantly reduced in the Arthroflow group, from 25% to 3.6% (p=0.001). Whitelaw et al (2001) compared the haemodynamic effects of 6 different mechanical thromboprophylactic devices on calf pump function and found that there was no statistically significant difference in increases in peak velocity between the most effective device (AirCast VenaFlow) and

active or passive dorsiflexion (Whitelaw et al., 2001). An early prospective randomized controlled trial of 290 patients who underwent total hip replacement, confirmed that not only did AV foot pumps influence popliteal venous velocities, but also reduced asymptomatic DVT to rates comparable to levels achieved with low molecular weight heparin (Warwick et al., 1998). Since then, intermittent pneumatic compression has been found to reduce symptomatic DVT (from 16.7% in control group, to 7.3% in IPC) and pulmonary embolism (from 2.8% to 1.2%) at meta-analysis level of 70 studies including over 16000 medial and surgical patients (Ho & Tan, 2013). It is also effective in reducing VTE in patients with hip fracture, patients undergoing total knee replacement and following stroke, all of which are considered high risk VTE groups (Handoll et al., 2000) (Kakkos et al., 2012) (Dennis et al., 2013).

Considering that active toe movement influences venous haemodynamics in healthy volunteers to a similar extent to intermittent pneumatic compression foot pump devices, it was anticipated that this may also reduce asymptomatic DVT in patients with casts (B. A. Hickey et al., 2014) (Whitelaw et al., 2001). One of the main reasons why AToM failed to reduce DVT rates may relate to the relative infrequent performance of exercises by patients. In the AToM study, participants in intervention group performed 60 sets of movements 4 times per day, which equates to 240 sets in a 24 hour period. In comparison to patients using A-V foot pumps in the study by Warwick et al (1998) used them for a median of 15 hours in a 24 hour period at a 20 second cycle, equating to approximately 2700 compressions (Warwick et al., 1998). This is more than 10 times the number of compressions participants in the AToM

study would have performed. To achieve this with AToM, assuming patients sleep for approximately 8 hours and would be awake for 16 hours per day, participants would need to perform almost 170 sets of AToM per hour to achieve a similar amount of compression. Considering that many patients in this study were unable to achieve even the minimum suggested number of active toe movements (240 in 24 hours) and that none of them reported performing more than 600 active toe movement exercises in a 24 hour period it is unlikely that a patient would be able activate their calf pump as frequently as an AV foot pump. Therefore, although the effect of active toe movement on popliteal velocities is similar to the effect of AV foot compression devices, there is a great difference in the number of calf pump activations performed (Whitelaw et al., 2001).

In view that the patient demographics in each study group were similar, it appears that the randomization sequence was effective. This is important, because unknown confounding factors are likely to have also been similar between groups (Bederman et al., 2010). This study was also free of thromboprophylaxis industry bias, being sponsored by AO UK. Follow up was almost 80%, which is higher than many previous randomized controlled studies of thromboprophylaxis in a similar patient group (Jørgensen et al., 2002) (Spannagel & Kujath, 1993) (Lapidus et al., 2007). It has previously been acknowledged that co-ordination of studies to reduce the risk of VTE is challenging due the fact that patients with lower limb casts present and are treated in several areas within the hospital e.g. Emergency department, fracture clinic (Nokes & Keenan, 2009). Although the intervention was

relatively simple, this means the findings of the study have good external validity. It has previously been suggested that prospective randomized controlled trials are at risk of lacking external validity due to highly intensive or regimented intervention protocols (Castillo, Scharfstein, & MacKenzie, 2012). Although only 33% of patient in the intervention group reported achieving the minimum recommended number of active toe movements, this is an effectiveness study which assesses how the treatment works in the real world (Bederman et al., 2010). Although multiple practitioners performed assessment for DVT, all had a minimum of 5 years experience in the NHS performing these scan as part of their clinical practice. None of the ultrasound scans for DVT were likely to be false positives in view that all were still positive on repeat scanning after a week interval. All assessors of DVT were blinded to the patient randomization group.

There are several limitations of this study. Firstly, only 100 (26%) patients were recruited after screening 342 for eligibility, due to the current NICE guidelines that recommend chemical thromboprophylaxis for patients with lower limb trauma treated with cast with additional risk factors. This meant that none of the patients who underwent surgery could be included (n= 105), with an additional 72 patients being prescribed LMWH for other reasons. Patients over the age of 60 years of age (n=97) during the study period because this is also considered to increase VTE risk in patients with lower limb trauma treated with cast (National Clinical Guideline Centre – Acute and Chronic Conditions (UK), 2010).

6.2.2 Incidence and anatomical location of VTE in patients with foot and ankle trauma and casts

In this study, it was found that 27% of patients with lower limb trauma and cast immobilization, who are considered to be low risk for deep vein thrombosis go on to develop an asymptomatic DVT. This is an important finding, because it cannot be predicted which of these asymptomatic DVT's will become symptomatic (Nokes & Keenan, 2009). Comparing the findings of this study with the control group DVT incidence in previous studies into the effect of LMWH in patients treated with casts reveals that patients considered as 'low risk' sustain a similar number of DVT's. For example, Spannagel et al found a 16.5% DVT rate in 127 patients, Jorgensen found an incidence of 17% of 106 patients and Lassen found an incidence of 18% of 191 patients (Spannagel & Kujath, 1993) (Jørgensen et al., 2002) (Lassen et al., 2002). These studies included patients with both bony and soft tissue injuries, which is similar to the injury demographic in the present study. Studies limited to patients with ankle fractures reveal asymptomatic DVT rates of 23% in those treated non-operatively, and 28% in those treated operatively (Sultan et al., 2014) (Lapidus et al., 2007). Patients with Achilles tendon injuries have consistently been found to have the highest incidence of asymptomatic DVT, which occur in between 33% and 42% of patients (Nilsson-Helander, Thurin, Karlsson, & Eriksson, 2009)(Lapidus and Rosfors 2007) (Domeij-Arverud et al., 2013).

Some studies have reported lower rates of asymptomatic DVT. The lowest rate was by Kock et al, in which only 4.3% of 163 patients with fracture of sprains developed this complication (Kock et al., 1995). Patil et al found that only 5% of 100 patients with isolated stable ankle fractures treated non-operatively developed asymptomatic DVT on Ultrasound scanning within 7 weeks of injury. It was concluded that this DVT incidence was low and therefore routine thromboprophylaxis was not recommended (Patil, Gandhi, Curzon, & Hui, 2007). There are several differences between this study and that by Patil, including that the majority of patients in their study were weight bearing (81%), and DVT scans were performed earlier following injury. The low incidence in the study by Patil may also be related to a higher false negative rate. Gehling et al also reported very low rates of asymptomatic DVT in 111 patients with lateral ligament injuries treated with cast immobilization for at least 1 week. Despite additional risk factors for DVT in 72% of these patients only 1 patient sustained an asymptomatic DVT when screened with venography. The median age of patients in this study was 26 years, and it has previously been suggested that this may be one of the reasons why the DVT rate was low (Nokes & Keenan, 2009). However, the mean age of patients who sustained DVT in this study was only 36 years (range 21 to 53), with 40% (n=8) of patients being aged 30 or younger.

Considering all of the aforementioned literature, it appears that asymptomatic DVT occurs in 15% of patients with lower limb trauma treated with cast immobilization (145/985). Symptomatic deep vein thrombosis is less common. Considering all prospective randomized controlled trials of low molecular

weight heparin to date in this patient population, symptomatic DVT occurred in 2.5% of patients (10/390) in the control group of studies which reported symptomatic DVT as an outcome measure (Lassen et al., 2002) (Lapidus et al., 2007) (Jørgensen et al., 2002). In non-randomized observational studies, the incidence of symptomatic venous thromboembolism has consistently been shown to be lower than this. In a large study of 2759 patients with lower limb injuries treated non-surgically, across 37 teaching hospitals and 413 non teaching hospitals over a 7 month period, it was found that 1% of patients sustained a symptomatic DVT (Riou et al., 2007). Although this study focused on adult patients not undergoing surgery, it included patients with both high and low VTE risk and also a variety of injuries ranging from minor injuries such as ankle sprains (34%) to severe injuries such as complete Achilles tendon rupture or ankle fracture (22%). Furthermore, LMWH thromboprophylaxis was provided to 61% of patients in this study, the decision being left to the treating clinician rather than any pre defined risk assessment score. Also, only 54% of patients in this study were treated with rigid cast immobilization i.e. resin or plaster cast, with the remaining patients treated with orthoses, taping and bandages. The important finding on multivariate analysis was that severe injury (complete Achilles tendon rupture or fracture), rigid cast immobilization, non-weight bearing and patients over age 50 years had higher risk of VTE (Riou et al., 2007). Therefore, the incidence of symptomatic VTE in a patient treated with a rigid cast as in this study is likely to be higher than 1%. In a single centre retrospective study, clinical records were reviewed for 381 consecutive patients treated with lower limb casts for any traumatic injury over a 1-year period. None received

thromboprophylaxis (Thomas & Van Kampen, 2011). The only exclusions were patients who had cancer or previous VTE. Patients who would normally be considered high risk for VTE, such as patients who underwent surgery or had Achilles tendon ruptures were included. The important finding from this study was that the overall symptomatic VTE rate was 1.84% (n=7). 4 patients (1%) sustained non-fatal pulmonary embolism. Considering the 4 patients in this study who sustained PE, 3 of them would have been provided with LMWH if they had been risk assessed as per NICE guidelines (one Achilles rupture, two underwent surgery). In view of this, it was concluded that thromboprophylaxis should be considered in those considered at high VTE risk. One patient however, was a 40 year old man with a non-operatively treated ankle fracture treated in non-weight bearing cast. Apart from smoking, he had no apparent risk factors and therefore it is unlikely this could have been prevented (0.2%) (Thomas & Van Kampen, 2011). For patients with ankle fracture who undergo surgical fixation, the incidence of symptomatic DVT and non-fatal PE is even higher (2.66 and 0.32% respectively) (Pelet, Roger, Belzile, & Bouchard, 2012).

In a prospective study with greater numbers (n=1200) found a symptomatic VTE incidence of 0.6% within 3 month of injury in patients with non-operatively treated fractures of the tibia, ankle, patella and foot (some operated). The only exclusions were patients with major trauma, active cancer or previous VTE. Of the 7 patients who sustained symptomatic VTE, 2 were proximal DVT's, 3 were distal, 2 patients sustained non-fatal PE. In contrast to the conclusion in Thomas and Van Kampen's study (2010), it was felt that

routine thromboprophylaxis was not warranted or cost effective (Selby et al., 2014). It is important to recognize that a cost analysis was not performed in either of these studies.

Achilles tendon rupture is associated with a particularly high incidence of symptomatic VTE. For this reason, NICE guidelines recommend that these patients are provided with chemical thromboprophylaxis. The first report of the association between Achilles tendon rupture and VTE was over 40 years ago, in a series of 6 patients who sustained symptomatic venous thromboembolic events associated with being treated with cast immobilization (Micheli, 1975). 3 of these patients had cast immobilization for Achilles tendon rupture. 2 of these patients developed Pulmonary Emboli. Subsequently, Persson & Wredmark (1979) found a symptomatic VTE of 15% in their series of 20 patients who had non-operatively treated Achilles tendon ruptures (Persson & Wredmark, 1979). The focus of their paper was on ankle range of motion and calf strength and it appears the significance of the high incidence of symptomatic VTE was not appreciated, in view that this was not even mentioned in the discussion. More recently, Healy et al (2010a) reported the incidence of symptomatic VTE in a series of 205 patients with Achilles tendon total of partial ruptures. 78% of these patients were treated non-operatively. 6% (n=13) of patients sustained a symptomatic VTE (3 pulmonary emboli, 4 proximal DVT, 6 distal DVT), of which only one had received chemical thromboprophylaxis (Healy et al., 2010). Domeij-Averaud et al (2013) found that the asymptomatic DVT incidence was over 60% in a series of 24 patients with Achilles tendon rupture treated operatively (Domeij-Arverud et al., 2013).

Although the incidence of asymptomatic DVT in patients with lower limb trauma treated with cast immobilization is very high (approximately 1 in 4 patients), the incidence of symptomatic VTE appears to range from 0.6% to 15% depending on the injury (Selby et al., 2014) (Persson & Wredmark, 1979). In practice, the significance of this depends on what is perceived as an acceptable level of risk, which will differ between individuals depending on perspective. This perspective may differ between a stakeholder paying for thromboprophylaxis and a patient. It is important that patients with lower limb trauma and cast immobilization are provided with accurate information on which they can make decisions on taking thromboprophylaxis. Unless patients believe it is important, their compliance is likely to be low, which will impact on the efficacy of any proposed prophylactic method.

Another important finding of this study, is that all DVT's occurred in the veins of the lower limb that had been injured and treated in cast. This simple observation is not reported in previous studies into the incidence of DVT in patients treated with casts. As originally suggested by Virchow, thrombosis results from a triad of stasis, endothelial dysfunction and hypercoagulability (Virchow, 1856). In view that all DVT's occurred in the limb which had been injured and casted, it is more likely that the former two components of this triad are more important. The energy transfer to the limb resulting in bony or soft tissue trauma is likely to result in endothelial damage. Venous stasis may result from pain, swelling occluding veins or the cast itself. A previous study by Solis and Saxby examined the incidence of below knee DVT in patients who underwent elective foot and ankle surgery. In their series of 201

consecutive patients, 3.5% of patients were found to have asymptomatic calf DVT on duplex follow up at between 6 and 18 days postoperatively. 86% of DVT's occurred in the operated leg (Solis & Saxby, 2002). 33% of patients in this study had postoperative immobilization of the foot and ankle, which did not have a statistically significant association with DVT on logistic regression. This suggests that endothelial dysfunction may play a more important role in the pathogenesis of deep vein thrombosis than stasis. This requires further investigation.

There are several limitations of this study. Due to the variety of injuries included, the duration of cast treatment was variable. As a result it was not possible to perform all Doppler scans at a specific time interval following injury. For logistical reasons it was not always possible for patients to wait to have bilateral lower limb Doppler ultrasound scans on day of cast removal. In these cases, patients had their scans arranged for their next fracture clinic or Physiotherapy appointment at the Hospital in order to minimize inconvenience. Some asymptomatic DVT's may have resolved during this time, and therefore the finding of a 25% DVT rate may under-estimate the true incidence. The loss to follow up was 19%, which may have also resulted in an underestimate of asymptomatic DVT incidence. None of these patients lost to follow up sustained symptomatic VTE events. Historically, venography was the gold standard for diagnosis of DVT in leg after Orthopaedic surgery, now Doppler accuracy has increased it has become the gold standard. (Domeij-Arverud et al., 2013). It is unlikely that any of the DVT's in this study were false positives because all DVT's were present on repeat scanning at 1 week after diagnosis.

6.3 The association between mobility and subsequent development of Deep Vein Thrombosis in patients with lower limb trauma and cast treatment

Patients with lower trauma and cast treatment are often assumed to be immobile. Although some patients with lower limb trauma and cast treatment may find mobilization challenging, due to pain, anxiety or difficulty non-weight bearing, mobility of these patients has never previously been objectively studied (Harvey & Runner, 2011). In this study, using objective measurement of mobility, less than 5% of patients with lower limb trauma treated with non-weight bearing below knee cast are immobile during the first 5 days of cast treatment (no steps or moves for 3 consecutive days). The remaining 95% were not immobile. Only 9 patients (13%) recorded 0 moves, steps or miles for at least one day.

In theory, reduced mobility can lead to venous stasis in the lower limbs, however the association between mobility level and thrombogenesis is poorly documented (Emed et al., 2010). In a study by Gibbs et al (1957) sixty years ago, it was found during post mortem that the incidence of lower limb venous thrombosis was greater in patients who had been confined to bed for longer than 3 days (Gibbs, 1957). In practice, it is difficult to determine the association between mobility and DVT because the definition of immobility differs substantially between studies. In a review of the literature by Hull et al

(2013) it was found that definitions of immobility vary widely between studies of immobility, from walking within hospital room or to the bathroom for 3 or more days, to total bed rest (with bathroom privileges) for 4 days or more within 2 months of VTE event (Hull, 2013). They concluded that definitions need refining (Hull, 2013). Although a systematic review of cohort and case control studies in medical patients concluded that immobilization i.e. bed rest is associated with increased risk of VTE, the assumption that reduction in mobility is an independent risk factor for VTE may not be true because it is difficult to exclude an influence on VTE by the underlying conditions causing the reduction in mobility (Pottier et al., 2009). In contrast, Warlow et al (1976) found that in patients with acute stroke, the incidence of DVT was unaltered by early mobilization i.e. bed rest less than 4 days compared with those who were in bed for longer durations (Warlow, Ogston, & Douglas, 1976).

In this study, 26% (n=18) of the 68 patients were found to have asymptomatic DVT on bilateral lower limb venous ultrasound after a mean cast duration of 42 days (SD 14). One was above knee (Popliteal vein). However, there was no statistically significant difference in moves or steps between Group 1 (No DVT) and Group 2 (DVT). No DVT's occurred in the 3 patients who were recorded as being immobile.

Although there was no association between mobility and DVT, it appears that a mean step count of 863 found in this study is not normal. For example, students have been shown to walk an average of 10,000 steps per day when

measured using ActiGraph wGT3X-BT for 7 consecutive days (Clemente, Nikolaidis, Martins, & Mendes, 2016). Sisson et al (2010) reported similar findings in their study of healthy volunteers using the Actigraph AM-7164, recording on average 9876 +/- 107 steps per day (uncensored). Even when steps taken at an intensity of <500 counts per minute (indicating inactivity intensity) were excluded, volunteers still recorded 6540 +/- 106 steps per day (Sisson et al., 2010). Le Masurier et al (2003) found that healthy participants walking at speeds of between 54 and 107 metres per minute walked between 461 and 660 steps in only 5 minutes of walking (Le Masurier & Tudor-Locke, 2003). In the largest study of physical activity (the National Health and Nutrition Examination Survey (NHANES), 14446 adults of mean age 48 years had steps measured using the actigraph AM-7164 accelerometer worn at the hip for continuous monitoring for 7 days, for a minimum of 10hrs per day. 13.3% of patients recorded a 'basal' physical activity category (<2500 steps/day), 22.7% met the limited category (2500-4999 steps per day), 27% were 'low active' (5000-7499), 19.3% were somewhat active (7500-9999), 9.6% were active (10,000-12,499) and 8.1% were 'highly active' (>12,500 steps per day). Based on this, all patients would be considered in the 'basal' physical category (Sisson et al., 2010).

Some studies have objectively measured mobility in Orthopaedic patients. Lutzkner et al (2016) reported in their study that patients awaiting total knee replacement walk an average of 5371 steps per day (SD 2820) (C. Lützner, Beyer, Kirschner, & Lützner, 2016). Similarly, in a study of patient mobility before and after TKR, it was found that patients ambulate less at 2 months

following surgery as compared to pre-operatively. However this was not a statistically significant reduction (4,993 gait cycles per day preoperatively compared to 4730 cycles postoperative) (Brandes, Ringling, Winter, Hillmann, & Rosenbaum, 2010). Even after transtibial amputation, Desveaux et al reported that patients walk an average of 3809 +/- 2189 steps per day (Desveaux et al., 2016). These patients appear more mobile than those in the present study, which found that mean daily accelerometer recorded moves were 1043 (SD 1019), mean daily steps were 863 (SD 848) and mean miles recorded were 0.48 (SD 0.48). Pre injury mobility levels were not available for comparison. There is however some evidence that lower limb casts may reduce mobility. Recently, Ceroni et al measured activity in 50 children who were had a long leg non-weight bearing cast, using the Actigraph 7164 uniaxial accelerometer and found a 62.4% reduction in mobility compared to controls (Ceroni, Martin, Lamah, et al., 2012b). Maggio et al (2016) measured activity-related energy expenditure in a series of 34 adolescents with lower limb casts using the same device and found a 16% reduction when compared with healthy controls, indicating they were less active (Maggio, Martin, & Ceroni, 2016). The only study of Orthopaedic patients which reports lower mobility than found in the present study is by Davenport et al (2015), who found that patients who underwent hip fracture surgery, postoperative mobility was only 36 steps per day (+/- 80.4). These patients were all admitted from their own home or low level care facility pre-fracture, so it could be anticipated that those from higher level care institutes such as nursing homes may mobilise even less (Davenport et al., 2015). Immobility to this extent may contribute to development of venous thrombosis.

Accelerometers and pedometers can both be used to detect 'steps' and have previously been shown to produce similar results when compared to actual video recorded steps taken under controlled treadmill conditions, between walking speeds of 1.1 – 1.8 m/s. However, at slow walking speeds of 0.9 m/s, accelerometers are significantly more sensitive and accurate. Participants in this study were between ages 20 an 55 years, which is comparable to our study group. Considering that many patients in our study would walk less then 0.9 m/s, use of an accelerometer was more appropriate (Le Masurier & Tudor-Locke, 2003). In a study of gait in 14 amputees walking with prostheses, Houdijk et al (2008) found that the DynaPort accelerometer was highly accurate in measurement of step count when compared with video recording (Pearson correlation co-efficient of 0.99, p<0.001). However, the DynaPort MiniMod accelerometer and accompanying GaitMonitor software are much more expensive than MOVband accelerometer, approximately $3,000 .(Houdijk, Appelman, Van Velzen, Van der Woude, & Van Bennekom, 2008). The majority of studies into physical activity use the Actigraph brand, however we were limited by costs constraints (Montoye et al., 2016). Under free living conditions, consumer level activity monitors, worn for 48 consecutive hours, including the Fitbit One, Fitbit Zip, Jawbone UP, Misfit Shine, Nike Fuelband, Striv Smart Pedometer and Withings Pulse were all shown to have strong correlation (r > 0.9) for measuring step count in comparison with research grade devices such as the ActiGraph GT3X+ and the BodyMedia SenseWear) (Ferguson, Rowlands, Olds, & Maher, 2015). More recently, the MOVband has been shown to have strong positive correlation with the Actigraph GT1M

research grade accelerometer in both adults and children (Williamson et al., 2014) (Fennell et al., 2016).

At present there is no current consensus on the best method for measurement of physical activity using accelerometer data (Sabia et al., 2014). However, one important aspect of data collection is minimum wear time. In an analysis of 1000 days of accelerometer data from 1000 participants in the National Health and Nutrition Examination Study data set (NHANES), shorter wear times were found to be associated with increased errors of physical activity measurement (Herrmann et al., 2014). Subsequently, it was recommended that accelerometers should be worn for greater than 12h/day to ensure accurate estimates of daily physical activity (Herrmann et al., 2014). Rich et al calculated reliability coefficients in 7704 participants who wore the Actigraph GT1M accelerometer and determined that devices should be worn for at least 10 hours per day for at least 2 days in order to reliably estimate physical activity (Rich, Geraci, et al., 2013b). Therefore, most studies using accelerometers to measure physical activity use a minimum of 4 days for a minimum of 10hrs per day (Skender et al., 2016). In the present study, patients wore the accelerometers for 24hr/day for 5 consecutive days, which should provide a valid measure of physical activity.

For those who completed this study and attended for DVT ultrasound scans (n=78), only 4 (5%) accelerometers were lost and 6 accelerometers (8%) failed to capture any data (13% total failure). This rate of return is high in comparison to previous studies and is due to positioning the accelerometer

within the cast. For example, Sharma et al (2012) attempted to study the first 7 days of mobility in women following child birth and found that only 36% of 200 recruited patients returned the pedometer by post (Sharma et al., 2012). Similarly, in a series of 10034 participants asked to wear an accelerometer at hip level for 7 consecutive days, only 66.5% achieved the minimum wear time of at least 10 hrs per day for 2 or more days (Rich, Cortina-Borja, et al., 2013a). Previous authors have also found difficulty with compliance when mobility-tracking devices have been applied to other regions of the body. For example, Ceroni et al (2012) measured post-operative mobility in 50 children following surgery and non-weight bearing long leg cast, during which all participants were asked to wear an Actigraph 7164 uniaxial accelerometer at the iliac crest of the right hip with an elastic belt for 10 days. 28% (n = 14) of patients were excluded because they failed to wear the accelerometer for at least 5 days or the instrument malfunctioned (Ceroni, Martin, Lamah, et al., 2012b). Maggio et al measured physical activity in 50 adolescents with lower limb casts for fracture using ActiGraph 7164 attached to the iliac crest, but had to exclude 32% (n=16) due to wear time of less than 5 days or instrument malfunctions (Maggio et al., 2016). Harrison et al (2011) reported a similar experience in 48 pregnant women using the Actigraph GT1 M accelerometer where 38% either failed to wear the accelerometer or had incomplete data (Harrison, Thompson, Teede, & Lombard, 2011). Harding et al (2014) reported a lower total failure rate using the Actigraph GT1M accelerometer in 57 patients who underwent hip or knee arthroplasty. Patients were asked to wear the accelerometer for 10hrs per day for 4 days and only 12% of patients were excluded because they either failed to wear the accelerometer for long

enough (n=5) or there was a technical problem with the monitor (n=2) (Harding, Holland, Delany, & Hinman, 2014). Hager et al (2015) achieved similar rates of failure (16%) in 457 adolescent girls asked to wear an Actical accelerometer at the ankle for 7 consecutive days (Hager et al., 2015).

As discussed, it was considered important to apply the accelerometer to the cast this in order to increase compliance with wearing the accelerometer in free-living conditions. In order to standardize the accelerometer position in the cast, all accelerometers were fitted by one author (BH), as it has previously been suggested that even subtle differences in positioning of devices can influence results (Rispens et al., 2014). However, many recent studies have suggested that site of accelerometer attachment may not be so influential on results. Examples include the activPAL3c triaxial accelerometer, which can be worn on either thigh, either upper or lower without significantly affecting step count measurements (Stanton, Guertler, Duncan, & Vandelanotte, 2016). The ActiGraph GT3X accelerometer, for which Dieu et al found no significant difference in activity counts for x,y or z axes when devices were worn simultaneously on dominant and non-dominant wrists for 24 hrs (Dieu et al., 2016). Similarly, Stepwatch accelerometer (Cyma, Seattle, Washington) step counts worn simultaneously at each ankle had excellent agreement, with disagreement of <1 step per minute at walking speeds of between 1 and 3mph (Foster et al., 2005). Reliability of the Actigraph GT3X+ monitor has also been found to have average Intraclass Correlations of >0.86 when results were compared between measurements at wrist, hip and ankle (just above lateral malleolus) in 40 healthy volunteers (Ozemek, Kirschner,

Wilkerson, Byun, & Kaminsky, 2014). The differences between these newer studies and older studies such as previously discussed by Taraldsen et al (2011) may be due to the differences in accelerometer technology (Taraldsen et al., 2011). For example the ActivPAL used by Taraldsen et al was a uniaxial accelerometer, whereas modern accelerometers, including the ActivPAL 3c and MOVband use triaxial accelerometry, which may be less affected by device positioning.

The main strength of this study is that objective measures were used to prospectively assess patient mobility in a consecutive series of patients with acute lower limb trauma treated with below knee cast. It was felt that this was more appropriate in view that the overall correlation between questionnaire-assessed and accelerometer-assessed physical activity was low in a large cohort study (Spearman's r = 0.3) (Sabia et al., 2014). To standardize data collection one author applied all accelerometers and a minimum wear time of a consecutive 120 hours was used, which enables valid conclusions to be made from the data. Accelerometer failures and losses were less than 13%, which is better than achieved in the majority of other studies in to physical activity using accelerometers. Particular attention has been given to reporting of the methods, providing specific details of the accelerometer used, according to recent guidance from Montoye et al (2016) (Montoye et al., 2016). Although this was a proof of principle study, it is likely that it was powered to detect significant differences in physical activity between groups. Assuming a 20% difference in steps per day between DVT and no DVT groups, with baseline activity levels of 1000 steps per day (SD 500), 28

participants would have needed to be recruited for power 0.8 at the 0.05%

level of significance (Davenport et al., 2015). Our loss to follow up was higher

than anticipated, mainly because 22% of recruited participants failed to attend

for their lower limb ultrasound to assess for deep vein thrombosis.

Conclusion

Using objective measurement, this study reveals that the majority of patients

(95%) with acute lower limb trauma treated with non-weight bearing below

knee cast are not immobile during the first 5 days after cast application.

Immobility was not associated with subsequent development of DVT.

Although it is likely that patients with lower limb trauma treated with casts are

less mobile than their pre injury states, physical activity levels do not appear

to provide discriminatory value in predicting which patients will develop deep

vein thrombosis. In view of this, patient mobility should not be used to stratify

these patients into risk of DVT.

6.4 Can we use biomarkers of coagulation to predict which patients with foot and ankle injury will develop deep vein thrombosis?

None of the plasma biomarker levels tested in this study (Tissue Factor, Interleukin 6, VCAM-1 and D-Dimer) were able to predict development of subsequent DVT. Although they are involved in venous thrombosis, they have no useful role risk stratification of patients with acute foot and ankle injury.

Tissue factor, also known as Thromboplastin or Coagulation factor III is a transmembrane glycoprotein (Mackman, 2006) found in the vascular adventitia (Girard & Nicholson, 2001). Vessel wall tissue factor is the principal initiator of thrombosis (Day et al., 2005) (Khorana et al., 2008) (Kretz, Vaezzadeh, & Gross, 2010), acting as the co-factor for factor VII. The combination of these results in activated VIIa, which activates factors X and IX (Chouhan et al., 1999). It is also found in the blood, stored in alpha granules of platelets and becomes expressed on the surface after platelet activation (Müller et al., 2003). The majority of blood-borne tissue factor is derived from monocytes and is functionally 'inactive' until 'decrypted' to act as a catalytic co-factor for factor VIIa (Bogdanov & Versteeg, 2015). In a rabbit model, Himber et al demonstrated that inhibition of tissue factor inhibited venous thrombosis propagation (Himber et al., 2003). However, despite these findings, there are limited numbers of studies which have investigated the

association between TF and subsequent development of VTE.

Khorana et al used a custom made ELISA test to measure plasma tissue factor antigen in 11 patients with Pancreatic cancer and found a significant association between TF antigen levels and subsequent development of symptomatic VTE (p=0.036) (Khorana et al., 2008). TF antigen levels in the two patients who subsequently developed VTE were 377 pg/mL and 504 pg/mL. For comparison, the highest TF level in the present study was 68 pg/mL. This patient did not develop DVT. In a study of patients with lower limb trauma treated with cast, Walenga et al (2014) measured tissue factor antigen at time of injury, between day 10 to 14 post injury and on cast removal. Interestingly, at time of injury, levels were not elevated above normal (<35pg/mL). Also, there were no significant differences in TF levels between fracture, Achilles and soft tissue injury groups (Walenga et al., 2014). In the present study, 18 patients (23%) had TF levels >35pg/mL, 3 of these subsequently developed DVT. In the present study, tissue factors were 23.92pg/ml in patients who did not develop DVT, compared with 20.33pg/ml in those who did (p=0.422). In contrast to the findings of this study, Walenga et al (2014) found that those who developed VTE had significantly higher tissue factor levels (median 49.05 pg/mL vs 14.86 pg/mL, p = 0.003). This difference was present at baseline, between 10 to 14 days post injury and at time of cast removal (Walenga et al., 2014). In contrast, Johnson et al (2009) measured several types of tissue factor in a series of 19 patients who underwent total knee replacement. Interestingly, plasma tissue factor levels did not increase significantly. However, the peripheral blood mononuclear cell functional tissue

factor procoagulant activity did increase on day 1 and 2 post surgery (G. J. Johnson et al., 2009).

DVT is associated with an inflammatory response (Mosevoll et al., 2015) (Hou et al., 2012), however, it unclear whether this is a cause or a consequence of thrombosis. Cheng et al found that IL-6 levels significantly increased on day 1 after total knee replacement when compared to pre-operative levels, suggesting that tissue injury activates inflammation (Cheng, Giebaly, Campbell, Rumley, & Lowe, 2014). In view that IL-6 creates a prothrombotic state by increasing the expression of tissue factor (Kerr, Stirling, & Ludlam, 2001), it is logical to consider that tissue injury may result in venous thrombosis. In a study of 717 patients with first acute symptomatic DVT, all had IL-6 blood test on USS confirmation of DVT. IL-6 was measured using ELISA (R&D systems). Higher IL-6 levels were found to be significantly associated with extent of DVT and clinical symptoms at diagnosis (Rabinovich, Cohen, Cushman, Kahn, BioSOX Investigators, 2015).

In this study, median IL-6 levels were 3.91 pg/mL in those who did not subsequently develop DVT and were 4.59 pg/mL in those who did develop DVT (p=0.764) (range 0 to 84.68 pg/mL). Similarly, Mosevall et al (2015) used the R&D systems Luminex assay to measure inflammatory markers in plasma of patients suspected of having lower limb DVT. They found no significant difference between IL-6 levels in those with (1.240 pg/ml), compared to those without (2.020 pg/ml) on subsequent venous USS. Furthermore, IL-6 levels in

the 21 patients found to have DVT on USS, were not significantly higher than 20 normal control patients without DVT (1.240 pg/ml vs 3.470 pg/ml. p=0.1967) (Mosevoll et al., 2015). In a series of 40 patients with phlebographically proven lower limb DVT, Roumen-Klappe et al measured IL-6 on day of presentation and compared levels to a group of 33 controls. They also measured IL-6 on the subsequent 5 days following DVT. Interestingly, they found that IL-6 levels were significantly higher in the group with DVT (15pg/mL, range <3 to 70 pg/mL) as compared with the control group (<3 pg/mL, range <3 to 11 pg/mL), but subsequently decreased during the following 5 days, to 5.5 pg/mL by day 5 (p <0.01). This indicates that that the raised IL-6 levels were the result of thrombosis rather than the cause (Roumen-Klappe et al., 2002). At 32 months after DVT, patients continue to have increased levels of IL-6 compared to controls, suggesting a persistent chronic sub-clinical response (Bittar et al., 2015). In view of that there were no significant differences in IL-6 between those who did and did not develop DVT This may represent that none had DVT at the time of measurement. It appears that IL-6 levels are raised in response to DVT rather than being the cause.

VCAM-1 levels are increased at sites of endothelial inflammation and are involved in leukocyte adhesion and migration across vascular endothelium (Yang et al., 2013) (Bouman et al., 2014). In a study of 135 patients suspected of having DVT, Bozic et al (2002) used R&D Systems quantitative ELISA to measure plasma VCAM-1. The 39% percent of patients who were subsequently found to have DVT on lower limb doppler ultrasound, had

significantly higher VCAM-1 levels (392 micrograms/litre vs 417 (p=0.03).

However, VCAM-1 was not as accurate as D-dimer in diagnosis using ROC

analysis (0.6 vs > 0.8 depending on D-dimer method used) (Božič, Blinc, &

Stegnar, 2002). In a similar recent study of 89 patients suspected of having

DVT, Mosevoll et al (2015) measured VCAM-1 using R&D systems Luminex

assay. VCAM-1 levels were significantly higher in the 21 patients who were

subsequently found to have DVT on lower limb ultrasound (850.161 ng/ml,

range 104.311 to 1571.607 vs 635.436, range 290.605 to 2793.862,

p=0.0009). Furthermore, in comparison to 20 control patients, VCAM-1 levels

were also significantly higher in patients with DVT (Mosevoll et al., 2015). In

the present study, median VCAM-1 levels were 552.98 ng/mL in those who

did not develop DVT, as compared with 496.84 ng/mL in those who did

(p=0.111). VCAM-1 levels ranged from 412.63 to 823.15 ng/mL. In view that

the same number of patients sustained DVT in this study as reported in the

study by Mosevoll et al, it is likely to be adequately powered to detect

statistically significant differences in VCAM-1 between groups who did and did

not subsequently sustain DVT (Mosevoll et al., 2015). In a recent mouse

model, thrombin was recently shown to induce the expression of VCAM-1,

suggesting that VCAM-1 is increased prior to thrombus formation (Bertin,

Lemarié, Robins, & Blostein, 2015). However, levels of VCAM-1 may be

increased before levels of D-Dimer rise. The difference with this study is that

VCAM-1 was measured within 3 days of injury, as opposed to at time of

diagnosis of DVT. This may have been too early, before a prothrombotic state

had occurred.

Fibrin clots form to cause haemostasis, and are subsequently broken down by the action of plasmin into d-dimer products (Hou et al., 2012). Many studies have shown that patients who undergo lower limb venous ultrasound and subsequently found to have deep vein thrombosis (DVT), also have significantly higher d-dimer levels than those with normal imaging (Božič et al., 2002). However, the challenge of using d-dimer to diagnose DVT is that it can be elevated in many situations, including following surgery, trauma, pregnancy, liver disease and increasing age (Hou et al., 2012) (Sudo et al., 2009). It is therefore useful for excluding VTE but is not specific enough to diagnose it (Coleman & Wakefield, 2012). In combination with pre test clinical probability scores such as the Wells score, the diagnostic accuracy of this test is improved, to the extent that patients with low clinical suspicion and low d-dimer can exclude DVT and negate the requirement for further imaging of the lower limb venous system (Perrier, 2006). Recently, a d-dimer result of <500ng/ml was shown to have a negative predictive value of 99.48% irrespective of clinical suspicion of DVT (Michiels et al., 2015). In patients who have undergone surgery, cut off levels for excluding DVT are higher. Abraham et al (1999) found that a d-dimer cut off level of <2808ng/mL on day 1 post total hip or knee arthroplasty was associated with a significantly lower incidence of subsequent asymptomatic DVT (USS proven) on postoperative day 7 (8% vs 21.4%) (Abraham et al., 1999). Yoo et al measured d-dimer on day 3 following total hip replacement or surface replacement in 221 patients and found a significant correlation with the finding of DVT on ultrasound/venogram at day 7 postoperative. A cut off value of 2640 ng/mL had a negative predictive value of 98.8% (Yoo, Cho, Ghanem, Ramteke, &

Kim, 2009). Sudo et al (2009) suggested a cut off level of 17700 ng/mL after hip or knee arthroplasty (Sudo et al., 2009).

In view that raised D-dimer may indicate a thrombotic state, some authors have attempted to use this to predict who will subsequently develop clinical signs of DVT. For example, in a study of 99 patients who underwent THR or TKR, d-dimer was measured pre and postoperatively at intervals. Patients also had DVT ultrasound scan at day 4 and 10 postoperatively. 15% were found to have a DVT. D-dimer levels were significantly higher postoperatively as compared to pre-operative levels. Those who were subsequently found to have DVT had statistically significantly higher d-dimer levels than those who did not, at days 4, 7, 10 and 14 postoperatively. Interestingly, there was no significant difference in d-dimer levels on day 1 postoperative between those who subsequently developed DVT and those that did not (Sudo et al., 2009). An et al measured d-dimer in 177 patients who underwent THR or TKR and found that d-dimer levels peaked 2 weeks postoperatively. None of these patients developed a symptomatic DVT (An et al., 2016). Similarly, Yoshioka et al (2010) found that d-dimer measurements within the first 3 days following spinal surgery were not predictive of findings of DVT on screening USS between days 7-10 postoperative. Interestingly, there was a statistically significant difference in d-dimer levels between those who did and did not have DVT, when measured on day 7 postoperative due to a rise in d-dimer in those with DVT. These studies suggests that there is a delay in prothrombotic state and subsequent rise in d-dimer following surgery (Yoshioka et al., 2010). In this study of patients with lower limb cast treatment for soft tissue injury or

fracture, blood samples were taken at baseline (time of randomisation), between day 10 and 14 after injury and again at time of cast removal. 18.6% of 188 patients who did not receive thromboprophylaxis developed VTE, which is similar to findings of this study. Similarly, all DVT's occurred in the injured leg. Interestingly, thrombin-antithrombin complex (TAT), which represents thrombin generation, was normal at time of injury and not significantly different at baseline between patients who went on to develop VTE compared with those who did not. However, when compared at between day 10-14 post injury, it was significantly higher in the group who were subsequently found to have DVT (Walenga et al., 2014). In the present study, D-Dimer levels were only 203.5 ng/mL in those who did not develop DVT and 236 ng/mL in those who did (p=0.490). Levels ranged from 31 to 1184 ng/mL. Considering the aforementioned literature, it is possible that blood samples in this study were taken prior to the prothrombotic state occurring. Although it is not possible to draw direct comparisons between absolute D-dimer levels in this study and those found by others due to differences in methods used to quantify D-dimer, it is evident that levels in the present study are relatively low (Crowther et al., 2004).

Limitations

In this study, quantitative levels of Tissue factor, IL-6, VCAM-1 and D-dimer were measured. For some of these tests, such as for Tissue factor it would have been useful to measure activity, because levels and activity may be independent. Also, Tissue factor pathway inhibitor (TFPI) was not considered,

so it is possible that the thrombogenic effect of exposed subendothelial tissue factor secondary to injury may have been prevented by TFPI (Maroney & Mast, 2015). Finally, blood samples were only taken at time of recruitment i.e. within 3 days of injury. It may have provided additional understanding if further samples had been taken at intervals, which may have enabled trends in levels of biomarkers to be assessed.

7 Conclusion

LMWH reduces the incidence of both asymptomatic and symptomatic VTE events. However, there is a risk of major bleeding (0.11%) which needs to be carefully considered against the benefit of prevention of symptomatic VTE events. Considering that the number needed to prevent symptomatic VTE was found to be 82, 11 symptomatic VTE events would be prevented for every major bleed. The clinical and financial implications of this require further prospective study. It is also vital to identify which patients in casts are most likely to develop VTE, to enable thromboprophylaxis to be prescribed accurately to those at highest risk. Multi centre studies are necessary to achieve definitive answers. Current guidelines will make such studies difficult, due to recommendation for thromboprophylaxis in many patients with casts and perceived additional VTE risk factors.

Regular active toe movement by patients treated with lower limb cast for acute foot and ankle trauma did not significantly influence calf pump function or reduce the incidence of asymptomatic deep vein thrombosis. All cases of deep vein thrombosis occurred in the limb, which had been injured and treated in cast. The majority (90%) of venous thrombosis involved the peroneal veins. Advice to patients to perform regular active toe movement alone appears insufficient to reduce the risk of development of asymptomatic deep vein thrombosis.

27% of patients with lower limb trauma treated with below knee cast immobilization will sustain an asymptomatic deep vein thrombosis, even in the absence of any other apparent risk factors. 15% of these DVT's involve the veins at the level of the popliteal vein or more proximal and required treatment with anticoagulation. All cases of deep vein thrombosis occurred in the limb that had been injured and treated in cast. The effect of asymptomatic deep vein thrombosis in patients with lower limb trauma and cast treatment requires further study to determine the long-term outcomes on venous function.

The majority of patients (95%) with acute lower limb trauma treated with non-weight bearing below knee cast are not immobile during the first 5 days after cast application. Immobility was not associated with subsequent development of DVT. Although it is likely that patients with lower limb trauma treated with casts are less mobile than their pre injury states, physical activity levels do not appear to provide discriminatory value in predicting which patients will develop deep vein thrombosis. In view of this, mobility of these patients should not be used to stratify these patients into risk of DVT.

In this study of patients with acute foot and ankle trauma, there was no association between levels of plasma Tissue factor, IL-6, VCAM-1 or D-dimer and subsequent development of DVT. These appear to have no predictive role when measured within the first 3 days of injury. Further study is required to determine which biomarkers of thrombosis can be used and when these

should be measured in order to identify patients that will subsequently develop DVT.

In conclusion, weak evidence exists for the effectiveness of chemical thromboprophylaxis in prevention of deep vein thrombosis in patients with foot and ankle trauma treated with below knee cast. There is no evidence that mechanical thromboprophylaxis is effective. DVT appears to solely occur in the lower limb that has been injured and treated in cast, but the development cannot be predicted using level of mobility or markers of tissue injury.

8 Further work

Future work should aim to prospectively validate VTE risk assessment tools, to enable risk stratification of patients and provide prophylaxis to those at greatest risk. Long term outcome of cast related asymptomatic and symptomatic DVT requires further evaluation, to determine their association with post thrombotic syndrome and clinical relevance of these outcomes. Mechanical prophylactic strategies should focus on the prevention of stasis in the leg that has been injured and treated with cast, the effect of neuromuscular calf pump function stimulators on DVT occurrence requires study.

Bibliography

Abdul Sultan, A., West, J., Tata, L. J., Fleming, K. M., Nelson-Piercy, C., & Grainge, M. J. (2013). Risk of first venous thromboembolism in pregnant women in hospital: population based cohort study from England. *Bmj*, *347*, f6099. http://doi.org/10.1136/bmj.f6099

Abraham, P., Ternisien, C., Hubert, L., Pidhorz, L., & Saumet, J. L. (1999). Does venous microemboli detection add to the interpretation of D-dimer values following orthopedic surgery? *Ultrasound in Medicine & Biology*, *25*(4), 637–640.

Agutter, P. S., Malone, P. C., & Silver, I. A. (2012). Experimental Validation of Methods for Prophylaxis against Deep Venous Thrombosis: A Review and Proposal. *Thrombosis*, *2012*, 156397. http://doi.org/10.1155/2012/156397

Aldington, S., Pritchard, A., Perrin, K., James, K., Wijesinghe, M., & Beasley, R. (2008). Prolonged seated immobility at work is a common risk factor for venous thromboembolism leading to hospital admission. *Internal Medicine Journal*, *38*(2), 133–135. http://doi.org/10.1111/j.1445-5994.2007.01597.x

Alikhan, R., Cohen, A. T., Combe, S., Samama, M. M., Desjardins, L., Eldor, A., et al. (2004). Risk factors for venous thromboembolism in hospitalized patients with acute medical illness: analysis of the MEDENOX Study. *Archives of Internal Medicine*, *164*(9), 963–968. http://doi.org/10.1001/archinte.164.9.963

An, T. J., Engstrom, S. M., Oelsner, W. K., Benvenuti, M. A., Polkowski, G. G., & Schoenecker, J. G. (2016). Elevated d-Dimer Is Not Predictive of Symptomatic Deep Venous Thrombosis After Total Joint Arthroplasty. *The Journal of Arthroplasty*. http://doi.org/10.1016/j.arth.2016.02.059

Anderson, F. A., & Spencer, F. A. (2003). Risk factors for venous thromboembolism. *Circulation*, *107*(23 Suppl 1), I9–16. http://doi.org/10.1161/01.CIR.0000078469.07362.E6

Andrews, B., Sommerville, K., Austin, S., Wilson, N., & Browse, N. L. (1993). Effect of foot compression on the velocity and volume of blood flow in the deep veins. *The British Journal of Surgery*, *80*(2), 198–200.

Antonopoulos, C. N., Sfyroeras, G. S., Kakisis, J. D., Moulakakis, K. G., & Liapis, C. D. (2014). The role of soluble P selectin in the diagnosis of venous thromboembolism. *Thrombosis Research*, *133*(1), 17–24. http://doi.org/10.1016/j.thromres.2013.08.014

Austin, R. T. (1983). Treatment of broken legs before and after the introduction of gypsum. *Injury*, *14*(5), 389–394. http://doi.org/10.1111/j.1538-7836.2009.03293.x

Baglin, T., Douketis, J., Tosetto, A., Marcucci, M., Cushman, M., Kyrle, P., et al. (2010). 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*, *8*(11), 2436–2442. http://doi.org/10.1111/j.1538-7836.2010.04022.x

Barg, A., Henninger, H. B., & Hintermann, B. (2011). Risk factors for symptomatic deep-vein thrombosis in patients after total ankle replacement who received routine chemical thromboprophylaxis. *The Journal of Bone and Joint Surgery. British Volume*, *93*(7), 921–927. http://doi.org/10.1302/0301-620X.93B7.26257

Beasley, R., Raymond, N., Hill, S., Nowitz, M., & Hughes, R. (2003). eThrombosis: the 21st Century variant of venous thromboembolism associated with immobility. *European Respiratory Journal*, *21*(2), 374–376. http://doi.org/10.1183/09031936.03.00039403

Bederman, S. S., Chundamala, J., & Wright, J. G. (2010). Randomized clinical trials in orthopaedic surgery: strategies to improve quantity and quality. *The Journal of the American Academy of Orthopaedic Surgeons*, *18*(8), 454–463.

Bembenek, J., Karlinski, M., Kobayashi, A., & Czlonkowska, A. (2011). Early stroke-related deep venous thrombosis: risk factors and influence on outcome. *Journal of Thrombosis and Thrombolysis*, *32*(1), 96–102. http://doi.org/10.1007/s11239-010-0548-3

Bergendal, A., Bremme, K., Hedenmalm, K., Lärfars, G., Odeberg, J., Persson, I., et al. (2012). Risk factors for venous thromboembolism in pre-and postmenopausal women. *Thrombosis Research*, *130*(4), 596–601. http://doi.org/10.1016/j.thromres.2012.05.024

Bertin, F. R., Lemarié, C. A., Robins, R. S., & Blostein, M. D. (2015). Growth arrest-specific 6 regulates thrombin-induced expression of vascular cell adhesion molecule-1 through forkhead box O1 in endothelial cells. *Journal of Thrombosis and Haemostasis*, *13*(12), 2260–2272. http://doi.org/10.1111/jth.13156

Bělohlávek, J., Dytrych, V., & Linhart, A. (2013). Pulmonary embolism, part I: Epidemiology, risk factors and risk stratification, pathophysiology, clinical presentation, diagnosis and nonthrombotic pulmonary embolism. *Experimental and Clinical Cardiology*, *18*(2), 129–138.

Bittar, L. F., Mazetto, B. de M., Orsi, F. L. A., Collela, M. P., De Paula, E. V., & Annichino-Bizzacchi, J. M. (2015). Long-term increased factor VIII levels are associated to interleukin-6 levels but not to post-thrombotic syndrome in patients with deep venous thrombosis. *Thrombosis Research*, *135*(3), 497–501. http://doi.org/10.1016/j.thromres.2014.12.024

Blom, J. W., Doggen, C. J. M., Osanto, S., & Rosendaal, F. R. (2005). Malignancies, prothrombotic mutations, and the risk of venous thrombosis. *Jama*, *293*(6), 715–722. http://doi.org/10.1001/jama.293.6.715

Bogdanov, V. Y., & Versteeg, H. H. (2015). "Soluble Tissue Factor" in the 21st Century: Definitions, Biochemistry, and Pathophysiological Role in Thrombus Formation. *Seminars in Thrombosis and Hemostasis*, *41*(7), 700–707. http://doi.org/10.1055/s-0035-1556049

Bouman, A. C., Cheung, Y. W., Spronk, H. M., Schalkwijk, C. G., Cate, Ten, H., Wolde, ten, M., & Cate-Hoek, Ten, A. J. (2014). Biomarkers for post thrombotic syndrome: a case-control study. *Thrombosis Research*, *134*(2), 369–375. http://doi.org/10.1016/j.thromres.2014.06.010

Božič, M., Blinc, A., & Stegnar, M. (2002). D-dimer, other markers of haemostasis activation and soluble adhesion molecules in patients with different clinical probabilities of deep vein thrombosis. *Thrombosis Research*, *108*(2-3), 107–114. http://doi.org/10.1016/S0049-3848(03)00007-0

Brandes, M., Ringling, M., Winter, C., Hillmann, A., & Rosenbaum, D. (2010). Changes in physical activity and health-related quality of life during the first year after total knee arthroplasty. *Arthritis Care & Research*, n/a–n/a. http://doi.org/10.1002/acr.20384

Briel, M., Bassler, D., Wang, A. T., Guyatt, G. H., & Montori, V. M. (2012). The dangers of stopping a trial too early. *The Journal of Bone and Joint Surgery (American)*, *94 Suppl 1*, 56–60. http://doi.org/10.2106/JBJS.K.01412

Broderick, B. J., Corley, G. J., Quondamatteo, F., Breen, P. P., Serrador, J., & OLaighin, G. (2010). Venous emptying from the foot: influences of weight bearing, toe curls, electrical stimulation, passive compression, and posture. *Journal of Applied Physiology*, *109*(4), 1045–1052. http://doi.org/10.1152/japplphysiol.00231.2010

Caprini, J. A. (2005). Thrombosis Risk Assessment as a Guide to Quality Patient Care. *Disease-a-Month*, *51*(2-3), 70–78. http://doi.org/10.1016/j.disamonth.2005.02.003

Caprini, J. A., Arcelus, J. I., & Reyna, J. J. (2001). Effective risk stratification of surgical and nonsurgical patients for venous thromboembolic disease. *Seminars in Hematology*, *38*(2 Suppl 5), 12–19.

Castillo, R. C., Scharfstein, D. O., & MacKenzie, E. J. (2012). Observational studies in the era of randomized trials: finding the balance. *The Journal of Bone and Joint Surgery (American)*, *94 Suppl 1*, 112–117. http://doi.org/10.2106/JBJS.L.00242

Cecchi, R., Lazzaro, A., Catanese, M., Mandarelli, G., & Ferracuti, S. (2012). Fatal thromboembolism following physical restraint in a patient with schizophrenia. *International Journal of Legal Medicine*, *126*(3), 477–482. http://doi.org/10.1007/s00414-012-0670-1

Ceroni, D., Martin, X. E., Delhumeau, C., Farpour-Lambert, N. J., De Coulon, G., Dubois-Ferrière, V., & Rizzoli, R. (2013). Recovery of Decreased Bone Mineral Mass After Lower-Limb Fractures in Adolescents. *The Journal of Bone and Joint Surgery (American)*, *95*(11), 1037. http://doi.org/10.2106/JBJS.L.00809

Ceroni, D., Martin, X., Delhumeau, C., Rizzoli, R., Kaelin, A., & Farpour-Lambert, N. (2012a). Effects of Cast-Mediated Immobilization on Bone Mineral Mass at Various Sites in Adolescents with Lower-Extremity Fracture. *The Journal of Bone and Joint Surgery (American)*, *94*(3). http://doi.org/10.2106/JBJS.K.00420

Ceroni, D., Martin, X., Lamah, L., Delhumeau, C., Farpour-Lambert, N., De Coulon, G., & Ferrière, V. D. (2012b). Recovery of physical activity levels in adolescents after lower limb fractures: a longitudinal, accelerometry-based activity monitor study. *???*, *13*(1), 1–1. http://doi.org/10.1186/1471-2474-13-131

Chang, H.-C. L., Burbridge, H., & Wong, C. (2013). Extensive deep vein thrombosis following prolonged gaming ('gamer's thrombosis'): a case report. *Journal of Medical Case Reports*, *7*(1), 1–1. http://doi.org/10.1186/1752-1947-7-235

Chen, L., & Soares, D. (2006). Fatal pulmonary embolism following ankle fracture in a 17-year-old girl. *The Journal of Bone and Joint Surgery. British Volume*, *88*(3), 400–401. http://doi.org/10.1302/0301-620X.88B3.17152

Cheng, K., Giebaly, D., Campbell, A., Rumley, A., & Lowe, G. (2014). Systemic effects of polymethylmethycrylate in total knee replacement: A prospective case-control study. *Bone & Joint Research*, *3*(4), 108–116. http://doi.org/10.1302/2046-3758.34.2000230

Chouhan, V. D., Comerota, A. J., Sun, L., Harada, R., Gaughan, J. P., & Rao, A. K. (1999). Inhibition of tissue factor pathway during intermittent pneumatic compression: A possible mechanism for antithrombotic effect. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *19*(11), 2812–2817.

Chow, V., Ng, A. C. C., Seccombe, L., Chung, T., Thomas, L., Celermajer, D. S., et al. (2014). Impaired 6-min walk test, heart rate recovery and cardiac function post pulmonary embolism in long-term survivors. *Respiratory Medicine*, *108*(10), 1556–1565. http://doi.org/10.1016/j.rmed.2014.08.002

Clemente, F. M., Nikolaidis, P. T., Martins, F. M. L., & Mendes, R. S. (2016). Physical Activity Patterns in University Students: Do They Follow the Public Health Guidelines? *PLoS ONE*, *11*(3), e0152516. http://doi.org/10.1371/journal.pone.0152516

Coleman, D. M., & Wakefield, T. W. (2012). Biomarkers for the diagnosis of deep vein thrombosis. *Expert Opinion on Medical Diagnostics*, *6*(4), 253–257. http://doi.org/10.1517/17530059.2012.692674

Corley, G. J., Birlea, S. I., Breen, P., & Olaighin, G. (2009). Popliteal blood flow and plantar flexion force due to neuromuscular electrical stimulation (NMES) of the calf muscle pump are strongly associated with NMES intensity. *Conference Proceedings : ... Annual International Conference of the IEEE Engineering in Medicine and Biology Society. IEEE Engineering in Medicine and Biology Society. Conference*, *2009*, 3051–3054. http://doi.org/10.1109/IEMBS.2009.5333580

Craik, J. D., Clark, A., Hendry, J., Sott, A. H., & Hamilton, P. D. (2015). The effect of ankle joint immobilization on lower limb venous flow. *Foot & Ankle International*, *36*(1), 18–23. http://doi.org/10.1177/1071100714552823

Cranley, J. J., Canos, A. J., & Sull, W. J. (1976). The diagnosis of deep venous thrombosis. Fallibility of clinical symptoms and signs. *Archives of Surgery (Chicago, Ill. : 1960)*, *111*(1), 34–36.

Crowther, M. A., Cook, D. J., Griffith, L. E., Meade, M., Hanna, S., Rabbat, C., et al. (2004). Neither baseline tests of molecular hypercoagulability nor D-dimer levels predict deep venous thrombosis in critically ill medical-surgical patients. *Intensive Care Medicine*, *31*(1), 48–55. http://doi.org/10.1007/s00134-004-2467-2

Davenport, S. J., Arnold, M., Hua, C., Schenck, A., Batten, S., & Taylor, N. F. (2015). Physical Activity Levels During Acute Inpatient Admission After Hip Fracture are Very Low. *Physiotherapy Research International : the Journal for Researchers and Clinicians in Physical Therapy*, *20*(3), 174–181. http://doi.org/10.1002/pri.1616

Day, S. M., Reeve, J. L., Pedersen, B., Farris, D. M., Myers, D. D., Im, M., et al. (2005). Macrovascular thrombosis is driven by tissue factor derived primarily from the blood vessel wall. *Blood*, *105*(1), 192–198. http://doi.org/10.1182/blood-2004-06-2225

De Bastos, M., Stegeman, B. H., Rosendaal, F. R., Van Hylckama Vlieg, A., Helmerhorst, F. M., Stijnen, T., & Dekkers, O. M. (2014). Combined oral contraceptives: venous thrombosis. *The Cochrane Database of Systematic Reviews*, (3), CD010813. http://doi.org/10.1002/14651858.CD010813.pub2

Decramer, A., Lowyck, H., & Demuynck, M. (2008). Parameters influencing thromboprophylaxis management of a lower leg trauma treated with a cast/splint. *Acta Orthopaedica Belgica*, *74*(5), 672–677.

Delis, K. T., Slimani, G., Hafez, H. M., & Nicolaides, A. N. (2000). Enhancing venous outflow in the lower limb with intermittent pneumatic compression. A comparative haemodynamic analysis on the effect of foot vs. calf vs. foot and calf compression. *European Journal of Vascular & Endovascular Surgery*, *19*(3), 250–260. http://doi.org/10.1053/ejvs.1999.1048

Dennis, M., Sandercock, P., Reid, J., Graham, C., Forbes, J., & Murray, G. (2013). Effectiveness of intermittent pneumatic compression in reduction of risk of deep vein thrombosis in patients who have had a stroke (CLOTS 3): a multicentre randomised controlled trial. *Lancet*, *382*(9891), 516–524. http://doi.org/10.1016/S0140-6736(13)61050-8

Desveaux, L., Goldstein, R. S., Mathur, S., Hassan, A., Devlin, M., Pauley, T., & Brooks, D. (2016). Physical Activity in Adults with Diabetes Following Prosthetic Rehabilitation. *Canadian Journal of Diabetes*, *40*(4), 336–341. http://doi.org/10.1016/j.jcjd.2016.02.003

Dieu, O., Mikulovic, J., Fardy, P. S., Bui-Xuan, G., Béghin, L., & Vanhelst, J. (2016). Physical activity using wrist-worn accelerometers: comparison of dominant and non-dominant wrist. *Clinical Physiology and Functional Imaging*, n/a–n/a. http://doi.org/10.1111/cpf.12337

Domeij-Arverud, E., Latifi, A., Labruto, F., Nilsson, G., & Ackermann, P. W. (2013). Can foot compression under a plaster cast prevent deep-vein thrombosis during lower limb immobilisation? *The Bone & Joint Journal*, *95-B*(9), 1227–1231. http://doi.org/10.1302/0301-620X.95B9.31162

Eekhoff, E. M., Rosendaal, F. R., & Vandenbroucke, J. P. (2000). Minor events and the risk of deep venous thrombosis. *Thrombosis and Haemostasis*, *83*(3), 408–411.

Egger, M., Davey Smith, G., Schneider, M., & Minder, C. (1997). Bias in meta-analysis detected by a simple, graphical test. *Bmj*, *315*(7109), 629–634.

Eisenberger, A., & Westhoff, C. (2014). Hormone replacement therapy and venous thromboembolism. *The Journal of Steroid Biochemistry and Molecular Biology*, *142*, 76–82. http://doi.org/10.1016/j.jsbmb.2013.08.016

Elpern, E., Killeen, K., Patel, G., & Senecal, P. A. (2013). The application of intermittent pneumatic compression devices for thromboprophylaxis: AN observational study found frequent errors in the application of these mechanical devices in ICUs. *The American Journal of Nursing*, *113*(4), 30–6– quiz 37. http://doi.org/10.1097/01.NAJ.0000428736.48428.10

Elsner, A., Schiffer, G., Jubel, A., Koebke, J., & Andermahr, J. (2007). The venous pump of the first metatarsophalangeal joint: clinical implications. *Foot & Ankle International*, *28*(8), 902–909. http://doi.org/10.3113/FAI.2007.0902

Emed, J., Mossison, D. R., Rosiers, Des, L., & Kahn, S. R. (2010). Definition of immobility in studies of thromboprophylaxis in hospitalized medical patients: A systematic review. *Journal of Vascular Nursing*, *28*(2), 54–66. http://doi.org/10.1016/j.jvn.2009.12.003

Ettema, H. B., Kollen, B. J., Verheyen, C. C. P. M., & Buller, H. R. (2008). Prevention of venous thromboembolism in patients with immobilization of the lower extremities: a meta-analysis of randomized controlled trials. *Journal of Thrombosis and Haemostasis*, *6*(7), 1093–1098. http://doi.org/10.1111/j.1538-7836.2008.02984.x

Exter, Den, P. L., Van der Hulle, T., Lankeit, M., Huisman, M. V., & Klok, F. A. (2013). Long-term clinical course of acute pulmonary embolism. *Blood Reviews*, *27*(4), 185–192. http://doi.org/10.1016/j.blre.2013.06.003

Fennell, C., Kobak, M., Glickman, E., & Barkley, J. (2016). Validation of a Low-cost Commercially-available Accelerometer During Low and High Physical Activity Conditions in Children. *Medicine and Science in Sports and Exercise*, 778.

Ferguson, T., Rowlands, A. V., Olds, T., & Maher, C. (2015). The validity of consumer-level, activity monitors in healthy adults worn in free-living conditions: a cross-sectional study. *The International Journal of Behavioral Nutrition and Physical Activity*, *12*(1), 42. http://doi.org/10.1186/s12966-015-0201-9

Fleming, P., Fitzgerald, P., Devitt, A., Rice, J., & Murray, P. (2000). The effect of the position of the limb on venous impulse foot pumps. *The Journal of Bone and Joint Surgery. British Volume*, *82*(3), 433–434.

Foster, R. C., Lanningham-Foster, L. M., Manohar, C., McCrady, S. K., Nysse, L. J., Kaufman, K. R., et al. (2005). Precision and accuracy of an ankle-worn accelerometer-based pedometer in step counting and energy expenditure. *Preventive Medicine*, *41*(3-4), 778–783. http://doi.org/10.1016/j.ypmed.2005.07.006

Friedman, A. M., Ananth, C. V., Lu, Y.-S., D'Alton, M. E., & Wright, J. D. (2013). Underuse of Postcesarean Thromboembolism Prophylaxis. *Obstetrics & Gynecology*, *122*(6), 1197–1204. http://doi.org/10.1097/AOG.0000000000000007

Fuchs, S., Heyse, T., Rudofsky, G., Gosheger, G., & Chylarecki, C. (2005). Continuous passive motion in the prevention of deep-vein thrombosis: a randomised comparison in trauma patients. *The Journal of Bone and Joint Surgery. British Volume*, *87*(8), 1117–1122. http://doi.org/10.1302/0301-620X.87B8.15680

Fujisawa, M., Naito, M., Asayama, I., Kambe, T., & Koga, K. (2003). Effect of calf-thigh intermittent pneumatic compression device after total hip arthroplasty: comparative analysis with plantar compression on the effectiveness of reducing thrombogenesis and leg swelling. *Journal of Orthopaedic Science*, *8*(6), 807–811. http://doi.org/10.1007/s00776-003-0706-y

Galanaud, J.-P., Sevestre-Pietri, M.-A., Bosson, J.-L., Laroche, J.-P., Righini, M., Brisot, D., et al. (2009). Comparative study on risk factors and early outcome of symptomatic distal versus proximal deep vein thrombosis: results from the OPTIMEV study. *Thrombosis and Haemostasis*, *102*(3), 493–500. http://doi.org/10.1160/TH09-01-0053

Gardner, A. M., & Fox, R. H. (1983). The venous pump of the human foot--preliminary report. *Bristol Medico-Chirurgical Journal (1963)*, *98*(367), 109–112.

Gardner, A. M., Fox, R. H., Lawrence, C., Bunker, T. D., Ling, R. S., & MacEachern, A. G. (1990). Reduction of post-traumatic swelling and compartment pressure by impulse compression of the foot. *The Journal of Bone and Joint Surgery. British Volume*, *72*(5), 810–815. http://doi.org/10.1136/emermed-2012-201302.3

Garino, J. P., Lotke, P. A., Kitziger, K. J., & Steinberg, M. E. (1996). Deep venous thrombosis after total joint arthroplasty. The role of compression ultrasonography and the importance of the experience of the technician. *The Journal of Bone and Joint Surgery (American)*, *78*(9), 1359–1365.

Geersing, G. J., Toll, D. B., Janssen, K. J. M., Oudega, R., Blikman, M. J. C., Wijland, R., et al. (2010). Diagnostic Accuracy and User-Friendliness of 5 Point-of-Care D-Dimer Tests for the Exclusion of Deep Vein Thrombosis. *Clinical Chemistry*, *56*(11), 1758–1766. http://doi.org/10.1373/clinchem.2010.147892

Ghanima, W., Wik, H. S., Tavoly, M., Enden, T., & Jelsness-Jørgensen, L.-P. (2017). Late consequences of venous thromboembolism: Measuring quality of life after deep vein thrombosis and pulmonary embolism. *Thrombosis Research*. http://doi.org/10.1016/j.thromres.2017.07.025

Gibbs, N. M. (1957). Venous thrombosis of the lower limbs with particular reference to bed-rest. *The British Journal of Surgery*, *45*(191), 209–236.

Girard, T. J., & Nicholson, N. S. (2001). The role of tissue factor/factor VIIa in the pathophysiology of acute thrombotic formation. *Current Opinion in Pharmacology*, *1*(2), 159–163.

Goldhaber, S. Z., & Bounameaux, H. (2012). Pulmonary embolism and deep vein thrombosis. *Lancet*, *379*(9828), 1835–1846. http://doi.org/10.1016/S0140-6736(11)61904-1

Golomb, B. A., Chan, V. T., Denenberg, J. O., Koperski, S., & Criqui, M. H. (2014). Risk marker associations with venous thrombotic events: a cross-sectional analysis. *BMJ Open*, *4*(3), e003208–e003208. http://doi.org/10.1136/bmjopen-2013-003208

Griffin, M., Nicolaides, A. N., Bond, D., Geroulakos, G., & Kalodiki, E. (2010). The efficacy of a new stimulation technology to increase venous flow and prevent venous stasis. *European Journal of Vascular and Endovascular Surgery : the Official Journal of the European Society for Vascular Surgery*, *40*(6), 766–771. http://doi.org/10.1016/j.ejvs.2010.06.019

Grodstein, F., Stampfer, M. J., Goldhaber, S. Z., Manson, J. E., Colditz, G. A., Speizer, F. E., et al. (1996). Prospective study of exogenous hormones and risk of pulmonary embolism in women. *The Lancet*, *348*(9033), 983–987. http://doi.org/10.1016/S0140-6736(96)07308-4

Haenen, J. H., Janssen, M. C., van Langen, H., van Asten, W. N., Wollersheim, H., van 't Hof, M. A., et al. (1999). The postthrombotic syndrome in relation to venous hemodynamics, as measured by means of duplex scanning and strain-gauge plethysmography. *Ymva*, *29*(6), 1071–1076.

Hager, E. R., Treuth, M. S., Gormely, C., Epps, L., Snitker, S., & Black, M. M. (2015). Ankle Accelerometry for Assessing Physical Activity Among Adolescent Girls: Threshold Determination, Validity, Reliability, and Feasibility. *Research Quarterly for Exercise and Sport*, *86*(4), 397–405. http://doi.org/10.1080/02701367.2015.1063574

Halanski, M., & Noonan, K. J. (2008). Cast and splint immobilization: complications. *The Journal of the American Academy of Orthopaedic Surgeons*, *16*(1), 30–40.

Handoll, H. H., Farrar, M. J., McBirnie, J., Tytherleigh-Strong, G., Awal, K. A., Milne, A. A., & Gillespie, W. J. (2000). Heparin, low molecular weight heparin and physical methods for preventing deep vein thrombosis and pulmonary embolism following surgery for hip fractures. *The Cochrane Database of Systematic Reviews*, (2), CD000305. http://doi.org/10.1002/14651858.CD000305

Hanslow, S. S., Grujic, L., Slater, H. K., & Chen, D. (2006). Thromboembolic disease after foot and ankle surgery. *Foot & Ankle International*, *27*(9), 693–695. http://doi.org/10.1177/107110070602700907

Harding, P., Holland, A. E., Delany, C., & Hinman, R. S. (2014). Do activity levels increase after total hip and knee arthroplasty? *Clinical Orthopaedics and Related Research*, *472*(5), 1502–1511. http://doi.org/10.1007/s11999-013-3427-3

Harenberg, J., Kallenbach, B., Martin, U., Dempfle, C. E., Zimmermann, R., Kübler, W., & Heene, D. L. (1990). Randomized controlled study of heparin and low molecular weight heparin for prevention of deep-vein thrombosis in medical patients. *Thrombosis Research*, *59*(3), 639–650.

Harrison, C. L., Thompson, R. G., Teede, H. J., & Lombard, C. B. (2011). Measuring physical activity during pregnancy. *The International Journal of Behavioral Nutrition and Physical Activity*, *8*(1), 19. http://doi.org/10.1186/1479-5868-8-19

Harvey, C. V., & Runner, M. (2011). Venous Thromboembolism After Fibula Fracture. *Orthopaedic Nursing*, *30*(3), 182–191. http://doi.org/10.1097/NOR.0b013e318219ae94

Healy, B., Beasley, R., & Weatherall, M. (2010). Venous thromboembolism following prolonged cast immobilisation for injury to the tendo Achillis. *The Journal of Bone and Joint Surgery. British Volume*, *92*(5), 646–650. http://doi.org/10.1302/0301-620X.92B5.23241

Heit, J. A., Kobbervig, C. E., James, A. H., Petterson, T. M., Bailey, K. R., & Melton, L. J. (2005). Trends in the incidence of venous thromboembolism during pregnancy or postpartum: a 30-year population-based study. *Annals of Internal Medicine*, *143*(10), 697–706.

Herrmann, S. D., Barreira, T. V., Kang, M., & Ainsworth, B. E. (2014). Impact of accelerometer wear time on physical activity data: a NHANES semisimulation data approach. *British Journal of Sports Medicine*, *48*(3), 278–282. http://doi.org/10.1136/bjsports-2012-091410

Hickey, B. A., Cleves, A., Alikhan, R., Pugh, N., Nokes, L., & Perera, A. (2016a). The effect of active toe movement (AToM) on calf pump function and deep vein thrombosis in patients with acute foot and ankle trauma treated with cast – A prospective randomized study. *Foot and Ankle Surgery*, 1–6. http://doi.org/10.1016/j.fas.2016.04.007

Hickey, B. A., Cleves, A., Alikhan, R., Pugh, N., Nokes, L., & Perera, A. (2017). Can we use biomarkers of coagulation to predict which patients with foot and ankle injury will develop deep vein thrombosis? *Foot and Ankle Surgery*, 1–5. http://doi.org/10.1016/j.fas.2017.08.002

Hickey, B. A., Morgan, A., Pugh, N., & Perera, A. (2014). The effect of lower limb cast immobilization on calf muscle pump function: a simple strategy of exercises can maintain flow. *Foot & Ankle International*, *35*(5), 429–433. http://doi.org/10.1177/1071100714530884

Hickey, B. A., Watson, U., Cleves, A., Alikhan, R., Pugh, N., Nokes, L., & Perera, A. (2016b). Does thromboprophylaxis reduce symptomatic venous thromboembolism in patients with below knee cast treatment for foot and ankle trauma? A systematic review and meta-analysis. *Foot and Ankle Surgery*, 1–9. http://doi.org/10.1016/j.fas.2016.06.005

Higgins, J. P. T., Altman, D. G., Gøtzsche, P. C., Jüni, P., Moher, D., Oxman, A. D., et al. (2011). The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *Bmj*, *343*, d5928. http://doi.org/10.1136/bmj.d5928

Himber, J., Wohlgensinger, C., Roux, S., Damico, L. A., Fallon, J. T., Kirchhofer, D., et al. (2003). Inhibition of tissue factor limits the growth of venous thrombus in the rabbit. *Journal of Thrombosis and Haemostasis : JTH*, *1*(5), 889–895.

Ho, K. M., & Tan, J. A. (2013). Stratified meta-analysis of intermittent pneumatic compression of the lower limbs to prevent venous thromboembolism in hospitalized patients. *Circulation*, *128*(9), 1003–1020. http://doi.org/10.1161/CIRCULATIONAHA.113.002690

Holst, A. G., Jensen, G., & Prescott, E. (2010). Risk Factors for Venous Thromboembolism: Results From the Copenhagen City Heart Study. *Circulation*, *121*(17), 1896–1903. http://doi.org/10.1161/CIRCULATIONAHA.109.921460

Horner, D. (2011). Towards evidence-based emergency medicine: best BETs from the Manchester Royal Infirmary. Evidence exists to guide thromboembolic prophylaxis in ambulatory patients with temporary lower limb immobilisation. *Emergency Medicine Journal*, *28*(8), 718–720. http://doi.org/10.1136/emermed-2011-200396-3

Hortobágyi, T., Dempsey, L., Fraser, D., Zheng, D., Hamilton, G., Lambert, J., & Dohm, L. (2000). Changes in muscle strength, muscle fibre size and myofibrillar gene expression after immobilization and retraining in humans. *The Journal of Physiology*, *524 Pt 1*, 293–304.

Hostler, D., Callaway, C. W., Newman, D. H., & D'Cruz, B. (2007). Thrombin-Antithrombin Appearance in Out-of-Hospital Cardiac Arrest. *Prehospital Emergency Care*, *11*(1), 9–13. http://doi.org/10.1080/10903120601023420

Hou, H., Ge, Z., Ying, P., Dai, J., Shi, D., Xu, Z., et al. (2012). Biomarkers of deep venous thrombosis. *Journal of Thrombosis and Thrombolysis*, *34*(3), 335–346. http://doi.org/10.1007/s11239-012-0721-y

Houdijk, H., Appelman, F. M., Van Velzen, J. M., Van der Woude, L. H. V., & Van Bennekom, C. A. M. (2008). Validity of DynaPort GaitMonitor for assessment of spatiotemporal parameters in amputee gait. *Journal of Rehabilitation Research and Development*, *45*(9), 1335–1342. http://doi.org/10.1682/JRRD.2007.12.0209

Hull, R. D. (2013). Relevance of Immobility and Importance of Risk Assessment Management for Medically Ill Patients. *Clinical and Applied Thrombosis/Hemostasis*, *19*(3), 268–276. http://doi.org/10.1177/1076029612452781

Hvid, L. G., Ortenblad, N., Aagaard, P., Kjaer, M., & Suetta, C. (2011). Effects of ageing on single muscle fibre contractile function following short-term immobilisation. *The Journal of Physiology*, *589*(19), 4745–4757. http://doi.org/10.1113/jphysiol.2011.215434

Ingle, B. M., Hay, S. M., Bottjer, H. M., & Eastell, R. (1999). Changes in bone mass and bone turnover following ankle fracture. *Osteoporosis International : a Journal Established as Result of Cooperation Between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA*, *10*(5), 408–415. http://doi.org/10.1007/s001980050247

Inoue, K. (2006). Venous Thromboembolism in Earthquake Victims. *Disaster Management & Response*, *4*(1), 25–27. http://doi.org/10.1016/j.dmr.2005.09.001

Iorio, A., Kearon, C., Filippucci, E., Marcucci, M., Macura, A., Pengo, V., et al. (2010). Risk of recurrence after a first episode of symptomatic venous thromboembolism provoked by a transient risk factor: a systematic review. *Archives of Internal Medicine*, *170*(19), 1710–1716. http://doi.org/10.1001/archinternmed.2010.367

Isma, N., Svensson, P. J., Gottsäter, A., & Lindblad, B. (2009). Prospective analysis of risk factors and distribution of venous thromboembolism in the population-based Malmö Thrombophilia Study (MATS). *Thrombosis Research*, *124*(6), 663–666. http://doi.org/10.1016/j.thromres.2009.04.022

Izumi, M., Ikeuchi, M., Mitani, T., Taniguchi, S., & Tani, T. (2010). Prevention of venous stasis in the lower limb by transcutaneous electrical nerve stimulation. *European Journal of Vascular and Endovascular Surgery : the Official Journal of the European Society for Vascular Surgery*, *39*(5), 642–645. http://doi.org/10.1016/j.ejvs.2009.11.035

Jackson, E., Curtis, K. M., & Gaffield, M. E. (2011). Risk of venous thromboembolism during the postpartum period: a systematic review. *Obstetrics & Gynecology*, *117*(3), 691–703. http://doi.org/10.1097/AOG.0b013e31820ce2db

Jameson, S. S., Augustine, A., James, P., Serrano-Pedraza, I., Oliver, K., Townshend, D., & Reed, M. R. (2011). Venous thromboembolic events following foot and ankle surgery in the English National Health Service. *The Journal of Bone and Joint Surgery. British Volume*, *93*(4), 490–497. http://doi.org/10.1302/0301-620X.93B4.25731

Jameson, S. S., Bottle, A., Malviya, A., Muller, S. D., & Reed, M. R. (2010). The impact of national guidelines for the prophylaxis of venous thromboembolism on the complications of arthroplasty of the lower limb. *The Journal of Bone and Joint Surgery. British Volume*, *92*(1), 123–129. http://doi.org/10.1302/0301-620X.92B1.22751

Jameson, S. S., Rankin, K. S., Desira, N. L., James, P., Muller, S. D., Reed, M. R., & Rangan, A. (2014). Pulmonary embolism following ankle fractures treated without an operation - an analysis using National Health Service data. *Injury*, *45*(8), 1256–1261. http://doi.org/10.1016/j.injury.2014.05.009

Janssen, M. C., Wollersheim, H., Haenen, J. H., van Asten, W. N., & Thien, T. (1998). Deep venous thrombosis: a prospective 3-month follow-up using duplex scanning and strain-gauge plethysmography. *Clinical Science (London, England : 1979)*, *94*(6), 651–656.

Johnson, B. F., Manzo, R., Bergelin, R., & Strandness, D. E. (1997). Flow characteristics in the deep veins of the leg during quiet respiration and with compression of the plantar veins. *Journal of Vascular Investigation*, 80–86.

Johnson, G. J., Leis, L. A., & Bach, R. R. (2009). Tissue factor activity of blood mononuclear cells is increased after total knee arthroplasty. *Thrombosis and Haemostasis*, *102*(4), 728–734. http://doi.org/10.1160/TH09-04-0261

Jørgensen, P. S., Warming, T., Hansen, K., Paltved, C., Vibeke Berg, H., Jensen, R., et al. (2002). Low molecular weight heparin (Innohep) as thromboprophylaxis in outpatients with a plaster cast: a venografic controlled study. *Thrombosis Research*, *105*(6), 477–480.

Kahn, S. R. (2006). The post-thrombotic syndrome: progress and pitfalls. *British Journal of Haematology*, *134*(4), 357–365. http://doi.org/10.1111/j.1365-2141.2006.06200.x

Kahn, S. R., & Ginsberg, J. S. (2004). Relationship between deep venous thrombosis and the postthrombotic syndrome. *Archives of Internal Medicine*, *164*(1), 17–26. http://doi.org/10.1001/archinte.164.1.17

Kahn, S. R., Shrier, I., Julian, J. A., Ducruet, T., Arsenault, L., Miron, M.-J., et al. (2008). Determinants and time course of the postthrombotic syndrome after acute deep venous thrombosis. *Annals of Internal Medicine*, *149*(10), 698–707.

Kakkos, S. K., Warwick, D., Nicolaides, A. N., Stansby, G. P., & Tsolakis, I. A. (2012). Combined (mechanical and pharmacological) modalities for the prevention of venous thromboembolism in joint replacement surgery. *The Journal of Bone and Joint Surgery. British Volume*, *94*(6), 729–734. http://doi.org/10.1302/0301-620X.94B6.28128

Kaplan, R. E., Czyrny, J. J., Fung, T. S., Unsworth, J. D., & Hirsh, J. (2002). Electrical foot stimulation and implications for the prevention of venous thromboembolic disease. *Thrombosis and Haemostasis*, *88*(2), 200–204.

Kerr, R., Stirling, D., & Ludlam, C. A. (2001). Interleukin 6 and haemostasis. *British Journal of Haematology*, *115*(1), 3–12.

Khorana, A. A., Francis, C. W., Menzies, K. E., Wang, J. G., Hyrien, O., Hathcock, J., et al. (2008). Plasma tissue factor may be predictive of venous thromboembolism in pancreatic cancer. *Journal of Thrombosis and Haemostasis*, *6*(11), 1983–1985. http://doi.org/10.1111/j.1538-7836.2008.03156.x

Killewich, L. A., Sandager, G. P., Nguyen, A. H., Lilly, M. P., & Flinn, W. R. (1995). Venous hemodynamics during impulse foot pumping. *Ymva*, *22*(5), 598–605.

Kim, Y.-H., & Kim, J.-S. (2007). The 2007 John Charnley Award. Factors leading to low prevalence of DVT and pulmonary embolism after THA: analysis of genetic and prothrombotic factors. *Clinical Orthopaedics and Related Research*, *465*, 33–39. http://doi.org/10.1097/BLO.0b013e318156bfac

Kock, H. J., Schmit-Neuerburg, K. P., Hanke, J., Rudofsky, G., & Hirche, H. (1995). Thromboprophylaxis with low-molecular-weight heparin in outpatients with plaster-cast immobilisation of the leg. *The Lancet*, *346*(8973), 459–461.

Kretz, C. A., Vaezzadeh, N., & Gross, P. L. (2010). Tissue factor and thrombosis models. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *30*(5), 900–908. http://doi.org/10.1161/ATVBAHA.108.177477

Kügler, C., Strunk, M., & Rudofsky, G. (2001). Venous pressure dynamics of the healthy human leg. Role of muscle activity, joint mobility and anthropometric factors. *Journal of Vascular Research*, *38*(1), 20–29.

Lapidus, L. J., Ponzer, S., Elvin, A., Levander, C., Lärfars, G., Rosfors, S., & De Bri, E. (2007). Prolonged thromboprophylaxis with Dalteparin during immobilization after ankle fracture surgery: a randomized placebo-controlled, double-blind study. *Acta Orthopaedica*, *78*(4), 528–535. http://doi.org/10.1080/17453670710014185

Lassen, M. R., Borris, L. C., & Nakov, R. L. (2002). Use of the low-molecular-weight heparin reviparin to prevent deep-vein thrombosis after leg injury requiring immobilization. *The New England Journal of Medicine*, *347*(10), 726–730. http://doi.org/10.1056/NEJMoa011327

Le Gall, C., Jacques, E., Medjebeur, C., Darques, L., Briand, F., Haddad, J., & Bleichner, G. (2006). Low molecular weight heparin self-injection training: assessment of feasibility, tolerance and economic analysis in emergency departments. *European Journal of Emergency Medicine : Official Journal of the European Society for Emergency Medicine*, *13*(5), 264–269.

Le Masurier, G. C., & Tudor-Locke, C. (2003). Comparison of pedometer and accelerometer accuracy under controlled conditions. *Medicine and Science in Sports and Exercise*, *35*(5), 867–871. http://doi.org/10.1249/01.MSS.0000064996.63632.10

Le Sage, S., Marianne McGee BSc, M. A. C., & Jessica D Emed RN, B. N. M. A. (2014). Knowledge of Venous Thromboembolism (VTE) prevention among hospitalized patients. *Journal of Vascular Nursing*, 1–9. http://doi.org/10.1016/j.jvn.2008.09.005

Lehtonen, H., Järvinen, T. L. N., Honkonen, S., Nyman, M., Vihtonen, K., & Järvinen, M. (2003). Use of a cast compared with a functional ankle brace after operative treatment of an ankle fracture. A prospective, randomized study. *The Journal of Bone and Joint Surgery (American)*, *85-A*(2), 205–211.

Ley, K. (2003). The role of selectins in inflammation and disease. *Trends in Molecular Medicine*, *9*(6), 263–268. http://doi.org/10.1016/S1471-4914(03)00071-6

Lippi, G., & Franchini, M. (2008). Pathogenesis of venous thromboembolism: when the cup runneth over. *Seminars in Thrombosis and Hemostasis*, *34*(8), 747–761. http://doi.org/10.1055/s-0029-1145257

Lubberts, B., Paulino Pereira, N. R., Kabrhel, C., Kuter, D. J., & DiGiovanni, C. W. (2016). What is the effect of venous thromboembolism and related complications on patient reported health-related quality of life? A meta-analysis. *Thrombosis and Haemostasis*, *116*(3), 417–431. http://doi.org/10.1160/TH16-02-0152

Lützner, C., Beyer, F., Kirschner, S., & Lützner, J. (2016). How Much Improvement in Patient Activity Can Be Expected After TKA? *Orthopedics*, *39*(3 Suppl), S18–23. http://doi.org/10.3928/01477447-20160509-15

Mackman, N. (2006). Role of tissue factor in hemostasis and thrombosis. *Blood Cells, Molecules & Diseases*, *36*(2), 104–107. http://doi.org/10.1016/j.bcmd.2005.12.008

Maggio, A. B., Martin, X. E., & Ceroni, D. (2016). Activity-related energy expenditure during lower limb cast immobilization in children. *Journal of Pediatric Orthopedics. Part B*, 1. http://doi.org/10.1097/BPB.0000000000000389

Malone, M. D., Cisek, P. L., Comerota, A. J., Holland, B., & Eid, I. G. (1999). High-pressure, rapid-inflation pneumatic compression improves venous hemodynamics in healthy volunteers and patients who are post-thrombotic. *Ymva*, *29*(4), 593–599.

Manly, D. A., Boles, J., & Mackman, N. (2011). Role of tissue factor in venous thrombosis. *Annual Review of Physiology*, *73*, 515–525. http://doi.org/10.1146/annurev-physiol-042210-121137

Mantha, S., Karp, R., Raghavan, V., Terrin, N., Bauer, K. A., & Zwicker, J. I. (2012). Assessing the risk of venous thromboembolic events in women taking progestin-only contraception: a meta-analysis. *Bmj*, *345*, e4944.

Marjoribanks, J., Farquhar, C., Roberts, H., Lethaby, A., & Lee, J. (2017). Long-term hormone therapy for perimenopausal and postmenopausal women. *The Cochrane Database of Systematic Reviews*, *1*, CD004143. http://doi.org/10.1002/14651858.CD004143.pub5

Maroney, S. A., & Mast, A. E. (2015). New insights into the biology of tissue factor pathway inhibitor. *Journal of Thrombosis and Haemostasis*, *13 Suppl 1*(S1), S200–7. http://doi.org/10.1111/jth.12897

McNally, M. A., Cooke, E. A., & Mollan, R. A. (1997). The effect of active movement of the foot on venous blood flow after total hip replacement. *The Journal of Bone and Joint Surgery (American)*, *79*(8), 1198–1201.

Menakaya, C. U., Pennington, N., Muthukumar, N., Joel, J., Ramirez Jimenez, A. J., Shaw, C. J., & Mohsen, A. (2013). The cost of outpatient venous thromboembolism prophylaxis following lower limb injuries. *The Bone & Joint Journal*, *95-B*(5), 673–677. http://doi.org/10.1302/0301-620X.95B5.30555

Mendoza, A., Hickey, A., Young, M., & Freedson, P. (2015). The Relationship Between Intensity And Accelerometer Output From The Sqord, Zamzee, And Movband Activity Trackers. *Medicine and Science in Sports and Exercise*, *47*, 431. http://doi.org/10.1249/01.mss.0000477609.71863.35

Micheli, L. J. (1975). Thromboembolic complications of cast immobilization for injuries of the lower extremities. *Clinical Orthopaedics and Related Research*, (108), 191–195.

Michiels, J. J., Maasland, H., Moossdorff, W., Lao, M., Gadiseur, A., & Schroyens, W. (2015). Safe Exclusion of Deep Vein Thrombosis by a Rapid Sensitive ELISA D-dimer and Compression Ultrasonography in 1330 Outpatients With Suspected DVT. *Angiology*, 0003319715616007. http://doi.org/10.1177/0003319715616007

Milne, A. A., Stonebridge, P. A., Bradbury, A. W., & Ruckley, C. V. (1994). Venous function and clinical outcome following deep vein thrombosis. *The British Journal of Surgery*, *81*(6), 847–849. http://doi.org/10.1302/0301-620X.92B5

Mirkazemi, C., Bereznicki, L. R., & Peterson, G. M. (2012). Are the national orthopaedic thromboprophylaxis guidelines appropriate? *ANZ Journal of Surgery*, *82*(12), 913–917. http://doi.org/10.1111/j.1445-2197.2012.06203.x

Mont, M. A., Jacobs, J. J., Boggio, L. N., Bozic, K. J., Valle, Della, C. J., Goodman, S. B., et al. (2011, December). Preventing venous thromboembolic disease in patients undergoing elective hip and knee arthroplasty. *The Journal of the American Academy of Orthopaedic Surgeons.*

Montoye, A. H. K., Moore, R. W., Bowles, H. R., Korycinski, R., & Pfeiffer, K. A. (2016). Reporting accelerometer methods in physical activity intervention studies: a systematic review and recommendations for authors. *British Journal of Sports Medicine*, bjsports–2015–095947. http://doi.org/10.1136/bjsports-2015-095947

Morris, R. J., & Woodcock, J. P. (2004). Evidence-Based Compression. *Annals of Surgery*, *239*(2), 162–171. http://doi.org/10.1097/01.sla.0000109149.77194.6c

Morris, R. J., & Woodcock, J. P. (2010). Intermittent Pneumatic Compression or Graduated Compression Stockings for Deep Vein Thrombosis Prophylaxis? *Annals of Surgery*, *251*(3), 393–396. http://doi.org/10.1097/SLA.0b013e3181b5d61c

Mosevoll, K. A., Lindås, R., Tvedt, T. H. A., Bruserud, Ø., & Reikvam, H. (2015). Altered plasma levels of cytokines, soluble adhesion molecules and matrix metalloproteases in venous thrombosis. *Thrombosis Research*, *136*(1), 30–39. http://doi.org/10.1016/j.thromres.2015.04.002

Müller, I., Klocke, A., Alex, M., Kotzsch, M., Luther, T., Morgenstern, E., et al. (2003). Intravascular tissue factor initiates coagulation via circulating microvesicles and platelets. *FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology*, *17*(3), 476–478. http://doi.org/10.1096/fj.02-0574fje

National Clinical Guideline Centre – Acute and Chronic Conditions (UK). (2010). Venous Thromboembolism: Reducing the Risk of Venous Thromboembolism (Deep Vein Thrombosis and Pulmonary Embolism) in Patients Admitted to Hospital. London: Royal College of Physicians (UK).

Nesheiwat, F., & Sergi, A. R. (1996). Deep venous thrombosis and pulmonary embolism following cast immobilization of the lower extremity. *The Journal of Foot and Ankle Surgery : Official Publication of the American College of Foot and Ankle Surgeons*, *35*(6), 590–4–discussion 602. http://doi.org/10.1016/S1067-2516(96)80135-9

Newcombe, R. G. (1998). Interval estimation for the difference between independent proportions: comparison of eleven methods. *Statistics in Medicine*, *17*(8), 873–890. http://doi.org/10.1002/(SICI)1097-0258(19980430)17:8<873::AID-SIM779>3.0.CO;2-I

Newcombe, R. G. (2006). Confidence intervals for an effect size measure based on the Mann-Whitney statistic. Part 1: general issues and tail-area-based methods. *Statistics in Medicine*, *25*(4), 543–557. http://doi.org/10.1002/sim.2323

Nilsson-Helander, K., Thurin, A., Karlsson, J., & Eriksson, B. I. (2009). High incidence of deep venous thrombosis after Achilles tendon rupture: a prospective study. *Knee Surgery, Sports Traumatology, Arthroscopy*, *17*(10), 1234–1238. http://doi.org/10.1007/s00167-009-0727-y

Nizankowska-Mogilnicka, E., Adamek, L., Grzanka, P., Domagala, T. B., Sanak, M., Krzanowski, M., & Szczeklik, A. (2003). Genetic polymorphisms associated with acute pulmonary embolism and deep venous thrombosis. *European Respiratory Journal*, *21*(1), 25–30. http://doi.org/10.1183/09031936.03.00034302

Noboa, S., Le Gal, G., Lacut, K., Mercier, B., Leroyer, C., Nowak, E., et al. (2008). Family history as a risk factor for venous thromboembolism. *Thrombosis Research*, *122*(5), 624–629. http://doi.org/10.1016/j.thromres.2007.12.026

Nokes, T. J. C., & Keenan, J. (2009). Thromboprophylaxis in patients with lower limb immobilisation - review of current status. *British Journal of Haematology*, *146*(4), 361–368. http://doi.org/10.1111/j.1365-2141.2009.07737.x

O'Donovan, K. J., O'Keeffe, D. T., Grace, P. A., & Lyons, G. M. (2005). Accelerometer based calf muscle pump activity monitoring. *Medical Engineering & Physics*, *27*(8), 717–722. http://doi.org/10.1016/j.medengphy.2005.02.002

Oger, E. (2000). Incidence of venous thromboembolism: a community-based study in Western France. EPI-GETBP Study Group. Groupe d'Etude de la Thrombose de Bretagne Occidentale. *Thrombosis and Haemostasis*, *83*(5), 657–660.

Ozemek, C., Kirschner, M. M., Wilkerson, B. S., Byun, W., & Kaminsky, L. A. (2014). Intermonitor reliability of the GT3X+ accelerometer at hip, wrist and ankle sites during activities of daily living. *Physiological Measurement*, *35*(2), 129–138. http://doi.org/10.1088/0967-3334/35/2/129

Pannucci, C. J., Shanks, A., Moote, M. J., Bahl, V., Cederna, P. S., Naughton, N. N., et al. (2012). Identifying patients at high risk for venous thromboembolism requiring treatment after outpatient surgery. *Annals of Surgery*, *255*(6), 1093–1099. http://doi.org/10.1097/SLA.0b013e3182519ccf

Parsonage, I. (2009). Venous thromboembolism in patients with below-knee plaster casts. *Emergency Nurse : the Journal of the RCN Accident and Emergency Nursing Association*, *16*(10), 32–35. http://doi.org/10.7748/en2009.03.16.10.32.c6852

Parsons, N. R., Hiskens, R., Price, C. L., Achten, J., & Costa, M. L. (2011). A systematic survey of the quality of research reporting in general orthopaedic journals. *The Journal of Bone and Joint Surgery. British Volume*, *93*(9), 1154–1159. http://doi.org/10.1302/0301-620X.93B9.27193

Pascarella, L. (2014). Intermittent pneumatic compression is effective in reducing venous thromboembolism risk in hospitalised patients. *Evidence-Based Medicine*, *19*(3), 104. http://doi.org/10.1136/eb-2013-101583

Patil, S., Gandhi, J., Curzon, I., & Hui, A. C. W. (2007). Incidence of deep-vein thrombosis in patients with fractures of the ankle treated in a plaster cast. *The Journal of Bone and Joint Surgery. British Volume*, *89*(10), 1340–1343. http://doi.org/10.1302/0301-620X.89B10.19241

Pelet, S., Roger, M.-E., Belzile, E. L., & Bouchard, M. (2012). The incidence of thromboembolic events in surgically treated ankle fracture. *The Journal of Bone and Joint Surgery (American)*, *94*(6), 502–506. http://doi.org/10.2106/JBJS.J.01190

Perrier, A. (2006). Review: The Wells clinical prediction guide and D-dimer testing predict deep vein thrombosis. *Evidence-Based Medicine*, *11*(4), 119–119. http://doi.org/10.1136/ebm.11.4.119

Persson, A., & Wredmark, T. (1979). The treatment of total ruptures of the Achilles tendon by plaster immobilisation. *International Orthopaedics*, *3*(2), 149–152.

Philbrick, J. T., & Becker, D. M. (1988). Calf deep venous thrombosis. A wolf in sheep's clothing? *Archives of Internal Medicine*, *148*(10), 2131–2138.

Piazza, G. (2013). Venous Thromboembolism and Cancer. *Circulation*, *128*(24), 2614–2618. http://doi.org/10.1161/CIRCULATIONAHA.113.002702

Pomp, E. R., le Cessie, S., Rosendaal, F. R., & Doggen, C. J. M. (2007). Risk of venous thrombosis: obesity and its joint effect with oral contraceptive use and prothrombotic mutations. *British Journal of Haematology*, *139*(2), 289–296. http://doi.org/10.1111/j.1365-2141.2007.06780.x

Pottier, P., Hardouin, J. B., Lejeune, S., Jolliet, P., Gillet, B., & Planchon, B. (2009). Immobilization and the risk of venous thromboembolism. A meta-analysis on epidemiological studies. *Thrombosis Research*, *124*(4), 468–476. http://doi.org/10.1016/j.thromres.2009.05.006

Prandoni, P., & Kahn, S. R. (2009). Post-thrombotic syndrome: prevalence, prognostication and need for progress. *British Journal of Haematology*, *145*(3), 286–295. http://doi.org/10.1111/j.1365-2141.2009.07601.x

Psatha, M., Wu, Z., Gammie, F. M., Ratkevicius, A., Wackerhage, H., Lee, J. H., et al. (2011). A longitudinal MRI study of muscle atrophy during lower leg immobilization following ankle fracture. *Journal of Magnetic Resonance Imaging*, *35*(3), 686–695. http://doi.org/10.1002/jmri.22864

Rabinovich, A., Cohen, J. M., Cushman, M., Kahn, S. R., BioSOX Investigators. (2015). Association between inflammation biomarkers, anatomic extent of deep venous thrombosis, and venous symptoms after deep venous thrombosis. *Journal of Vascular Surgery: Venous and Lymphatic Disorders*, *3*(4), 347–353.e1. http://doi.org/10.1016/j.jvsv.2015.04.005

Rectenwald, J. E., Myers, D. D., Hawley, A. E., Longo, C., Henke, P. K., Guire, K. E., et al. (2005). D-dimer, P-selectin, and microparticles: novel markers to predict deep venous thrombosis. A pilot study. *Thrombosis and Haemostasis*, *94*(6), 1312–1317. http://doi.org/10.1160/TH05-06-0426

Ricci, M. A., Fisk, P., Knight, S., & Case, T. (1997). Hemodynamic evaluation of foot venous compression devices. *Ymva*, *26*(5), 803–808.

Rich, C., Cortina-Borja, M., Dezateux, C., Geraci, M., Sera, F., Calderwood, L., et al. (2013a). Predictors of non-response in a UK-wide cohort study of children's accelerometer-determined physical activity using postal methods. *BMJ Open*, *3*(3), e002290. http://doi.org/10.1136/bmjopen-2012-002290

Rich, C., Geraci, M., Griffiths, L., Sera, F., Dezateux, C., & Cortina-Borja, M. (2013b). Quality control methods in accelerometer data processing: defining minimum wear time. *PLoS ONE*, *8*(6), e67206. http://doi.org/10.1371/journal.pone.0067206

Riou, B., Rothmann, C., Lecoules, N., Bouvat, E., Bosson, J.-L., Ravaud, P., et al. (2007). Incidence and risk factors for venous thromboembolism in patients with nonsurgical isolated lower limb injuries. *The American Journal of Emergency Medicine*, *25*(5), 502–508. http://doi.org/10.1016/j.ajem.2006.09.012

Rispens, S. M., Pijnappels, M., van Schooten, K. S., Beek, P. J., Daffertshofer, A., & van Dieën, J. H. (2014). Consistency of gait characteristics as determined from acceleration data collected at different trunk locations. *Gait & Posture*, *40*(1), 187–192. http://doi.org/10.1016/j.gaitpost.2014.03.182

Roach, R. E. J., Lijfering, W. M., Helmerhorst, F. M., Cannegieter, S. C., Rosendaal, F. R., & van Hylckama Vlieg, A. (2013). The risk of venous thrombosis in women over 50 years old using oral contraception or postmenopausal hormone therapy. *Journal of Thrombosis and Haemostasis*, *11*(1), 124–131. http://doi.org/10.1111/jth.12060

Roberts, C. (2012). Towards evidence-based emergency medicine: best BETs from the Manchester Royal Infirmary. BET 3: thromboprophylaxis significantly reduces venous thromboembolism rate in ambulatory patients immobilised in below-knee plaster cast. *Emergency Medicine Journal*, *29*(5), 424–425. http://doi.org/10.1136/emermed-2012-201302.4

Roberts, L. N., & Arya, R. (2011). Deep vein thrombosis and pulmonary embolism: diagnosis, treatment and prevention. *Clinical Medicine (London, England)*, *11*(5), 465–466.

Roberts, L. N., Porter, G., Barker, R. D., Yorke, R., Bonner, L., Patel, R. K., & Arya, R. (2013). Comprehensive VTE Prevention Program Incorporating Mandatory Risk Assessment Reduces the Incidence of Hospital-Associated Thrombosis. *CHEST Journal*, *144*(4), 1276. http://doi.org/10.1378/chest.13-0267

Roderick, P., Ferris, G., Wilson, K., Halls, H., Jackson, D., Collins, R., & Baigent, C. (2005). Towards evidence-based guidelines for the prevention of venous thromboembolism: systematic reviews of mechanical methods, oral anticoagulation, dextran and regional anaesthesia as thromboprophylaxis. *Health Technology Assessment (Winchester, England)*, *9*(49), iii–iv– ix–x– 1–78.

Rogers, F. B., Hammaker, S. J., Miller, J. A., Lee, J. C., Chandler, R., Edavettal, M. M., et al. (2011). Does prehospital prolonged extrication (entrapment) place trauma patients at higher risk for venous thromboembolism? *The American Journal of Surgery*, *202*(4), 382–386. http://doi.org/10.1016/j.amjsurg.2010.10.013

Roumen-Klappe, E. M., Heijer, den, M., van Uum, S. H. M., van der Ven-Jongekrijg, J., van der Graaf, F., & Wollersheim, H. (2002). Inflammatory response in the acute phase of deep vein thrombosis. *Ymva*, *35*(4), 701–706.

Sabia, S., van Hees, V. T., Shipley, M. J., Trenell, M. I., Hagger-Johnson, G., Elbaz, A., et al. (2014). Association between questionnaire- and accelerometer-assessed physical activity: the role of sociodemographic factors. *American Journal of Epidemiology*, *179*(6), 781–790. http://doi.org/10.1093/aje/kwt330

Salzman, E. W., McManama, G. P., Shapiro, A. H., Robertson, L. K., Donovan, A. S., Blume, H. W., et al. (1987). Effect of optimization of hemodynamics on fibrinolytic activity and antithrombotic efficacy of external pneumatic calf compression. *Annals of Surgery*, *206*(5), 636–641.

Samuels, P. B., & Webster, D. R. (1952). The role of venous endothelium in the inception of thrombosis. *Annals of Surgery*, *136*(3), 422–438.

Schulman, S., Lindmarker, P., Holmström, M., Lärfars, G., Carlsson, A., Nicol, P., et al. (2006). Post-thrombotic syndrome, recurrence, and death 10 years after the first episode of venous thromboembolism treated with warfarin for 6 weeks or 6 months. *Journal of*

Thrombosis and Haemostasis : JTH, *4*(4), 734–742. http://doi.org/10.1111/j.1538-7836.2006.01795.x

Selby, R., Geerts, W. H., Kreder, H. J., Crowther, M. A., Kaus, L., & Sealey, F. (2014). Symptomatic venous thromboembolism uncommon without thromboprophylaxis after isolated lower-limb fracture: the knee-to-ankle fracture (KAF) cohort study. *The Journal of Bone and Joint Surgery (American)*, *96*(10), e83–e83. http://doi.org/10.2106/JBJS.M.00236

Selby, R., Geerts, W. H., Kreder, H. J., Crowther, M. A., Kaus, L., & Sealey, F. (2015). A double-blind, randomized controlled trial of the prevention of clinically important venous thromboembolism after isolated lower leg fractures. *Journal of Orthopaedic Trauma*, *29*(5), 224–230. http://doi.org/10.1097/BOT.0000000000000250

Sharma, R., Atkin, H., Mackillop, L., & Paterson-Brown, S. (2012). Assessment of the mobility of mothers postpartum to identify those at greatest risk of venous thromboembolism. *Journal of Obstetrics and Gynaecology : the Journal of the Institute of Obstetrics and Gynaecology*, *32*(5), 461–463. http://doi.org/10.3109/01443615.2012.676694

Silverstein, M. D., Heit, J. A., Mohr, D. N., Petterson, T. M., O'Fallon, W. M., & Melton, L. J. (1998). Trends in the incidence of deep vein thrombosis and pulmonary embolism: a 25-year population-based study. *Archives of Internal Medicine*, *158*(6), 585–593.

Sisson, S. B., Camhi, S. M., Church, T. S., Tudor-Locke, C., Johnson, W. D., & Katzmarzyk, P. T. (2010). Accelerometer-determined steps/day and metabolic syndrome. *American Journal of Preventive Medicine*, *38*(6), 575–582. http://doi.org/10.1016/j.amepre.2010.02.015

Skender, S., Ose, J., Chang-Claude, J., Paskow, M., Brühmann, B., Siegel, E. M., et al. (2016). Accelerometry and physical activity questionnaires - a systematic review. *BMC Public Health*, *16*(1), 515. http://doi.org/10.1186/s12889-016-3172-0

Slaybaugh, R. S., Beasley, B. D., & Massa, E. G. (2003). Deep venous thrombosis risk assessment, incidence, and prophylaxis in foot and ankle surgery. *Clinics in Podiatric Medicine and Surgery*, *20*(2), 269–289. http://doi.org/10.1016/S0891-8422(03)00007-7

Snijders, T., Wall, B. T., Dirks, M. L., Senden, J. M. G., Hartgens, F., Dolmans, J., et al. (2013). Muscle disuse atrophy is not accompanied by changes in skeletal muscle satellite cell content. *Clinical Science (London, England : 1979)*, *126*(8), 557–566. http://doi.org/10.1042/CS20130295

Solis, G., & Saxby, T. (2002). Incidence of DVT following surgery of the foot and ankle. *Foot & Ankle International*, *23*(5), 411–414. http://doi.org/10.1177/107110070202300507

Sonnevi, K., Bergendal, A., Adami, J., Lärfars, G., & Kieler, H. (2013). Self-reported family history in estimating the risk of hormone, surgery and cast related VTE in women. *Thrombosis Research*, *132*(2), 164–169. http://doi.org/10.1016/j.thromres.2013.06.003

Spannagel, U., & Kujath, P. (1993). Low molecular weight heparin for the prevention of thromboembolism in outpatients immobilized by plaster cast. *Seminars in Thrombosis and Hemostasis*, *19 Suppl 1*, 131–141.

Spyropoulos, A. C., Anderson, F. A., Fitzgerald, G., Decousus, H., Pini, M., Chong, B. H., et al. (2011). Predictive and associative models to identify hospitalized medical patients at risk for VTE. *CHEST Journal*, *140*(3), 706–714. http://doi.org/10.1378/chest.10-1944

Stanton, R., Guertler, D., Duncan, M. J., & Vandelanotte, C. (2016). Agreement between activPAL3c accelerometers placed at different thigh positions. *Gait & Posture*, *48*, 230–236. http://doi.org/10.1016/j.gaitpost.2016.06.013

Stegeman, B. H., de Bastos, M., Rosendaal, F. R., van Hylckama Vlieg, A., Helmerhorst, F. M., Stijnen, T., & Dekkers, O. M. (2013). Different combined oral contraceptives and the risk

of venous thrombosis: systematic review and network meta-analysis. *Bmj*, *347*, f5298.

Sudo, A., Wada, H., Nobori, T., Yamada, N., Ito, M., Niimi, R., et al. (2009). Cut-off values of D-dimer and soluble fibrin for prediction of deep vein thrombosis after orthopaedic surgery. *International Journal of Hematology*, *89*(5), 572–576. http://doi.org/10.1007/s12185-009-0323-4

Sugwara, J., Hayashi, K., Kaneko, F., Yamada, H., Kizuka, T., & Tanaka, H. (2004). Reductions in Basal Limb Blood Flow and Lumen Diameter after Short-Term Leg Casting. *Medicine and Science in Sports and Exercise*, *36*(10), 1689–1694. http://doi.org/10.1249/01.MSS.0000142410.45142.28

Sultan, M. J., Zhing, T., Morris, J., Kurdy, N., & McCollum, C. N. (2014). Compression stockings in the management of fractures of the ankle: a randomised controlled trial. *The Bone & Joint Journal*, *96-B*(8), 1062–1069. http://doi.org/10.1302/0301-620X.96B8.32941

Sweetland, S., Green, J., Liu, B., Berrington de González, A., Canonico, M., Reeves, G., et al. (2009). Duration and magnitude of the postoperative risk of venous thromboembolism in middle aged women: prospective cohort study. *Bmj*, *339*, b4583. http://doi.org/10.1136/bmj.b4583

Taraldsen, K., Askim, T., Sletvold, O., Einarsen, E. K., Gruner Bjastad, K., Indredavik, B., & Helbostad, J. L. (2011). Evaluation of a Body-Worn Sensor System to Measure Physical Activity in Older People With Impaired Function. *Physical Therapy*, *91*(2), 277–285. http://doi.org/10.2522/ptj.20100159

Taylor, K., Paul, D. R., Martin, A. K., Beitey, S. P., Croston, S. L., Goc Karp, G., et al. (2014). Physical Activity Measurement Validity of Motion Sensor Technologies in Laboratory Settings, *8*(2).

Tepper, N. K., Whiteman, M. K., Marchbanks, P. A., James, A. H., & Curtis, K. M. (2016). Progestin-only contraception and thromboembolism: A systematic review. *Contraception*, *94*(6), 678–700. http://doi.org/10.1016/j.contraception.2016.04.014

Testroote, M., Morrenhof, W., & Janzing, H. (2011a). Prevention of venous thromboembolism in patients with below-knee immobilisation of the leg--survey of current practice in The Netherlands. *Acta Chirurgica Belgica*, *111*(1), 32–35.

Testroote, M., Stigter, W. A. H., Janssen, L., & Janzing, H. M. J. (2014). Low molecular weight heparin for prevention of venous thromboembolism in patients with lower-leg immobilization. *The Cochrane Database of Systematic Reviews*, *4*, CD006681. http://doi.org/10.1002/14651858.CD006681.pub3

Testroote, M., Stigter, W., de Visser, D. C., & Janzing, H. (2011b). Low molecular weight heparin for prevention of venous thromboembolism in patients with lower-leg immobilization. *The Cochrane Database of Systematic Reviews*, (4), CD006681. http://doi.org/10.1002/14651858.CD006681.pub2

Thom, J. M., Thompson, M. W., Ruell, P. A., Bryant, G. J., Fonda, J. S., Harmer, A. R., et al. (2001). Effect of 10-day cast immobilization on sarcoplasmic reticulum calcium regulation in humans. *Acta Physiologica Scandinavica*, *172*(2), 141–147. http://doi.org/10.1046/j.1365-201X.2001.00853.x

Thomas, S., & Van Kampen, M. (2011). Should orthopedic outpatients with lower limb casts be given deep vein thrombosis prophylaxis? *Clinical and Applied Thrombosis/Hemostasis : Official Journal of the International Academy of Clinical and Applied Thrombosis/Hemostasis*, *17*(4), 405–407. http://doi.org/10.1177/1076029610371472

Tick, L., Doggen, C. J. M., Rosendaal, F. R., Faber, W. R., Bousema, M. T., Mackaay, A. J. C., et al. (2010). 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*, *8*(12), 2685–2692. http://doi.org/10.1111/j.1538-7836.2010.04065.x

Tick, L., Kramer, M. H. H., Rosendaal, F. R., Faber, W. R., & Doggen, C. J. M. (2008). Risk factors for post-thrombotic syndrome in patients with a first deep venous thrombosis. *Journal of Thrombosis and Haemostasis*, *6*(12), 2075–2081. http://doi.org/10.1111/j.1538-7836.2008.03180.x

Van Hylckama Vlieg, A., & Rosendaal, F. R. (2014). Risk of venous thrombosis associated with different types of combined oral contraceptive preparations. *Polskie Archiwum Medycyny Wewnetrznej*, *124*(3), 85–87.

Van Stralen, K. J., Rosendaal, F. R., & Doggen, C. J. M. (2008). Minor injuries as a risk factor for venous thrombosis. *Archives of Internal Medicine*, *168*(1), 21–26. http://doi.org/10.1001/archinternmed.2007.5

Vandenbroucke, J. P., Koster, T., Briët, E., Reitsma, P. H., Bertina, R. M., & Rosendaal, F. R. (1994). Increased risk of venous thrombosis in oral-contraceptive users who are carriers of factor V Leiden mutation. *The Lancet*, *344*(8935), 1453–1457.

Venkatachalam, S., & Wright, K. U. (2012). Fatal pulmonary embolism following conservatively managed tendo achilles rupture-A case report. *International Journal of Surgery Case Reports*, *3*(1), 3–5. http://doi.org/10.1016/j.ijscr.2011.09.005

Virchow, R. L. K. (1856). Thrombose und Embolie. Gefässentzündung und septische Infektion. *Gesammelte Abhandlungen Zur Wissenschaftlichen Medicin (in German). Frankfurt Am Main: Von Meidinger & Sohn. Pp. 219–732.Matzdorff AC, Bell WR (1998). Thrombosis and Embolie (1846-1856). Canton, Massachusetts: Science History Publications*, 219–732.

Wakefield, T. W., Myers, D. D., & Henke, P. K. (2008). Mechanisms of venous thrombosis and resolution. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *28*(3), 387–391. http://doi.org/10.1161/ATVBAHA.108.162289

Walenga, J. M., Kaiser, P. C., Prechel, M. M., Hoppensteadt, D., Jeske, W. P., Misselwitz, F., et al. (2014). Sustained release of tissue factor following thrombosis of lower limb trauma. *Clinical and Applied Thrombosis/Hemostasis : Official Journal of the International Academy of Clinical and Applied Thrombosis/Hemostasis*, *20*(7), 678–686. http://doi.org/10.1177/1076029614545212

Wallace, R. G. H., Heyes, G. J., & Michael, A. L. R. (2011). The non-operative functional management of patients with a rupture of the tendo Achillis leads to low rates of re-rupture. *The Journal of Bone and Joint Surgery. British Volume*, *93*(10), 1362–1366. http://doi.org/10.1302/0301-620X.93B10.26187

Warlow, C., Ogston, D., & Douglas, A. S. (1976). Deep venous thrombosis of the legs after strokes. Part I--incidence and predisposing factors. *British Medical Journal*, *1*(6019), 1178–1181.

Warwick, D. J., Shaikh, A., Gadola, S., Stokes, M., Worsley, P., Bain, D., et al. (2013). Neuromuscular electrostimulation viathe common peroneal nerve promotes lower limb blood flow in a below-kneecast: A potential for thromboprophylaxis. *Bone & Joint Research*, *2*(9), 179–185. http://doi.org/10.1302/2046-3758.29.2000176

Warwick, D., Harrison, J., Glew, D., Mitchelmore, A., Peters, T. J., & Donovan, J. (1998). Comparison of the use of a foot pump with the use of low-molecular-weight heparin for the prevention of deep-vein thrombosis after total hip replacement. A prospective, randomized trial. *The Journal of Bone and Joint Surgery (American)*, *80*(8), 1158–1166.

Wells, G. A., Cranney, A., Peterson, J., Boucher, M., Shea, B., Robinson, V., et al. (2008).

Alendronate for the primary and secondary prevention of osteoporotic fractures in postmenopausal women. *The Cochrane Database of Systematic Reviews*, (1), CD001155. http://doi.org/10.1002/14651858.CD001155.pub2

White, R. H. (2012). Identifying Risk Factors for Venous Thromboembolism. *Circulation*, *125*(17), 2051–2053. http://doi.org/10.1161/CIRCULATIONAHA.112.102814

Whitelaw, G. P., Oladipo, O. J., Shah, B. P., DeMuth, K. A., Coffman, J., & Segal, D. (2001). Evaluation of intermittent pneumatic compression devices. *Orthopedics*, *24*(3), 257–261.

Wille-Jørgensen, P., Jorgensen, L. N., & Crawford, M. (2005). Asymptomatic postoperative deep vein thrombosis and the development of postthrombotic syndrome. A systematic review and meta-analysis. *Thrombosis and Haemostasis*, *93*(2), 236–241. http://doi.org/10.1267/THRO05020236

Williamson, M., Rebold, M., Carnes, A., Glickman, E., & Barkley, J. (2014). *Validity of a Novel low-cost accelerometer during free-living physical activity*. *American College of Sports Medicine Annual Meeting, Orlando, Florida* (p. 126).

Wilson, D., Cooke, E. A., McNally, M. A., Wilson, H. K., Yeates, A., & Mollan, R. A. B. (2002). Altered venous function and deep venous thrombosis following proximal femoral fracture. *Injury*, *33*(1), 33–39.

Wilson, E. (2007). Preventing deaths from VTE in hospital 2: thromboprophylaxis. *Nursing Times*, *103*(38), 26–27.

Wolff-Hughes, D. L., McClain, J. J., Dodd, K. W., Berrigan, D., & Troiano, R. P. (2016). Number of accelerometer monitoring days needed for stable group-level estimates of activity. *Physiological Measurement*, *37*(9), 1447–1455. http://doi.org/10.1088/0967-3334/37/9/1447

Wukich, D. K., & Waters, D. H. (2008). Thromboembolism Following Foot and Ankle Surgery: A Case Series and Literature Review. *The Journal of Foot and Ankle Surgery*, *47*(3), 243–249. http://doi.org/10.1053/j.jfas.2008.02.003

Yang, H., Zhao, F., Li, Y., Xu, M., Li, L., Wu, C., et al. (2013). VCAM-1-targeted core/shell nanoparticles for selective adhesion and delivery to endothelial cells with lipopolysaccharide-induced inflammation under shear flow and cellular magnetic resonance imaging in vitro. *International Journal of Nanomedicine*, *8*, 1897–1906. http://doi.org/10.2147/IJN.S44997

Yoo, M.-C., Cho, Y.-J., Ghanem, E., Ramteke, A., & Kim, K.-I. (2009). Deep vein thrombosis after total hip arthroplasty in Korean patients and D-dimer as a screening tool. *Archives of Orthopaedic and Trauma Surgery*, *129*(7), 887–894. http://doi.org/10.1007/s00402-008-0751-2

Yoshioka, K., Kitajima, I., Kabata, T., Tani, M., Kawahara, N., Murakami, H., et al. (2010). Venous thromboembolism after spine surgery: changes of the fibrin monomer complex and D-dimer level during the perioperative period. *Journal of Neurosurgery: Spine*, *13*(5), 594–599. http://doi.org/10.3171/2010.5.SPINE09883

Zee, A. A., van Lieshout, K., van der Heide, M., Janssen, L., & Janzing, H. M. (2017). Low molecular weight heparin for prevention of venous thromboembolism in patients with lower-limb immobilization. *The Cochrane Database of Systematic Reviews*, *8*(Suppl 1), CD006681. http://doi.org/10.1002/14651858.CD006681.pub4

Appendices

Appendix 1 Papers published

Hickey, B. A., Morgan, A., Pugh, N., & Perera, A. (2014). The effect of lower limb cast immobilization on calf muscle pump function: a simple strategy of exercises can maintain flow. *Foot & Ankle International*, *35*(5), 429–433. http://doi.org/10.1177/1071100714530884

Hickey, B. A., Cleves, A., Alikhan, R., Pugh, N., Nokes, L., & Perera, A. (2016). The effect of active toe movement (AToM) on calf pump function and deep vein thrombosis in patients with acute foot and ankle trauma treated with cast – A prospective randomized study. *Foot and Ankle Surgery*, 1–6. http://doi.org/10.1016/j.fas.2016.04.007

Hickey, B. A., Watson, U., Cleves, A., Alikhan, R., Pugh, N., Nokes, L., & Perera, A. (2016). Does thromboprophylaxis reduce symptomatic venous thromboembolism in patients with below knee cast treatment for foot and ankle trauma? A systematic review and meta-analysis. *Foot and Ankle Surgery*. http://dx.doi.org/10.1016/j.fas.2016.06.005

Hickey, B. A., Cleves, A., Alikhan, R., Pugh, N., Nokes, L., & Perera, A. (2017). Can we use biomarkers of coagulation to predict which patients with foot and ankle injury will develop deep vein thrombosis. *Foot and Ankle Surgery*. https://doi.org/10.1016/j.fas.2017.08.002