Universidade de Lisboa
Faculdade de Motricidade Humana

MANUAL LYMPHATIC DRAINAGE IN CHRONIC VENOUS DISEASE

Tese elaborada com vista à obtenção do Grau de Doutor em
Motricidade Humana na especialidade de Reabilitação

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Rute Sofia dos Santos Crisóstomo

Setembro 2014

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DEDICATORY

This work is dedicated to my family: Bárbara, António, Pedro, Liliana, Paulo, Ema, Joaquina and Joaquim.

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ABSTRACT

Title: Manual lymphatic drainage in chronic venous disease

Objective: To assess the efficacy of manual lymphatic drainage (MLD) in the treatment of patients with chronic venous disease (CVD).

Design: Three cross-sectional studies and one single-blind randomized controlled trial, were performed.

Methods: A total of 108 participants with CVD and 62 healthy participants were assessed in four studies. The first study assessed calf muscle pump function (CMPF) and architecture of gastrocnemius muscles by ultrasound in CVD and healthy participants; the second and third studies were performed with duplex ultrasound to assess venous hemodynamics during MLD; the fourth study, a randomized controlled study, assessed for efficacy of the MLD in CVD management.

Results: Ultrasound measures demonstrate changes in CMPF efficacy along a series of contractions as well as between CVD and healthy participants, although the method suffers from bias. MLD maneuvers increase superficial and deep venous flow, mostly when applied along the anatomical course of the major lower limb veins, but without differences between different MLD maneuvers. MLD decreases the symptoms and clinical severity (related to venous edema) of CVD, and improve dimension of pain of health-related quality of life in this condition, after four weeks of treatment and the effect is maintained after 4 weeks of follow up.

Conclusions: MLD applied with skin-stretching along the course of venous vessels increases venous return, and may be used as a conservative coadjutant option to treat patients with CVD.

Key Words: Chronic venous disease; manual lymphatic drainage; venous flow; health-related quality of life; calf muscle pump function.

RESUMO

Título: Drenagem linfática manual na doença venosa crónica

Objetivo: Avaliar a eficácia da drenagem linfática manual (DLM) no tratamento de utentes com doença venosa crónica (DVC).

Desenho do estudo: Foram realizados três estudos transversais e um estudo prospetivo, controlado e com ocultação simples.

Metodologia: Nos 4 estudos foram avaliados 108 participantes com DVC e 62 participantes saudáveis. No primeiro estudo foi avaliada a bomba muscular venosa da perna (BMVP) e a arquitetura dos músculos gémeos por ultrassonografia, em participantes com DVC e saudáveis. No segundo e terceiro estudos foram avaliadas por ultrassonografia vascular as variações hemodinâmicas venosas durante a DLM. No quarto estudo, o estudo prospetivo, avaliou-se a eficácia da DLM no tratamento de doentes com DVC.

Resultados: A avaliação por ultrassonografia identificou alterações na eficácia da BMVP durante uma série de 10 contrações nos participantes com DVC e nos participantes saudáveis, contudo, este método apresentou uma fiabilidade pobre. As manobras de DLM aumentaram o fluxo venoso, sobretudo quando aplicadas na localização anatómica das principais veias do membro inferior. A DLM aliviou sintomas e reduziu a dimensão da dor da qualidade de vida relacionada com a saúde e severidade clinica da DVC (sobretudo edema) no fim de 4 semanas de tratamento. Parte destes resultados mantiveram-se após 4 semanas de follow-up.

Conclusão: Tracionar a pele durante a DLM, ao longo do percurso das veias, aumenta o retorno venoso e revela-se como uma potencial estratégia conservadora e coadjuvante no tratamento de doentes com DVC.

Palavras-chave: Doença venosa crónica; drenagem linfática manual; fluxo venoso; qualidade de vida relacionada com a saúde; bomba muscular venosa da perna.

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LIST OF ABBREVIATORS

CEAP - Clinical Etiological Anatomical Classification

CIVIQ - Chronic Venous Insufficiency Questionnaire

CMPF - Calf Muscle Pump Function

CVD - Chronic Venous Disease

FV - Femoral Vein

GSV - Great Saphenous Vein

HRQL - Health-related quality of life

IC - Interval of Confidence

ICC - Intraclass Correlation Coefficient

MLD - Manual Lymphatic Drainage

PV - Popliteal Vein

SSV - Small Saphenous Vein

VCSS - Venous Clinical Severity Score

1 INTRODUCTION

In Portugal the prevalence of chronic venous disease (CVD) in males is 17.8% and in females is 34.1%, i.e., around 2.5 million cases, with around 70.000 new cases every year (Capitão et al., 1995).

Chronic venous insufficiency represents the most severe cases of CVD, and is related to a physiopathology that associates venous hypertension, vein valve damage, venous obstruction, calf muscle pump impairment, inflammations of tissues (skin, subcutaneous tissue and muscle) and veins, alterations of morphology and function of veins, with abnormal venous reflux, venous edema, changes of the skin and subcutaneous tissues, with ulcer representing the more severe stage of this condition (Eklof et al., 2004; Meissner et al., 2007b; Panny et al., 2009; Qiao et al., 2005; Rowland, 2001; Simka, 2007; Yamaki et al., 2010). It is estimated that 100.000 Portuguese citizens present an open (or active) venous ulcer (Oliveira et al., 2003), and 280.000 an open or healed venous ulcer, with 10% of healed ulcer at risk for recurrence (Bradbury, 2010; Pina et al., 2005).

The first problem referred from patients are the symptoms that, together with varicose veins, is present form mild to severe cases of CVD, strongly contributing to a the negative self-esteem that also characterizes this disease (Boisseau, 2007; Bradbury et al., 1999; Campbell et al., 2007; Cesarone et al., 2010; Darvall et al., 2012; Eklof et al., 2009; Koupidis et al., 2008). The diminished health-related quality of life (HRQL) of this condition in these patients, with functional impairments, reflected in their daily activities are well studied (Andreozzi et al., 2005; Darvall et al., 2012; Furtado et al., 2008; Koupidis et al., 2008). This negative impact is so

important that the previous view of this condition as an aesthetic problem has been abandoned for years.

The socioeconomic burden of this condition is very high. The indirect costs are substantial and are associated with the symptoms, functional impairment, emotional disturbances and negative impact in HRQL (Lamping et al., 2003). The direct costs of CVD treatment are almost entirely related to its high prevalence, morbidity, and chronicity (Labropoulos et al., 2009; Lamping et al., 2003). In developed countries, around 1-3% of the heath costs are due to CVD (Eklof et al., 2004). However, when patients with less severe stages of the disease are diagnosed and treated early, the physiopathology course of the disease can be prevented or even receded (Nullen, 2010).

Manual lymphatic drainage (MLD) is a low pressure form of skin-stretching massage, described as a conservative treatment option for the treatment of patients with CVD (Steins & Jünger, 2000) as a coadjutant of other treatments, like stockings and surgery for example (Molski et al., 2013; Molski et al., 2009). It is assumed that this technique may have an effect on superficial venous flow, when a special maneuver the so-called call-up maneuver from the Leduc method (Leduc & Leduc, 2000) is applied, although to a limited extent (Leduc et al., 2011).

This technique has been proposed as an option in the treatment of venous lymphedema associated to CVD (Mortimer, 2000; Raju et al., 2012). It is suggested by the literature that this technique should be applied in the course of the great saphenous vein (GSV) to treat patients with CVD, but the capacity of MLD to improve venous flow has been poorly studied (Leduc et al., 2000; Molski et al., 2009; Peyre & Robert, 2000). Despite controversial evidence regarding the ability to

reduce edema or lymphedema, MLD associated with other treatments, the so-called lymphatic decongestive therapy, may have an important role in improving health and the functional status in patients with edema associated to sport injury or related to breast cancer surgery, just to mention two common situations (Ebert et al., 2013; Huang et al., 2013; Vairo et al., 2009). However, the real efficacy of MLD to treat patients with CVD remains unknown.

The importance of calf muscle pump function (CMPF) in CVD development is well established (Araki et al., 1994; Kan & Delis, 2001; Moloney et al., 2007; O'Brien et al., 2012; Panny et al., 2009; Recek, 2013; Shiman et al., 2009; Simka, 2007; Yang et al., 1999). Whether MLD has any role in improving calf muscle pump function in CVD patients has not been explored before. Also, it seems very important that low cost and reliable methods for dynamic evaluation muscle pump function in CVD are developed, taking into account its critical importance.

The major aim of the work reported in this thesis was to evaluate the efficacy of MLD in the treatment of patients with CVD. To this end, the efficacy of MLD maneuvers (call-up and reabsorption) in increasing venous flow in both superficial and deep venous system of the lower limbs was evaluated. Also, the reproducibility of ultrasound evaluation of the CMPF was assessed in order to ascertain its potential role in testing the efficacy of MLD.

For this purpose, after this brief introduction where an overall view of the work is presented we proceed to the "Review of the Literature" section, where major concepts on which our investigation relied are explored, like the social and health impact of CVD, its diagnose and major physiopathology, the importance and the role of calf muscle pump in this disease and the possibility of assessments of this

function, the principles of MLD and the evidence supporting the use of this therapy as a conservative treatment of CVD, and the role of the conservative treatments in the intervention in this disease. In the section "Scope of the Problem and Hypothesis", the principles and objectives of this thesis are presented and our four basic hypotheses are established. The fourth section, "Instruments and Methods", describes the design for each of the four studies comprising this thesis, as well as the detail related with the participants, instruments and methodologies, and statistical analysis. The section "Results" presents the results and outcomes of the four studies in a descriptive manner and guided by the major objectives highlighted in previous sections. In the "Discussion" section offers an interpretation of the results based on existing evidence and taking into account the hypotheses that were formulated and divided in three subsections: "Ultrasound assessments of calf muscle pump function", "Hemodynamic effects of manual lymphatic drainage" and "Therapeutic efficacy of manual lymphatic drainage for treatment of patients with chronic venous disease". The "Conclusion" section presents the synthesis of the results of the thesis and reflects about clinical implications of the results and limitation of this work.

2 REVIEW OF LITERATURE

2.1 Health and social impact of CVD

2.1.1 Epidemiology

Chronic venous insufficiency represents the most severe stages of CVD and is characterized by the presence of edema, alterations of the skin and venous ulcer, either healed or active, and comprises the C_{3-6} of clinical etiological anatomical pathological (CEAP) classification (Eklof et al., 2009). Despite its frequency in the population, the prevalence of CVD is still underestimated. Epidemiological data estimate that this condition affects 1-17% of men and 1-40% of the women (Robertson et al., 2008), accounting to around 2.5 million people in the USA (Koupidis et al., 2008). The estimated prevalence of CVD varies according to its severity, being around 10%, 9%, 1.5% and 0.5% for CEAP clinical levels C_3, C_4, C_5 and C_6, respectively (Gloviczki et al., 2011). In Portugal, approximately 20.7-36.8%, of men and 40.8-62.4% of women aged 24 years or over suffer from CVD (Capitão et al., 1995), and around 50% of the total number of skin ulcers is of venous origin (Pina et al., 2005). In Europe, the prevalence of varicose veins (C_2, from clinical CEAP classification) vary in the range 7-40% in men and 25-32% in women (Robertson et al., 2008). Variation in estimations of CVD prevalence are likely explained by differences in gender, age, ethnic group, and variations in diagnostic criteria and methods (Robertson et al., 2008).

2.1.2 Functional and HRQL implications of CVD

Patients with CVD display impaired functional capacity (de Moura et al., 2012; Koupidis et al., 2008) and diminished HRQL (Andreozzi et al., 2005; Beebe-Dimmer et al., 2005; Furtado et al., 2008; Vasquez & Munschauer, 2008). The severity of CVD, HRQL scores, the clinical signs, and venous ultrasound findings are usually correlated (Passman et al., 2011).

The impact of CVD on HRQL is primarily seen in the physical items and in the emotional role, but in advance stages (presence of venous ulcers) the mental dimension might also become involved (Andreozzi et al., 2005). In its most severe stages, the impact of CVD in HRQL is similar to that of other chronic diseases, such as diabetes, cancer, chronic pulmonary disease, or other like heart failure (Andreozzi et al., 2005).

Patients with venous ulcer present severe pain, which is in relationship with impaired tissue healing ability, diminished HRQL, and lowered self-esteem and social interactions (Koupidis et al., 2008). The psychological effects of CVD may not be strictly related to ulceration itself but rather with the symptoms (80.5-69.4%), altered appearance and esthetical concerns (66.7%), lack of sleep (66.6%), functional impairment (58.3%), and disappointment with treatment outcomes (50%) (Koupidis et al., 2008). Also, patients with uncomplicated varicose veins often have severe symptoms that adversely affect their HRQL, irrespectively of the severity of the disease, refuting the view that this disease is mostly an aesthetic problem (Darvall et al., 2012). In this disease, 49% of men and 62% of women have symptoms related to CVD (Gloviczki et al., 2011), like pain, itching, tingling, cramps, restless legs,

swelling, heaviness, and fatigue (Darvall et al., 2012; Eklof et al., 2009). A recent survey reveals that 14.9% of the general Greek population refer symptoms and/or present signs related to CVD (Dimakakos et al., 2013).

Concerning the lower extremities, one-third of people with CVD report health or cosmetic problems that turn going out of home and to participate in social activities a burden, and they avoid wearing clothes exposing their legs or going on vacations to very warm places (Dimakakos et al., 2013). According to self-reports, functional status is diminished in these patients (de Moura et al., 2012; Passman et al., 2011) also because of some physical dysfunctions, like abnormalities in gait (van Uden et al., 2005), impaired balance, peripheral neuropathy (Newland et al., 2009; Shiman et al., 2009), weak calf muscles (plantar and dorsiflexors muscles) (Panny et al., 2009; Qiao et al., 2005; van Uden et al., 2005), or diminished ankle range of motion (Dix et al., 2003; Furtado et al., 2008; Panny et al., 2009). These dysfunctions are also associated with impaired muscle pump function in the lower extremity (Shiman et al., 2009), an important risk factor for venous ulceration (Kan et al., 2001).

2.1.3 Socio-economic impact of CVD

Severe CVD has a significant economic impact, mostly due to raised morbidity. During the last decade neither CVD-associated and inflation-adjusted mean hospital charge, nor length of hospital stay decreased, possibly as a result of poor advancements in prevention and treatment of this disease (Tsai et al., 2005). In advanced stages, venous ulcers require wound care, compression, chemical and

mechanical debridement and, in frequent cases, antibiotic therapy (Tsai et al., 2005). Western European countries spend 1.5-2% of their annual health budget in the treatment of this disease (Sandor, 2004). The economic burden is not just associated with clinical visits and outpatient treatments, but also with travel time, loss of work hours for patients and family, and physiological impairment related to analgesic and antibiotic use (Tsai et al., 2005). Limb amputation is a radical outcome of this disease: three in four amputations of venous origin are undertaken in ambulatory regimen, and many of these cases are also related to comorbidities, for instance diabetes and arterial vascular disease (Tsai et al., 2005). Other important complications, such as hemorrhage, thrombosis and pulmonary embolism, also compound the unhealthy profile of these patients (Dimakakos et al., 2013). Deep venous thrombosis may cause chronic conditions like post-thrombotic syndrome and CVD, increasing the costs of the treatment (Tsai et al., 2005). Preventing deep venous thrombosis and complications is one of the most important aims for reducing socio-economic burden associated to CVD (Tsai et al., 2005).

Despite its cost, the efficacy of conservative pharmacological treatment of CVD is usually poor, and should be combined with other strategies, such as the use of elastic garment compression (Gloviczki et al., 2011).

Similar procedures are recommended following surgery and sclerotherapy, in which case post-operative compression therapy and health education are crucial for treatment success (Bobridge et al., 2010; Dimakakos et al., 2013). There are several risk factors associated with CVD that patients should be informed of by the health professionals, like the use of hormonal contraceptives by women, daily routines (sitting or standing), pregnancy, age, obesity and heredity factors (i.e., family

history) (Beebe-Dimmer et al., 2005; Carpentier et al., 2004; Dimakakos et al., 2013; Tsai et al., 2005). Also important, advice regarding behavioral changes, engaging in so-called venous exercises and the proper use of the health care services should be offered to CVD patients (Dimakakos et al., 2013). Obtaining the right advice from health care professionals is an important measure for preventing and managing CVD (Dimakakos et al., 2013).

Because of the wide spectrum of factors that cause functional impairment in these patients and the high costs of treatment (Darvall et al., 2012; Eklof et al., 2009; Koupidis et al., 2008), the prevention of CVD by educational and prophylactic interventions has been shown to be clinically cost-effective, by avoiding disease progression to the last stages (Allegra, 2003; Tsai et al., 2005).

2.2 Pathology of CVD

CVD is caused by venous hypertension, valvular insufficiency and/or reflux (Ibegbuna et al., 2006; Lim & Davies, 2009; Meissner et al., 2007a). Venous hypertension might be accompanied by outflow obstruction (Meissner et al., 2007a) and affects the superficial, perforator and deep veins (Eklof et al., 2009; Ibegbuna et al., 2006). Insufficient lymphatic drainage or a dysfunction of calf muscle pump are very often associated with this disease (Sandor, 2010). Venous hypertension is related to structural (veins and valves), hemodynamic (obstruction, reflux, stasis), biomechanical (calf muscle pump strength and ankle range of motion) and biochemical factors (leucocyte-endothelial process inflammation) (Ibegbuna et al., 2006; Meissner et al., 2007a).

2.2.1 Etiology and anatomical location of CVD

The etiology of CVD can be described as primary, secondary (post-thrombosis) or congenital (Eklof et al., 2004). Although reflux is the only hemodynamic alteration in CVD, in secondary venous disease most of the cases present a mix of reflux and obstruction (Ibegbuna et al., 2006). It seems that 80% of cases of CVD have a secondary etiology of post-thrombotic pathology, and 20% are of primary cause, as a result of valvular incompetence (Meissner et al., 2007a).

In superficial veins the insufficiency is most often the result of a primary preexisting weakness in the vessel wall or valve, as a consequence of a direct injury, excessive venous distention caused by hormonal effects or high hydrostatic pressure, or secondary to venous obstruction (i.e., phlebitis) (Eberhardt & Raffetto, 2005; Gloviczki et al., 2011; Padberg, 2005; Sandor, 2010). Failure of valves (superficial veins) may increase superficial venous pressure, resulting in venous dilatation and varicose veins (Sandor, 2010). Although the primary mechanism of valvular incompetence in superficial veins is not fully known (Meissner et al., 2007a), it appears that first there are changes in mechanical properties of vein walls caused by increased collagen content and decreased amount of elastin and smooth muscle, leading to vessel enlargement and, secondarily, to valvular insufficiency (Meissner et al., 2007a).

The perforating veins can also become insufficient by primary incompetence of vein valves or secondary to venous obstruction (Delis, 2004). In these cases, there is reflux from deep to superficial venous system: with valve incompetence the reflux to saphenous veins may allow the re-entry of venous blood to the deep venous

system (Delis, 2004). In the case of secondary incompetence, the high pressures are transmitted to superficial veins causing the enlargement of dermal capillarity bed, increasing filtration to the interstitial space (Delis, 2004; Meissner et al., 2007a).

Deep veins insufficiency has been suggested to be most often the consequence of deep veins thrombosis, i.e., from secondary etiology (Sandor, 2010). However, primary deep venous incompetence is also common (8-22% of the cases (Labropoulos et al., 2009)) but is usually compensated by a strong CMPF (Labropoulos et al., 2009; Meissner et al., 2007a). It seems that outflow obstruction and reflux caused by valve damage may cause deep vein thrombosis and these two alterations together they increase the probability of the development of post-thrombotic syndrome (Labropoulos et al., 2009; Lopez et al., 2004).

Deep venous thrombosis may also occur because of an intrinsic venous process, such as a previous deep venous thrombosis episode with inadequate recanalization or venous stenosis, or because of extrinsic compression, as in May-Thurner syndrome (Eberhardt et al., 2005). Also, it can be caused by venous agenesis, such as in the Klippel-Trenaunay syndrome, trauma, surgical mishap, and tumors (Meissner et al., 2007a).

Congenital etiology of CVD, in which case the condition is already present at birth, also occurs, despite this it might only be recognized later in life, such as in the cases of the Klippel-Trenaunay (varicosities and venous malformations, capillary malformation, and limb hypertrophy) (Gloviczki et al., 2011) and Parkes-Weber (venous and lymphatic malformations, capillary malformations, and arteriovenous fistulas) syndromes (Eberhardt et al., 2005).

2.2.2 Physiopathology hypothesis for CVD

The exact mechanisms behind the development of CVD are not clear yet (Bergan et al., 2008; Meissner et al., 2007a). The major hypothetical sequence of events is that venous hypertension in the initial stages of the disease triggers inflammation affecting the vein walls and valves, and as the condition aggravates inflammatory changes spread to the skin and the muscles, causing dermal changes, like hyperpigmentation, subcutaneous tissue fibrosis (lipodermatosclerosis), and ulceration (Meissner et al., 2007a; Raju et al., 2012; Sandor, 2004, 2010) and tissue necrosis, in the most severe cases (Bergan et al., 2008).

Venous obstruction occurs because of the triad: blood stasis, changes in the vessel wall, and hypercoagulability (Lopez et al., 2004). This may occur as a phlebitis (obstructions of superficial venous system) or as deep venous thrombosis (obstructions of deep venous system), and can be diagnosed as acute or chronic (Labropoulos et al., 2009). The acute deep venous thrombosis may cause nociceptive pain, swelling and tenderness, and both phlebitis and deep venous thrombosis must be confirmed by venous ultrasonography (Meissner et al., 2007a). Hypertension that ensues opposes venous return, leading to luminal hypoxemia and vein wall distension, which impairs perfusion and causes endothelial hypoxia and leukocyte invasion of the wall (Lopez et al., 2004). A progressive aggravating remodeling process is then triggered consisting of hypoxemia-related venous/capillary wall injury, leukocytes accumulation and adhesion, progressive blockage of capillary blood flow, and ongoing damage of subcutaneous tissues and skin (lipodermosclerosis and skin ulceration) (Malone & Agutter, 2009).

Usually, venous reflux and obstruction occur together (Meissner et al., 2007a). Following venous obstruction recanalization occurs and blood flow through the vein is restored (Lopez et al., 2004). However, lysis of the clot or thrombus is usually only partial and the residual thrombus might undergo fibrosis and completely obstruct of the lumen of the vein, for example involving leaflets (Lopez et al., 2004). Then collateral blood circulation develops, and obstruction may be overcome (Meissner et al., 2007a). Sometimes, calf perforating veins may be an important collateral venous path when the popliteal vein is involved, causing CVD of a secondary etiology (Meissner et al., 2007a).

The initial hypertension in CVD may also be caused by valvular incompetence alone (Bergan et al., 2008). Varicose veins may result from endothelial changes (reduced elastin and smooth muscle content together with increased collagen) associated with hypoxia, causing weakened venous tone (Bergan et al., 2008). Other changes include downregulated apoptosis (Ascher et al., 2001), decrease energy for cells' metabolism and increased lysossomal activity (Stvrtinova & Ferencikova, 1992).

Also, venous hypertension in CVD (because of obstruction or reflux) may cause extravasion of macromolecules and red blood cells, leading to endothelial cell activation, leucocyte diapedesis, extracellular matrix alterations, and intensive collagen deposition (Amato et al., 2013; Serra et al., 2014). Changes in skin and other tissues interstitium also cause the release of growth factors and pro-inflammatory factors that stimulate increases in synthesis and deposition of collagen and other extracellular matrix components, while also affecting tissue remodeling by the metalloproteinases (Amato et al., 2013; Lowell et al., 1992; Sayers et al., 1993;

Serra et al., 2014). Also, numerous mast cells are present in venous ulcers, suggesting that this cells may also contribute to cytokine activation, tissue remodeling, and ulceration (Serra et al., 2014).

As a cause of CVD, the venous stasis hypothesis that postulates that venous blood pooling within dilated veins in the skin causes anoxia and cell death, and the arteriovenous shunting hypothesis, that postulates that abnormal arteriovenous communications may increase oxygen tension in varicose veins, were abandoned (Meissner et al., 2007a; Sandor, 2004).

The fibrin cuff and the white cell trapping hypothesis are the most accepted explanations for the physiopathology of CVD (Sandor, 2004). The enlarged interendothelial pores, stretched by raised intraluminal pressure, allow the passage of fibrinogen into the interstitial space where it is polimerized into fibrin, causing hypoxia and tissue injury (skin and subcutaneous tissue) (Meissner et al., 2007a). Associated to this perivascular cuff is leucocyte infiltration (around capillaries and post-capillaries venules) and deposition of collagen, that create a barrier to oxygen and nutrient diffusion, causing fibrosis, chronic inflammation (Meissner et al., 2007a), and damage of venous valves (Sandor, 2004).

Low capillary flow rate in CVD facilitates white cells trapping, the pulling of the capillaries, and the potent initiation of leucocytes adherence to the endothelium (Bergan et al., 2008). Perhaps white cells cause occlusion of capillaries, but the cause of local hypoxia and tissue injury are presumed to be mediated by toxic products released by trapped white cells (Eberhardt et al., 2005; Sandor, 2004, 2010).

The breakdown of the skin is also related to increased expression of tissue metalloproteinases, that also inhibits venous ulcer healing (Bergan et al., 2008;

Meissner et al., 2007a; Sandor, 2004). The mechanism responsible for wound healing is an orderly process that involves inflammation, re-epitalization, matrix deposition, and tissue remodeling, and these last two stages are controlled by reducing the activity of extracellular matrix metalloproteinases and by increasing inhibitors of these proteolytic enzymes (Amato et al., 2013; Serra et al., 2014). Matrix metalloproteinases are controlled by a diversity of signaling molecules, including pro-inflammatory cytokines and growth factors (TGF-β_1, that cause excessive fibrosis and inflammation) (Meissner et al., 2007a).

Also, varicose veins present reduced ability to undergo venoconstriction and show lessened compliance, which is explained by reduced amount of smooth muscle (Eberhardt et al., 2005; Sandor, 2004, 2010), reduced quantity of elastin, and increased collagen as the result of endothelial dysfunction in response to stasis and hypoxia (Lowell et al., 1992; Sayers et al., 1993).

Changes in the venous hemodynamics of the large veins of the lower extremity are transmitted into the microcirculation and eventually result in the development of venous microangiopathy (elongation, dilation, and tortuosity of capillary beds, thickening of basement membranes with increased collagen and elastic fibers, endothelial damage with widening of interendothelial spaces, and increased pericapillary edema) (Eberhardt et al., 2005; Sandor, 2004, 2010). The increased permeability of capillaries and high venous pressure leads to the accumulation of fluid, macromolecules (i.e., proteins like fibrinogen and α2-macroglobulin), and red blood cells extravasation into the interstitial space, causing injury to the subcutaneous tissue (Meissner et al., 2007a; Sandor, 2010; Smith,

2006), chronic inflammation with degradation of red blood cells, and sustained recruitment of leucocytes (Smith, 2006; Wilkinson et al., 1993).

Tissue remodeling in CVD is greatly dependent on the balance between inflammation and tissue injury and the process of healing and repair (Meissner et al., 2007a). The frequency and the intensity of triggering events will determine such balance and ultimately the severity of tissue injury (Meissner et al., 2007a). Risk factors have an important role in this inflammatory cascade (Vlajinac et al., 2012).

In addition to changes in the blood vessels and connective tissue, alterations in the lymphatic network and nervous system may occur (Boisseau, 2007; Eberhardt et al., 2005). Fragmentation and destruction of microlymphatics may further impair drainage from the extremities, whereas dysfunction of local nerve fibers may alter regulatory mechanisms (Eberhardt et al., 2005). As the capillary pressure elevates the content of proteins within the interstitial space, the skin and subcutaneous tissues become damaged, such as in lipodermatoesclerosis (Meissner et al., 2007a; Smith, 2006), and because of peripheral lymphatic system dysfunction, secondary lymphedema may occur, motivated by the high oncotic pressure in the interstitial space (Raju et al., 2012).

2.2.3 Symptoms in CVD

There are several symptoms that are associated with CDV, including pain, tightness, skin irritation, heaviness, muscle cramps, burning feelings, fatigue, soreness, tingling, restless leg, and feeling of swelling (Bradbury et al., 1999;

Campbell et al., 2007; Darvall et al., 2012; Eklof et al., 2009; Eklof et al., 2004; Gloviczki et al., 2011).

The number of symptoms reported by patients with CVD varies but are usually several (Boisseau, 2007; Bradbury et al., 1999; Darvall et al., 2012). Importantly, the number and severity of symptoms are not strictly related with CVD severity and sometimes severe symptoms, and those that have the largest impact on HRQL, are present in less severe cases (Darvall et al., 2012).

Despite indication for surgery for varicose veins following the clinical evaluation of one or more symptoms and of reflux in saphenous veins, some studies suggest that the majority of the symptoms in patients with varicose veins are non-venous related (Bradbury et al., 1999). Indeed, it seems very difficult to separate venous from non-venous causes of symptoms in CVD (Campbell et al., 2007).

Several studies show the presence of both neuropathic and nociceptive pain in patients with CVD (Boisseau, 2007; Shiman et al., 2009). In addition, and based on the physiopathology of chronic pain (Smart et al., 2011; Vranken, 2012), the possibility of patients with CVD presenting central sensitization of pain cannot be discarded.

Nociceptive pain announces a potential threat caused by noxious stimuli of chemical (inflammatory), mechanical or thermal nature, that activate primary afferent slow-conducting neurons (Aδ and C fibres) (Fornasari, 2012). Neuropathic pain is related to a primary lesion or dysfunction of the nervous system resulting from infection, trauma and other causes (Fornasari, 2012), that changes the function of neural circuitries (neural plasticity) (Vranken, 2012). Neuropathic pain involves several mechanisms, including the action of soluble mediators (e.g., cytokines, H^+,

nerve growth factor), alterations in calcium channels, sodium channels, hyperpolarisation-activated nucleotide-gated ion channels, potassium channels, phenotypic switches and sprouting of nerves endings, and the involvement of the sympathetic nervous system (Vranken, 2012).

The progress from acute to chronic pain is related to changes in the central nervous system and with the altered transmission and modulation of pain sensation following a lesion (Fornasari, 2012). Peripheral sensitization of sensory nerve fibers cause hypersensitivity to pain, and may be present in inflammatory and neuropathic pain as due to CVD, however central sensitization are not described in this patients (Fornasari, 2012; Shiman et al., 2009; Vranken, 2012).

In CVD, the adhesion of the leucocytes to the endothelial cells trigger an inflammatory process (Boisseau, 2007; Nicolaides, 2005). Leucocytes leave circulation because of inflammation (vascular media or intima), pre-inflammatory endothelial cell activation (hypoxia in lumen or wall vessel), or altered hemodynamics (decreasing or abnormal blood flow), damaging the microcirculation and enlarging the veins (Boisseau, 2007; Meissner et al., 2007a; Sandor, 2004). This may occur in deep vein thrombosis or venous reflux (Boisseau, 2007). Both leucocyte adhesion and its products, acting as signaling molecules, and the hypertension (decreased venous blood flow), acting as a mechanical factor, increase the permeability of capillaries, leading to CVD and edema (Boisseau, 2007; Meissner et al., 2007a; Nicolaides, 2005; Sandor, 2004). The abundant biochemical mediators released by leucocytes into vein and venule walls and into the interstitial space are presumed responsible for stimulating the nerve endings and causing nociceptive pain in CVD, rather than venous dilation per se, which seems to be painless (Boisseau,

2007). Unlike skin, veins possess only few nociceptive nerve endings (Boisseau, 2007). First, the mechanical stimulation of Aβ nerve fibers after priming stimulation of both Aδ e C nerve fibers by chemical stimuli, gives rise to a truly painful sensation followed by a diffuse and sustained pain (Boisseau, 2007). The pain transmitted by C fibers are more visceral-like, meaning that it is more sustained, diffuse, while also causing anxiety, and the readiness to feel pain may interfere with this subjective sensation of symptoms (Boisseau, 2007). With time, the nociceptors might become sensitized and pain pathway might suffer facilitation due to morphological (e.g., nerve endings sprouting) and/or neurophysiological changes causing peripheral neuropathy and neuropathic pain (Boisseau, 2007; Newland et al., 2009).

CVD may cause tissue damage in the leg, including the peripheral nerves (Reinhardt et al., 2000). Patients with CVD present a higher motor latency, a reduced vibration threshold, and diminished warm and cold perception, resulting from disturbances of Aα, Aβ, Aδ and C fibers (Reinhardt et al., 2000) and denervation (Shiman et al., 2009). Patients with venous ulcer and varicose veins present a decreased number of nerve fibers at the epidermis (Guest et al., 2004). Also, the fewer number of epidermal nerve fibers in chronic ulcers suggests that skin innervation may be important for healing (Guest et al., 2004). On the other hand, the application of nerve growth factor is associated with improved venous ulcer healing, suggesting that changes in these kinds of growth factors may be at the origin of CVD-associated peripheral neuropathy and neuropathic pain (Shiman et al., 2009).

Nevertheless, patients with CVD may present other comorbidities that make only the CVD-related pain difficult to assess (Shiman et al., 2009). For example,

peripheral neuropathy and pain might be due not to CVD, at least exclusively, but rather associated with comorbidities, like diabetes (Reinhardt et al., 2000).

2.2.4 Signs of CVD

Several signs are recognized to be associated with CVD, like telangiectasias and reticular veins, varicose veins, venous edema, skin pigmentation, corona phlebectatica, inflammation (eczema, cellulitis, dermatitis, for example), induration like lipodermatoesclerosis and atrophie blanche, and ulcer (healed or active), associated or not with symptoms of venous origin (Eklof et al., 2009; Eklof et al., 2004; Gloviczki et al., 2011). Nevertheless, CVD might exist without the presence of signs (Eklof et al., 2009; Eklof et al., 2004; Gloviczki et al., 2011). The signs are the consequence of the physiopathology of CVD, and are caused by hypertension, inflammation, and injury of the microcirculation, the skin or the subcutaneous tissue (Meissner et al., 2007a).

Reticular veins, also called blue veins, subdermal varices, and venulectasias, are dilated subdermal veins, usually 1 mm to less than 3 mm in diameter and with tortuous paths (Eklof et al., 2004). Telangiectasias, also called spider veins, hyphen webs, and thread veins, represent the confluence of dilated intradermal venules less than 1 mm in caliber (Eklof et al., 2004).

According to the latest guidelines, varicose veins (also called varix, varices, and varicosities (Eklof et al., 2004)) should be palpable in an upright position and represent abnormal veins with at least 3 mm in diameter, (Eklof et al., 2009; Eklof et al., 2004; Gloviczki et al., 2011). Varicose veins can be present as a result of

hypertension caused by reflux and/or obstruction, as discussed before (Labropoulos et al., 2009; Meissner et al., 2007a). The development of varicose veins most frequently involves the saphenous veins, saphenous tributaries, or nonsaphenous superficial leg veins (Eklof et al., 2004). Varicose veins are usually tortuous, but tubular saphenous veins with demonstrated reflux may be classified as varicose veins (Eklof et al., 2004).

Corona phlebectatica, also called malleolar flare and ankle flare, is commonly viewed as an early sign of CVD, and designates the accumulation of numerous small intradermal veins packed together on the medial or the lateral aspects of the ankle and foot (Eklof et al., 2004).

Venous edema occurs when there is imbalance between venous filtration, venous reabsorption and lymphatic reabsorption (Mortimer, 2000). About 90% of the venous filtration is reabsorbed again in to the venous system, and the remaining 10% of the venous filtration (proteins, plasma, and other components) is reabsorbed by the lymphatic circulation (Morgan, 2008; Raju et al., 2012). In CVD, the venous filtration is increased by means of venous hypertension and raised permeability due to inflammation (Morgan, 2008; Mortimer, 2000). In these conditions venous edema may occur (Mortimer, 2000; Raju et al., 2012). This is a pitting edema that get worse through the day and improves at night with resting and leg elevation, and that usually is accompanied with venous symptoms and signs (Mortimer, 2000; Raju et al., 2012). When edema is at the dorsum of the foot, is associated to squaring of the toes, to thick skin, and is of non-pitting edema type, it is assumed that a lymphatic compromise exists (Gloviczki et al., 2011). The lymphatic circulation may compensate for the excessive filtration, but lymph vessels also suffer damaged with

time (microlymphoangiopahty), because of chronic inflammation and accompanying subcutaneous and skin lesions (Eberhardt et al., 2005; Raju et al., 2012). Therefore, venous edema becomes compound with signs of lymphedema, with non-pitting edema and with hyperkeratosis, and is now associated with lymphatic insufficiency (Mortimer, 2000). Clinically, venous edema is perceived as an increase in volume of fluid in the skin and subcutaneous tissue, characteristically diminished by pressure (Eklof et al., 2004). Venous edema usually occurs around the ankle region, but may extend to the leg and foot (Eklof et al., 2004).

The presence of pigmentation means that the skin becomes darker and brownish (Eklof et al., 2004). This results from extravasation of red blood cells into the interstitial space (Meissner et al., 2007a). Blood extravasation and skin pigmentation is most noticed around the ankle, but may also be visible in the leg and foot (Eklof et al., 2004; Vasquez et al., 2010).

Atrophie blanche (white atrophy) is an induration of tissues. This skin alteration, that should not be confused with healed venous ulcers, is usually well localized and has the shape of a circular white and atrophic skin surrounded by dilated capillaries and sometimes hyperpigmentation (Eklof et al., 2004; Vasquez et al., 2010).

Lipodermatosclerosis is also a clinical sign of tissue induration, characterized by local chronic inflammation and fibrosis of skin and subcutaneous tissues at the lower region of the leg (also compromising the Achilles tendon), sometimes preceded by diffuse inflammatory edema of the skin, which may be painful and which often is referred to as hypodermitis (Eklof et al., 2004; Vasquez et al., 2010). Clinically, lipodermatosclerosis must be differentiated from lymphangitis, erysipelas,

or cellulitis by their characteristically different local signs and systemic characteristics (Eklof et al., 2004).

The eczema, is an inflammation process, erythematous dermatitis, which may progress to blistering, weeping, or scaling eruption of the leg skin, and may be located anywhere in the leg (Eklof et al., 2004; Vasquez et al., 2010). Eczema is very frequent in uncontrolled CVD, but may also be associated to sensitization to local therapy (Eklof et al., 2004).

Venous ulcers are the worst clinical sign of CVD and represent the loss of integrity of the skin, with a full-thickness defect and occur most frequently near the ankle region (Eklof et al., 2004), at the site of major perforating veins and the greatest hydrostatic pressure (Eberhardt et al., 2005). Venous ulcers are also characterized by failure to heal spontaneously and are sustained by CVD (Eklof et al., 2004).

2.3 CVD diagnosis

The diagnosis of CVD is based on history and physical examination of patients with the assistance of non-invasive tests, such as duplex ultrasound scanning (Eberhardt et al., 2005; Meissner et al., 2007a; Min et al., 2003; Nicolaides, 2000). Duration and valve closure time calculated by duplex ultrasound scan are used to diagnosis veins with insufficiency (Magnusson et al., 1995). The diagnose using the reflux time has been shown to be reproducible (Asbeutah et al., 2005).

With B-mode ultrasonography, veins' lumen should be imaged at transversal or/and longitudinal view, and the transducer should be adjusted for correct vein

imaging and to assess the presence/absence of acute or chronic thrombosis (Coleridge-Smith et al., 2006). Pulsed-waved spectral or color Doppler are used to assess the velocity and direction of venous flow and should be performed with a recommended Doppler range of 5-10 cm/s, with the wall filter at its lowest setting, and with the angle of insonation at 45-60º (Coleridge-Smith et al., 2006). During the examination (reflux and diameter), patients are at upright position and several methods are used to elicit reflux: release after calf squeeze, for proximal veins, and after foot squeeze, for calf veins, manual compression of vein clusters, pneumatic calf cuff deflation (the more reproducible method), active foot dorsiflexion and relaxation, and Valsalva maneuver, in this case to demonstrate saphenofemoral incompetence (Coleridge-Smith et al., 2006). Reflux is generally considered abnormal when its duration attains a cutoff time of 0.5 seconds in the case of the saphenous, tibial, deep femoral, and perforating veins, and 1 second, in the case of the femoral and popliteal veins (Eberhardt et al., 2005; Gloviczki et al., 2011; Min et al., 2003; Nicolaides, 2000). One study concludes that veins are normal if reflux time is less than 0.5 seconds, with sensitivity of 90%, and are insufficient when reflux increases to above 0.7 seconds, with specificity greater than 90% (Lurie & Pevec, 2000). The same study reports good reliability of reflux time measures, with correlation coefficients of r = 0.97 and r = 0.85, for immediate and late test-retest, respectively. Nevertheless, studies disagree whether this method allows to distinguish between different levels of venous insufficiency severity (Lurie et al., 2000).

On the other hand, the volume reflux index (the percentage of antegrade blood volume that flows backwardly (reflux) after a muscle calf

compression/contraction (Lurie et al., 2000)) is an accurate measure to evaluate the severity of venous insufficiency. This index is calculated by duplex ultrasound scanning taking into account the veins' cross-sectional area and blood mean velocity (Lurie et al., 2000).

Air-plethysmography can also be used to complement the diagnosing of CVD when duplex scanning is unable to provide definitive information about its physiopathology, also being a good test to assess CMPF (Gloviczki et al., 2011). Other imaging studies, such as computed tomography venography, magnetic resonance venography, ascending and descending contrast venography, and contrast ultrasonography are used selectively (Meissner et al., 2007a), such as in cases of endoluminal or extraluminal venous obstructions (like post-thrombotic syndrome and deep venous thrombosis, and pathologies as tumors, traumas, and some medical interventions). However, routinely, the duplex ultrasound scanning is the most economic, valid and reliable non-invasive method for diagnosing CVD (Gloviczki et al., 2011).

2.4 Calf muscle pump function

The venous blood return from periphery to heart via the venous system is linked to the action of a central pump (heart and respiratory cycle), periphery venous pump, a pressure gradient, and competent veins and/or venous valves (Shiman et al., 2009).

The calf muscle pump has an important role for the effective venous return and relies on dynamic interaction between the ankle joint, muscle fascia, muscles of

the calf and venous valves (Meissner et al., 2007a; Shiman et al., 2009). During muscle contractions, the venous blood is forced in direction to the heart and the valves prevent reflux during relaxation (Kan et al., 2001; Meissner et al., 2007b; Recek, 2013). As deep veins are tethered to surrounding tissues, muscle relaxation causes the veins to open, lead to a sudden drop in pressure within these vessels (Cavalheri et al., 2008; Clarke Moloney et al., 2006; Ibegbuna et al., 2006). The large pressure gradient that develops forces the blood to flow from superficial to deep veins trough perforator veins, decreasing venous pressure and allowing arterial flow (Meissner et al., 2007a; Shiman et al., 2009).

2.4.1 The three venous muscle pumps of the lower limb

There are three venous muscle pumps: foot, calf and thigh (Ludbrook, 1966; Meissner et al., 2007a).

The calf muscles, and possibly the thigh muscles, act as a pump, also called as ‘‘peripheral heart”, which can generate pressures of up to 300 mm Hg during exercise (Gaweesh, 2009). Nevertheless, it has been suggested that thigh muscle pump has a minor effect in venous return, compared to calf muscle pump (Ludbrook, 1966; Meissner et al., 2007a; Shiman et al., 2009). Calf muscles contraction can elevate the pressure to approximately 140 mm Hg and increase venous blood flow through the popliteal and the femoral veins (Recek, 2013). In competent veins, the centrifugal component during muscle relaxation lasts approximately 200 to 300 milliseconds and represents the physiological reflux, in incompetent veins the duration exceeds 500 milliseconds (Recek, 2013).

During gait, venous pressure in the leg decreases from around 100 mm Hg to around 22 mm Hg, due to combined action of the muscle contractions and plantar compression (Rowland, 2001). The plantar venous plexus is compressed during gait, increasing venous flow through the posterior tibial venous system into the popliteal vein (Meissner et al., 2007a; White et al., 1996). Despite these observations, it has been suggested that foot muscle pump has two possible mechanisms that operate during stance: firstly the weight bearing compression of the plantar veins, and secondly the contraction of the foot muscles (e.g., the abductor digiti minimi, abductor hallucis, extensor digitorum brevis, flexor digitorum brevis, and flexor hallucis brevis) around these veins. The two mechanisms, however, do not work synchronously, with plantar compression acting first then followed by the action of the muscle contractions at the foot (Corley et al., 2010). These two different foot pump mechanisms may both be present during the stance phase of the gait cycle, but would be active at slightly different moments (Corley et al., 2010). Also, certain clinical conditions of CVD could be explained by a conflict between the mechanisms of the foot pump and the leg pumps (Ricci et al., 2014). The knowledge about the interaction of the lower limb muscle pumps during contraction/relaxation as a mechanism for venous return is still quite poor (Meissner et al., 2007a).

The calf muscle pump contraction is assumed as the most important muscle pump of the lower limb (Alimi et al., 1994; Meissner et al., 2007b). Less efficient CMPF (involving especially the gastrocnemius and soleus muscles) has also been related with muscle inflammation, reduced muscle oxygen supply, muscle necrosis, myofibril atrophy (muscle fibers type I and II) and muscle denervation (Qiao et al., 2005; Yamaki et al., 2010). A study by Araki et al. (Araki et al., 1994) concluded

that venous insufficiency cannot fully explain venous ulceration, pointing to deficient calf muscle pump as a primary factor in CVD-related skin and tissue damage. Several studies show that early treatment, by exercising the muscle pump, can prevent the most severe forms of CVD (Nullen, 2010; Padberg et al., 2004). The important role of CMPF in the progression of CVD is well established, but in many individual cases impaired calf pump function may go undetected until most severe changes become evident (Bradbury, 2010). Therefore, assessable, accurate and non-invasive methods to evaluate CMPF are needed (Bradbury, 2010; Nicolaides, 2000; Padberg et al., 2004; Panny et al., 2009; Sandor, 2010).

2.4.2 Impairment of calf muscle pump and functional capacity

Calf muscle pump dysfunction might be caused by weakness of calf muscles, but may also be related to decreased range of motion around the ankle joint during walking and other movements (Back et al., 1995; Cavalheri et al., 2008; Panny et al., 2009; Yang et al., 1999), neuropathy, muscle denervation or muscle atrophy, or gait abnormalities (de Moura et al., 2012; Qiao et al., 2005; Shiman et al., 2009).

Ankle function plays an important role in mobility (de Moura et al., 2012). Distal leg muscles may exhibit reductions in strength and power with aging, and these affects walking, balance, and increases the risk of falling (Webber & Porter, 2010). Impaired ankle muscles strength has been associated with falls (Suzuki et al., 2001). Dorsiflexion power has been found to be closely associated with function in community-dwelling older women in terms of their ability to get up from and sit down on a chair and climb stairs (Suzuki et al., 2001). Plantarflexion strength has

been shown to be positively related to both preferred gait speed and fast gait speed (Suzuki et al., 2001). Patients with CVD present limited ankle range of motion (Back et al., 1995; Cavalheri et al., 2008; de Moura et al., 2012; Dix et al., 2003; van Uden et al., 2005). Diminished ankle mobility tends to aggravate as CVD progresses and is parallel with increasing severity of symptoms, thus further contributing to a poor CMPF (Back et al., 1995; Cavalheri et al., 2008). Together with decreased ankle range of motion, there is also decreased muscle strength of dorsi and plantarflexors (Panny et al., 2009; Yang et al., 1999), with decreased peak torque, power ability (de Moura et al., 2012), muscle resistance (number of heel rises) (van Uden et al., 2005), and total work performed by the ankle plantarflexors (Yang et al., 1999). Other functional alterations associated with CVD include decreased gait speed (de Moura et al., 2012; van Uden et al., 2005), decreased number of steps per week (in venous ulcer patients) (Clarke-Moloney et al., 2007), and generally impaired functional capacity and mobility (de Moura et al., 2012). Also, changes in ankle function alters foot pressure distribution during gait that becomes higher at the midfoot and lower at the toes (Shiman et al., 2009).

These functional alterations, specially the decreased strength of the calf muscles and reduced ambulation, contribute to venous hypertension (Back et al., 1995; Dix et al., 2003; Meissner et al., 2007a; Newland et al., 2009; Panny et al., 2009; Shiman et al., 2009). Dysfunction of the muscle pumps leads to venous blood not being effectively emptied out of the distal extremity (Panny et al., 2009). This rarely occurs as a "primary" disorder with neuromuscular conditions or muscle wasting syndromes; however, clinically significant muscle pump dysfunction often occurs in severe reflux or obstruction (Nicolaides, 2000). Muscle pump dysfunction

appears to be a major mechanism for the development of superficial venous incompetence and its complications, such as venous ulcers, and around 70% of patients with venous ulcer present calf muscle pump dysfunction (Araki et al., 1994; Bradbury, 2010; Eberhardt et al., 2005; Kan et al., 2001; O'Brien et al., 2012).

2.4.3 Air-plethysmography: the gold standard assessment of calf muscle pump

Venous hypertension usually results from reflux, obstruction, and poor ejection, and air-plethysmography is capable of defining the contribution of each of these components (Nicolaides, 2000).

Although duplex ultrasound scanning is the method most used to diagnose CVD, the evaluation of calf muscle pump is usually done by air-plethysmography (Asbeutah et al., 2005). This is carried out by assessing the ability of the calf muscle pump to eject blood from the leg veins after ten consecutive calf muscle contractions during tip-toe/heel-rise movement (Nicolaides, 2000). However, other studies used vascular ultrasound to estimate the capacity of muscle contraction to eject venous blood as an estimation of CMPF (Hitos et al., 2007; Moloney et al., 2007; Staubesand et al., 1995).

Calf air-plethysmography has been the gold standard measure to evaluate CMPF in venous insufficiency, showing good reproducibility (Asbeutah et al., 2005). This method is used for calculating the ejection fraction (the percent of blood volume expelled in one muscle contraction) and residual volume fraction (the percent of blood volume remaining within the calf at the end of ten tip-toe movements) (Nicolaides, 2000). A poor ejection fraction (< 40%) and a high residual volume

fraction (> 20/30%) are related to the most severe stages of CVD (Cavalheri et al., 2008; Nicolaides, 2000; Padberg et al., 2004). Also, the complications and severity of CVD, such as ulceration, have been shown to correlate with the severity of reflux assessed by vascular ultrasound (de Moura et al., 2012; Eberhardt et al., 2005). This technique provides quantitative information about several aspects of global venous function (Eberhardt et al., 2005). It may be used in the selection of interventions and for assessing the response to interventions directed to CMPF and to provide an overall measure of the efficacy of CVD treatments (Owens et al., 2000; Padberg et al., 2004).

2.4.4 Ultrasound assessment of hemodynamic component of calf muscle pump

Despite there being no strong correlation with CVD severity, the capacity to increase venous blood flow (flow velocities and flow volume) through the veins during a calf muscle contraction has been assessed in several studies that evaluate CMPF (Moloney et al., 2007; Sochart & Hardinge, 1999; Staubesand et al., 1995).

The reproducibility of venous volume flow (mean flow velocity × cross-sectional area of the vein) measurements by vascular ultrasound scanning improves with the use of sampling volumes that cover the entire venous lumen at points where the veins' profile is roughly circular, by using an incident angle of the ultrasound beam of 60 degrees, and measuring for 40-second intervals or longer (Lurie et al., 2002; Ogawa et al., 2002).

At rest, flow volume can reach [mean (standard deviation)]147 (70.2) mL/min in the superficial femoral vein, 86 (40.8) mL/min in the deep femoral vein,

and 38 (16.8) mL/min in the GSV (Ogawa et al., 2002). During contractions of lower limb muscles, it is possible to assess the increase in flow velocity and flow volume in the respective veins (Hitos et al., 2007; Moloney et al., 2007).

With ultrasonography, it is possible to separately assess the effect of plantarflexion and dorsiflexion during both active and passive movements (Table 1) (Hitos et al., 2007; Moloney et al., 2007).

2.4.5 Ultrasound assessment of muscle component of calf muscle pump

Muscle pump function relies on muscle ability to contract and generate pressure against the deep veins, pulling venous blood through these vessels (Meissner et al., 2007a; Shiman et al., 2009). Macroscopic arrangement of muscle fibres is known as a muscle's architecture (Lieber & Friden, 2000), also known as the arrangement of muscle fibres within a muscle relative to the axis of force generation (Lieber & Friden, 2001). Because muscle architecture is a primary determinant of muscle function, understanding this structure-function relationship is of great practical importance, by, for example, clarifying the physiological basis of force production (Lieber et al., 2000, 2001). Muscle force generation ability is related to the amount of muscle mass and in particular to its cross-sectional area (Lieber et al., 2000, 2001). In this case, ultrasound imaging can be employed to determine muscle thickness, which is an indicator of muscle mass and muscle cross-sectional area (Chi-Fishman et al., 2004; Lieber et al., 2000, 2001). Also, ultrasound imaging provides data of muscle fascicles orientation either in relaxed or contracted

conditions, a parameter that is a determinant of the amount of muscle force (Chi-Fishman et al., 2004).

In ultrasound imaging, muscle fibres themselves are hipoechogenic, but the perimysium, that covers muscle fascicles, and the epimysium, covering the whole muscle, are hyperechogenic and are used, together with the aponeurosis and tendons, as references for defining the muscle architecture (Woodhouse & McNally, 2011). The parameters most used of muscle architecture are the muscle length (distance from origin of the most proximal muscle fibres to the insertion of the most distal fibres), fibre length (expression on number of sarcomeres in series, and in most studies measured as the fascicle length), pennation angle (i.e., the fibre angle relative to force-generating axis, that usual range from 0º to 30º for the gastrocnemius muscle) and physiological cross-sectional area (the sum of the cross-sectional areas of all the muscle fibres within the muscles) (Lieber et al., 2000, 2001). The muscle fascicle length plays a role in force generation during high-speed muscle contractions, while fascicle pennation angle and muscle thickness are important factors for overall force generation ability. These parameters can be reliably evaluated with ultrasound (Raj et al., 2012). Gastrocnemius muscle, one of the most important muscles of the CMPF, is a relatively thick muscle with short muscle fascicles, showing high capacity to produce force with low muscle excursion during activity (Lieber et al., 2000, 2001). During gastrocnemius contraction, muscle fascicles shorten from around 126 to 67 mm and the pennation angle increases from 20º to 45º (Lieber et al., 2000, 2001).

Table 1 - Assessment of venous blood flow during calf muscle pump

VEIN ASSESSED	MOVEMENT	VENOUS BLOOD FLOW MEASURES/RESULTS
Popliteal vein	During active plantar flexion of patients with venous ulcer (Moloney et al., 2007).	Increased peak flow velocity to a median of 70.08 (28.73 - 206.2) cm/s.
	After exercise of calf and foot muscles (Hitos et al., 2007).	Increased venous flow velocity (7.3 - 20.2 cm/s) and flow volume (7.7 - 21.5 mL/s), calculated with formula: $\pi \times$ (diameter of popliteal vein)$^2 \div 4$, when compared to rest not using any compression or active movement (5.8 - 15.6 cm/s and 6.6 - 16.3 mL/s), at sitting position in healthy subject with risk of deep venous thrombosis by steadiness during an airplane travel.
	During active tip-toe movement at upright position (Breen et al., 2007).	Increased mean and peak flow velocity and flow volume during an active tip-toe movement at upright position, with accurate measures when automatic assessment of hemodynamic variables are achieved.
Great saphenous vein	After a series of passive and active dorsiflexion-plantarflexion of ankle (Staubesand et al., 1995).	Increased peak flow velocity (21.28 - 30.62 cm/s and 21.01 - 43.12 cm/s, respectively) comparing with rest blood flow velocity (12.28 - 14.30 cm/s), in patients with CVD.
Femoral vein	At lying position during (Sochart et al., 1999):	Increased peak flow velocity, in healthy subjects:
	– Passive dorsiflexion-plantarflexion	31.2 cm/s and 53.8 cm/s, respectively;
	– Active dorsiflexion-plantarflexion	35.6 cm/s and 59.0 cm/s, respectively;
	– Passive inversion-eversion	31.5 cm/s and 53.5 cm/s, respectively;
	– Active inversion-eversion	35.5 cm/s and 61.6 cm/s, respectively;
	– Passive combination for rotation of ankle	Reach 58.0 cm/s;
	– Active combination for rotation of ankle	Reach 70.2 cm/s.

There is scarce data relating muscle architecture with CMPF in healthy subjects and CVD patients. In one study, the volume of calf muscles of patients with venous ulcer was shown to be unrelated with the increase in venous blood peak flow velocity in popliteal vein during maximal plantar flexion contractions (Moloney et al., 2007). No other features of calf muscles architecture was evaluated in patients with CVD, despite existing changes in gastrocnemius muscles in these population, including disseminated myofibril atrophy (especially in fiber type I, accompanied by moderate to severe atrophy in fiber type II, associated to denervation and reinnervation of the gastrocnemius muscle), cell necrosis, inflammatory cell infiltration, proliferation and dilation of interfascicular veins (Qiao et al., 2005).

2.5 Manual lymphatic drainage

2.5.1 Brief history of manual lymphatic drainage

There are four recognized techniques of MLD: the Földi (Tan et al., 2011), Vodder (Kasseroller, 1998), Casley-Smith (Casley-Smith et al., 1998) and Leduc (Leduc et al., 1998).

Back in the nineteenth century, A. T. Still (1828-1917), the founder of Osteopathy, drew attention to the importance of the lymphatic system and of its connection with the venous circulation, but it was Winiwater, in 1890's, an Austrian Physician from Vienna, that described for the first time a lymphatic massage (drainage), associated to leg elevation, compression and exercise (Kasseroller, 1998; Morgan, 2008). After these initial approaches, the theme of lymphatic drainage and

of its application in the treatment of edema remained relatively abandoned, undergoing further developments later by Vodder, Asdonk, and Leduc, and more recently by Földi (Casley-Smith et al., 1998).

In 1920, Miller proposed a so-called thoracic pump technique that would presumably generate fluctuations in intrathoracic pressure and thus increasing lymph flow (Morgan, 2008).

In 1930, Vodder and his wife developed the technique that was called manual lymph drainage (Morgan, 2008), that was originally directed to an essentially normal lymphatic system (like traumatic edema) (Casley-Smith et al., 1998). In 1960, Asdnok, a German physician, tested the Vodder technique of MLD, establishing the indications, contraindication, and the effects of the technique (Morgan, 2008).

Földi combined Vodder's MLD with bandaging, exercise, and skin care and named such therapeutic approach the "complete decongestive physiotherapy", later renamed to as "complete decongestive therapy" (Coleridge-Smith et al., 2006; Morgan, 2008). His work, together with the knowledge about the physiology and pathophysiology of microcirculation and the development of the benzopyrone group of drugs, were one of the most important contributions for lymphedema treatment (Coleridge-Smith et al., 2006). The emphasis in self-care advocated by Casley-Smith, complemented the complete decongestive therapy with techniques of deep breath and self-drainage. (Casley-Smith et al., 1998).

Leduc in 1970's started to publish his research, giving a novel and scientific understanding about the effects of MLD on the lymphatic and venous systems, and about the two basic mechanisms that MLD and other treatments must address: the

reabsorption and evacuation of the lymph, respecting the physiology and pathophysiology of lymphatic system (Leduc et al., 1988; Leduc et al., 1998).

In February 1988, in New York, Földi, Leduc, Vodder and Casely-Smith agreed on the term "decongestive lymphatic therapy" as a suitable name to this group of treatment techniques.

The four methods of MLD show some differences, but the major basic principles are very similar. In short, the maneuvers should be applied softly (with specific exceptions), should consist of a skin-stretching form of massage (not sliding), should comply with the direction of lymph flow, should be done using the entire hand or exceptionally with fingers, and should begin at the proximal regions of the extremity (Casley-Smith et al., 1998; Foldi, 1998a, 1998b; Kasseroller, 1998; Leduc et al., 1998).

2.5.2 Manual lymphatic drainage - Leduc method

MLD is used as a conservative treatment of lymphedema, independently of the specific method (Koul et al., 2007). In the case of the Leduc method, MLD consists of a skin-stretching (Koul et al., 2007) form of massage that applies low pressure (<40 mm Hg) to the underlying tissues (Leduc et al., 1998) along the anatomical distribution of the superficial lymphatic vessels and ganglions, stimulating lymph flow (Leduc et al., 2011; Lee et al., 2011) and the reabsorption of interstitial fluid and macromolecules through the lymphatic circulation (Leduc et al., 2011; Leduc et al., 1998; Lee et al., 2011). At the lower extremity, the call-up maneuver, a technique belonging to the Leduc method, initiates with inciting (or call-

up) maneuvers in the inguinal region (ganglionar stimulation) and then progresses distally along the lower extremity down to the edematous region, again employing call-up maneuvers, in order to stimulate lymph flow by enhancing the contractility of lymphagions of lymph collectors (Leduc et al., 2011; Lee et al., 2011). The reabsorption maneuver, another Leduc technique, is then applied over the edema to drain the interstitial fluid and soluble macromolecules through the lymphatic circulation (Leduc et al., 1988; Leduc et al., 2011; Lee et al., 2011) by stretching the leak filaments (connections between connective tissue to endothelial cell of lymphatic capillaries) when the skin is mobilized (Leduc et al., 1988; Leduc et al., 2011; Lee et al., 2011). The whole procedure ends with a second round of call-up maneuvers which are then applied in the reverse direction, ending at the groin region, in the case of the lower limb (Leduc et al., 2011; Lee et al., 2011). Technically, the call-up maneuver initiates with the most proximal part of the hand and ends with the hands touching the skin while producing skin-stretching and is applied to promote the increase of lymph flow (Leduc et al., 2011; Lee et al., 2011). The reabsorption maneuver initiates with the most distal part of hand and ends with hands touching the skin while applying skin-stretching (Leduc et al., 2011; Lee et al., 2011).

2.5.3 Therapeutic efficacy of manual lymphatic drainage

There are several indications for the use of MLD other than lymphedema, like CVD, post thrombotic syndrome, chronic wounds, traumatic edema (iatrogenic, post-surgical, musculoskeletal injury), complex regional pain syndrome, and lypedema (Morgan, 2008).

The evidence of MLD for the treatment of edema (related to cancer or traumatic during sport activity) (Huang et al., 2013; Vairo et al., 2009) and in improving functional status (related to total knee arthroplasty) (Ebert et al., 2013) is unclear, but has been considered minor. Nevertheless, MLD might have an important role in CVD by improving HRQL, symptoms (Kim et al., 2012; Lasinski et al., 2012), and range of motion (Ebert et al., 2013) when edema/lymphedema is present. In palliative treatment, MLD improves pain and dyspnea (Huang et al., 2013).

Based on a systematic review, the importance of MLD for preventing the incidence of lymphedema is unclear (Huang et al., 2013). A meta-analysis shows that MLD does not provide further therapeutic benefit in reducing lymphedema related to breast cancer, when compared to the standard treatment or with compression therapy (Huang et al., 2013), but another study has demonstrated a benefit when employing MLD in these cases (McNeely et al., 2011). However, the small benefit of MLD must be evaluated together with its cost in terms of time and money spent by patients and health care systems and such cost-benefit evaluation favours the option for compression therapy by using multilayer bandages or compression hosiers and considering adding MLD only if the response to treatment is unsatisfactory (McNeely et al., 2011).

A simple lymphatic drainage, more simple in sequence, but using the same principles as MLD, in a way that can be applied by patients, with self-drainage (de Godoy et al., 2001; Williams et al., 2002) is less effective than MLD in reducing limb volume or lymphedema related to breast cancer, but can be used as a more economical MLD option (Huang et al., 2013).

It has been also suggested that MLD, despite the augmentation of lymph flow (increasing lymphagion contraction and lymphatic reabsorption), might also be responsible for increasing arteriolar blood flow, venous flow, redirection of flow towards collateral vessels, anastomoses, and perhaps stimulating angionesis, but this hypothetical effects of MLD need scientific evidence (Morgan, 2008).

Younger patients, those heavier in weight and higher in body mass index are more likely to show poor lymphedema treatment outcome after intensive decongestive therapy (Huang et al., 2013). When an elastic sleeve and multilayer bandaging are associated to MLD, there is a higher chance that the lymphedema treatment is successful (Huang et al., 2013).

2.5.4 Decongestive lymphatic therapy

Decongestive lymphatic therapy is the physical treatment for lymphedema by combining MLD with other treatments, like low-stretch bandaging and compression garments, exercise, and skin care, and sometimes also with intermittent pneumatic compression (Foeldi et al., 1989; Koul et al., 2007; 2009). This method may reveal itself as effective in the treatment of lymphedema of the lower limb as it is for that affecting the upper limb as a result of cancer (Devoogdt et al., 2010; Lasinski et al., 2012).

Most often, decongestive lymphatic therapy is applied along two phases: the first, is the edema reduction and intensive one, the second one, is the maintenance phase ("The diagnosis and treatment of peripheral lymphedema: 2013 Consensus

Document of the International Society of Lymphology," 2013; Fialka-Moser et al., 2013; Koul et al., 2007).

The first phase is composed of skin care, exercises, MLD (for approximately 1/2 hour), low pressure intermittent pneumatic compression therapy (one hour at a compression of 20 to 40 mm Hg), and multilayer bandages (worn day and night) ("The diagnosis and treatment of peripheral lymphedema: 2013 Consensus Document of the International Society of Lymphology," 2013; Leduc, 2008). In breast cancer-related lymphedema, the patients remain active and proceed with normal activities, but should avoid sport activities (Leduc, 2008). Also, a treatment frequency of five times per week for two to three weeks or, if necessary, four weeks is recommended (Fialka-Moser et al., 2013; Koul et al., 2007; Leduc, 2008).

In the second phase of treatment, that aims to conserve and optimize the results obtained in the first phase, is to recommended that patients receive MLD (1/2 hour), low pressure intermittent pneumatic compression therapy (1 hour), compression hosiery (Leduc, 2008), with low-stretch elastic stocking or sleeve, skin care, and continued exercise ("The diagnosis and treatment of peripheral lymphedema: 2013 Consensus Document of the International Society of Lymphology," 2013). Sometimes, other components, such as self-drainage, might be added (Fialka-Moser et al., 2013; Koul et al., 2007). The treatment is initially commenced daily for 2-3 weeks and depending on clinical evolution can be reduced to once a week (Leduc, 2008).

Sometimes, long term treatment is needed in the presence of some resistant, chronic edemas ("The diagnosis and treatment of peripheral lymphedema: 2013 Consensus Document of the International Society of Lymphology," 2013). This

treatment requires regular MLD (1 session per week), education about the practice of sports, and wearing compression hosiery ("The diagnosis and treatment of peripheral lymphedema: 2013 Consensus Document of the International Society of Lymphology," 2013).

Decongestive lymphatic therapy is often prescribed for patients with venous ulcer and when CVD are associated with mixed edema (lymphatic and venous origin), now combining MLD, compression bandages and stocking, physical therapy to improve calf muscle performance, and in few cases, intermittent pneumatic compression (Steins et al., 2000). In the case of CVD, wearing compression garments is essential for treatment efficacy ("The diagnosis and treatment of peripheral lymphedema: 2013 Consensus Document of the International Society of Lymphology," 2013; Steins et al., 2000).

2.5.5 Contraindications/Precautions

There are several contraindications and precautions for MLD and decongestive lymphatic therapy. It is suggested that cardiac, pulmonary and renal functions should be monitored because of temporary increase in blood flow and circulatory loading (Morgan, 2008).

As for the contraindications, the literature describes erysipelas, lymphatic systemic infection and lymphangitis, meaning inflammation of the lymphatic system, as absolute contraindications for MLD and decongestive lymphatic therapy (Lymphoedema Framework. Management of Lymphoedema. International consensus, 2006). Severe renal and heart failure are also contraindications for the use

of multilayer bandages and intermittent pneumatic compression (Leduc, 2008), whereas caution should be enforced when employing MLD in patients with severe cardiac insufficiency (Leduc, 2008; Leduc et al., 2011; Lymphoedema Framework. Management of Lymphoedema. International consensus, 2006). Unstable hypertension, thyroid dysfunction, hepatic cirrhosis with abdominal fluid (ascites), superior vena cava obstruction, untreated tuberculosis or malaria, are also contraindications for physical treatment (Leduc, 2008; Lymphoedema Framework. Management of Lymphoedema. International consensus, 2006). If swelling occurs for a long time after initial breast surgery, medical examination should be sought and any physical treatment will be stopped if inflammation occurs (Leduc, 2008). Also, Crohn disease, recent surgery, and diabetes are some additional clinical conditions that may be monitored for precaution (Morgan, 2008).

2.5.6 Manual lymphatic drainage in CVD

MLD has also been used as a conservative treatment for CVD, (Steins et al., 2000), mostly when venous lymphedema is present (Mortimer, 2000; Raju et al., 2012). In this case, MLD is recommended to be applied at the level of the root of superficial veins, like GSV (medial aspect of the lower limb) (Leduc et al., 2000; Molski et al., 2009; Peyre et al., 2000).

MLD applied before surgery in patients with CVD improves the clinical class of CEAP classification, HRQL, depression, anxiety, edema and symptoms (Molski et al., 2013; Molski et al., 2009). Nevertheless, foot volumetry and reflux volume index

only improve when MLD is associated to surgery and compression stockings (Molski et al., 2013; Molski et al., 2009).

There is also the assumption that MLD has an effect on blood flow in superficial veins, especially through the call-up maneuver (Leduc et al., 2000). However, the real impact of MLD in hemodynamics is unclear, although it has been suggested as being insignificant (Leduc et al., 2011). One study concluded that 5 to 15 minutes of MLD does not change cardiac output in patients with heart failure despite the near 100% increase in venous return after 5 minutes of MLD (Leduc et al., 2011).

However, there is little information about the acute effect of MLD on blood flow in the superficial and the deep veins of the lower extremity and if there are, in fact, differences in such effect between alternative MLD maneuvers, like between the call-up and reabsorption maneuvers.

2.6 The role of conservative treatments of CVD

The treatment of patients with CVD might focus on both the symptoms and secondary changes of the disease, such as, for instance, edema, skin and subcutaneous changes or ulcers (Gloviczki et al., 2011). Usually, initial treatment of CVD patients involves a non-invasive conservative treatment to reduce symptoms and help prevent the development of secondary complications and the progression of the disease (Eberhardt et al., 2005). Complementary, or posteriorly, some interventional or surgical treatments can be undertaken (Eberhardt et al., 2005; Gloviczki et al., 2011).

Behavioral education, like giving advices to raise the legs to minimize edema and reducing intra-abdominal pressure, for exercising, for using compressive stockings and proper care of the skin and wounds, together with pharmacological therapy, are the most common referred conservative treatments (Eberhardt et al., 2005; Gloviczki et al., 2011). The conservative pharmacological treatment with venoactive drugs may be indicated for patients with pain and edema and should be implemented in association with compression for healing venous ulcers (Gloviczki et al., 2011). If conservative treatment is unsuccessful or provides an unsatisfactory response, then further treatment, including surgery, should be considered based on anatomic and pathophysiological features (Eberhardt et al., 2005).

Interventional treatments, like sclerotherapy, ablative therapy with endovenous radiofrequency and laser, endovascular therapy, are less invasive than surgery for treating CVD (Eberhardt et al., 2005). It has been recommended to use these techniques to treat superficial incompetence (endovenous thermal ablation, as laser and radiofrequency) and varicose veins (sclerotherapy) (Gloviczki et al., 2011).

Surgical treatments are recommended in severe forms of CVD, like venous ulcers that did not heal after 6 months of treatment (Eberhardt et al., 2005). There are several surgical procedures described in the literature, like ligation, stripping and venous phlebectomy, subfascial endoscopic perforator surgery or valve reconstruction (Eberhardt et al., 2005).

In CVD, the compression, like that provided by stockings, is recommended as a primary treatment, except when patients are candidates for vein ablation, in which case compression is also suggested as an adjuvant treatment, particularly to prevent ulcer recurrence (Gloviczki et al., 2011). Compression therapy is recommended as a

complement to surgery (like stripping), and to venoactive drug treatment, in order to control edema and pain, and to enhance venous ulcer healing (Gloviczki et al., 2011). MLD is also recommended to be used before venous surgery, and should be complemented with compression stockings (Molski et al., 2013; Molski et al., 2009).

There are several conservative treatments described in the literature other than MLD and compression to treat and prevent complications associated with CVD, like exercise (Kahn et al., 2011; Padberg et al., 2004), intermittent pneumatic pressure (Lurie et al., 2008), kinesio taping (Aguilar-Ferrandiz et al., 2013a; Aguilar-Ferrandiz et al., 2013b), electrical muscle stimulation (Clarke Moloney et al., 2006; Izumi et al., 2010), transcutaneous electrical nerve stimulation (Izumi et al., 2010), hydrotherapy (Carpentier et al., 2014; Carpentier & Satger, 2009), health education (Knapp et al., 2011). Most of these techniques are complementary to compression therapy or pharmacological treatment.

3 SCOPE OF THE THESIS AND HYPOTHESIS

Despite the literature assuming that MLD might be a potential conservative treatment for CVD, in association with surgery (Molski et al., 2013; Molski et al., 2009), or combined with other physical therapy treatments (Steins et al., 2000), the real efficacy of MLD alone remains unknown. The exact mechanisms underlying the therapeutic effects of MLD are doubtful, and may be associated with increased lymph flow (Leduc et al., 2011; Lee et al., 2011) or with increased venous flow (Leduc et al., 2011).

Also, there is little information about the acute effect of the MLD maneuvers (reabsorption and call-up of the Leduc technique, both a low pressure, skin-stretching form of massage maneuver) on blood flow in the superficial and the deep veins of the lower extremity and if there are, in fact, differences between these two techniques in their effects. As for the lymphatic circulation, these MLD maneuvers may have an impact on venous blood flow both on superficial and deep veins of the lower extremity, but this effect is assumed to be mild (Leduc et al., 2011).

In addition, the best anatomical regions of the lower limb where of MLD maneuvers should be applied remains unknown, despite the indication that they should be applied along the route of GSV (Leduc et al., 2000; Molski et al., 2009; Peyre et al., 2000).

The major importance of CMPF in CVD physiopathology is well known (Panny et al., 2009; Shiman et al., 2009; Yamaki et al., 2010; Yang et al., 1999), but the efficacy of MLD in improving this function was not evaluated yet. Notwithstanding, several studies use the vascular ultrasound to estimate the capacity

of ejection of venous blood during muscle contractions (Hitos et al., 2007; Moloney et al., 2007; Staubesand et al., 1995) a dynamic characterization of CMPF in CVD patients is still missing. In particular, the relationship between calf muscles architecture and muscle pump function in CVD patients has not been established. Although the volume of calf muscles in patients with venous ulcer was shown not to be related with the capacity to increase venous blood flow velocity in popliteal vein during a maximal plantar flexion contraction (Moloney et al., 2007), there are several other muscle morphological features that determine muscle performance and that could be affected in CVD patients (Qiao et al., 2005).

The efficacy of CMPF is usually evaluated using air-plethysmography (Nicolaides, 2000). However, this method requires equipment not readily available in most clinical settings and the development of alternative method to accurately assess CMPF could stimulate further investigation about the role of muscle pump function in CVD (Bradbury, 2010; Nicolaides, 2000; Padberg et al., 2004; Panny et al., 2009; Sandor, 2010).

3.1 Objectives of the study

The objectives addressed in this thesis were the following:

1- To evaluate CMPF in CVD patients using ultrasound imaging and to relate this function with gastrocnemius muscle architecture.

2- To assess the effect of the two low-pressure manual skin-stretching maneuvers of MLD: the call-up and the reabsorption on both deep and superficial venous blood flow in patients with CVD.

3- To verify whether the effect of MLD on venous blood flow differs if applied to different anatomical regions of the lower limb or between deep and superficial veins.

4- To assess the long term effect of MLD in the conservative treatment of CVD.

3.2 Hypothesis

We defined the following hypothesis:

1- Calf muscles architecture is altered in CVD patients when compared with healthy subjects and these changes are related with the efficacy of the CMPF.

2- Call-up and reabsorption maneuvers of MLD technique will enhance venous blood flow to a similar extent and in both the superficial and the deep veins of the lower extremity.

3- Higher venous blood flow enhancement occurs when MLD techniques are applied to the medial aspect of the thigh and of the leg, thus in coincidence with the course of GSV.

4- MLD will improve HRQL, clinical and functional status of patients with CVD and that this effect will remain at the end of a short-term follow-up. We also anticipate that the effect of MLD on CVD severity and symptoms are enhanced by compliance to compression stockings.

4 INSTRUMENTS AND METHODS

4.1 Studies outline

This thesis is based on four separate studies, which are outlined in Table 2. The studies addressed each of the problems stated in Section 3. Three of the studies were of a cross-sectional design, including CVD and healthy control participants (one to assess reproducibility of the CMPF assessment, and two to assess hemodynamic effects of MLD), whereas the fourth study followed a prospective, single-blind randomized controlled trial (RCT) design, aimed at assessing the efficacy of MLD for the treatment of CVD.

The first study (Study I) was performed to assess CMPF dynamically in a group of CVD patients and healthy controls using ultrasound. This study also aimed to assess the relationship between architecture of the two heads of the gastrocnemius muscle and the blood pumping function of calf contractions.

The second study (Study II), was performed to assess the effect of the two low-pressure manual skin-stretching maneuvers: the call-up and the reabsorption on both deep and superficial venous blood flow in patients with CVD. Vascular ultrasonography was used to measure the cross-sectional area and blood velocity at the femoral vein (FV) and GSV during MLD maneuvers applied to the medial aspect of the thigh in CVD (patients with varicose veins, edema, skin and trophic changes) and healthy participants.

The third study (Study III) was performed to verify if venous blood flow enhancement is the same when MLD is performed over different anatomical regions

of the lower limb. MLD applied to the medial versus the lateral aspect of the thigh and of the leg. Vascular (veins' cross-sectional area) and hemodynamics changes (flow velocity and flow volume) in the FV, GSV, popliteal vein (PV), and small saphenous vein (SSV) were assessed with ultrasound imaging in CVD (edema, skin and trophic changes and healed ulcer) and healthy participants.

The fourth study (Study IV) was performed to assess the long term effect of MLD (10 sessions, during four weeks) in the conservative treatment of CVD, having as outcomes HRQL, severity of disease, symptoms (heaviness and fatigue), leg volume, calf muscle strength (torque, torque/body weight, total work, and average power for dorsiflexors and plantarflexors muscle), and ankle dynamic range of motion in patients with CVD. Also, how adherence to compression stockings moderates the effect of MLD in this group of patients was also evaluated, as using such stockings is a primary conservative treatment for CVD.

4.2 Ethics

Before study enrolment, all participants were informed about the purpose and procedures of the study and signed an informed consent (Appendix 1 to 4). All studies received ethical approval from the Ethics Council of Faculdade de Motricidade Humana da Universidade de Lisboa. Our randomized controlled trial was registered with ClinicalTrials.gov registry (register number: NCT01899482).

Table 2 - Objectives and Procedures of the studies

OBJECTIVES	PROCEDURES
Study I	
Respond to Hypothesis 1. To evaluate CMPF in CVD patients using ultrasound imaging and to relate this function with gastrocnemius muscle architecture.	Design: Cross-sectional design with a CVD and a healthy control group of participants. Outcomes and intervention: - Clinical class of CEAP Classification; - Severity of disease: VCSS; - Vascular ultrasound of popliteal vein for assessing CMPF, during 10 tip-toe movements (assessed twice with one week apart); - Gastrocnemius muscle architecture evaluated, with the ankle in plantarflexion, dorsiflexion and neutral position.
Study II	
Respond to Hypothesis 2. To assess the effect of the two low-pressure manual skin-stretching maneuvers: the call-up and the reabsorption on both deep and superficial venous blood flow in patients with CVD.	Design: Cross-sectional design with a CVD and a healthy control group of participants. Outcomes and intervention: - Clinical class of CEAP Classification; - Severity of disease: VCSS; - Venous hemodynamic in GSV and FV during MLD (call-up and reabsorption maneuver) applied at medial aspect of thigh.
Study III	
Respond to Hypothesis 3. To verify whether the effect of MLD on venous blood flow differs when applied to different anatomical regions of the lower limb and between deep and superficial veins.	Design: Cross-sectional design with a CVD and a healthy control group of participants. Outcomes and intervention: - Clinical class of CEAP Classification; - Severity of disease: VCSS; - Venous hemodynamics in GSV and FV during MLD applied at the medial and lateral aspects of the thigh, and in SSV and PV during MLD applied at the medial and lateral aspects of the leg.
Study IV	
Respond to Hypothesis 4. To assess the long term effect of MLD in the conservative treatment of CVD.	Design: A single blind randomized controlled trial with patients with CVD Outcomes and intervention: - CEAP Classification; - Severity of disease: VCSS; - Health-related quality of life and symptoms quantification; - Leg volume; - Ankle isokinetic dynamometer assessment; - The intervention in experimental group was one educational session and 10 MLD sessions during 4 weeks; - The intervention in control group was one educational session.

CVD: chronic venous disease. CMPF: calf muscle pump function. MLD: manual lymphatic drainage. VCSS: Venous Clinical Severity Score. CEAP: Clinical Etiological Anatomical Pathological classification. GSV: great saphenous Vein. SSV: small saphenous vein. FV: femoral vein. PV: popliteal vein.

4.3 Participants

For Study I, participants were recruited from Lisbon area, whereas for the other three studies, participants were recruited from Castelo Branco area. In our studies, participants were excluded because of few contraindications/precautions of MLD. Therefore, the exclusion criteria included: 1) the presence of severe cardiac insufficiency, 2) acute venous or arterial obstruction, 3) renal insufficiency, 4) uncompensated thyroid dysfunction, 5) neoplastic pathology, 6) systemic or limb infection, 6) age over 65 years. Because other conditions may influence the severity and functional status of CVD, participants were also excluded if they presented recent musculoskeletal injury of the lower limb, arterial insufficiency, peripheral neuropathy in the lower limb, and pregnancy.

In Study I, 15 healthy control participants (10 women and 5 men), and 16 participants (13 women and 3 men) with a diagnosis of CVD (CVD group), were enrolled. All CVD participants presented venous blood reflux in at least one vein of the lower extremity with a minimum duration of 0.5 sec, and CEAP clinical classification in the range C_{1-4}.

For Study II, 23 participants (13 women and 10 men) with a diagnosis of CVD (CVD group), most of whom were outpatients in a local health unit, and 18 healthy control participants (10 women and 8 men), were recruited. All CVD participants presented pathological venous blood reflux in the lower extremity and CEAP clinical classification in the range C_{1-5}. Seven subjects were excluded: two participants presented active ulcer (C_6), two participants were diagnosed with cardiac insufficiency, and three participants had severe arterial insufficiency. Three

participants from the CVD group had their GSV not evaluated due to previous surgery of this vein. In three other participants from this group, the FV was not evaluated because of time constraints.

In Study III, 57 participants were included, 28 patients (21 women and 7 men) with CVD, and 29 healthy control participants (17 women and 12 men). All CVD participants presented pathological venous blood reflux in the lower extremity and a CEAP classification in the range C_{3-5}. Twenty two participants were excluded: 11 participants presented a clinical CEAP classification below C_3, 2 participants presented active ulcer (C_6), 3 participants suffered from cardiac insufficiency, 3 participants had arterial insufficiency, and 3 participants were over 65 years of age. In 6 participants with CVD, data was not collected from the GSV due to previous surgical treatment. For the same reason, the SSV was also excluded from data collection in 5 participants of this group.

Study IV, a single blind randomized controlled clinical trial, was conducted in patients recruited from a school-based health community attendant service between October 2011 and July 2012. All the participants had CVD with C_{3-5}, according to the clinical class of CEAP classification. Out of the 125 prospective candidates, 50 met the eligibility criteria and were randomly allocated to the experimental (N = 25) or to the control group (N = 25). During the first visit, the participants were informed that they would be expected to complete 10 treatment sessions over a period of 4 weeks, and another 3 sessions for assessments, starting within the following 2 months. Those conducting the assessments were blinded to group allocation, and the physical therapists applying the treatment were unaware of the results of the assessments. After the first round of testing [Time 0 (T0)], all

participants attended an educational session. Participants in the control group did not receive any further treatment during the time of the study. All participants were instructed to maintain their habitual CVD-management scheme as well as their habitual daily-life activities during the study. Participants in CVD group were reassessed at the end of the MLD-treatment program [Time 1 (T1)], and after 4 weeks of follow-up [Time 2 (T2)]. Participants in the control group were tested with 4-weeks intervals between T0-T1, and T1-T2. The timeline of the study, including sampling, testing and intervention, is depicted in Figure 1. Participants in the control group were also provided with the MLD treatment after T2.

The demographic and clinical data of the participants are presented in Table 3 to 9. Some participants were assessed in more than one study.

4.4 Diagnose and Severity of disease

The presence of CVD relies on patient's history and physical examination (Gloviczki et al., 2011). It is recommended that the clinical evaluation (signs and symptoms) is carried out by the CEAP classification and the venous clinical severity score (VCSS) (Gloviczki et al., 2011; Kakkos et al., 2003). Complementary diagnostic testing, like duplex scanning, is also recommended (Gloviczki et al., 2011). Patients' health history is important because several comorbidities, like diabetes, arterial insufficiency, lower limb injury, may influence the clinical status of CVD patients, and is important also to assess the history of vascular or lower limb surgery, medication, and other treatments. Also, the presence of symptoms was always registered.

4.5 Duplex scanning: diagnose of CVD

Duplex scanning is a safe, non-invasive, cost-effective, and reliable exam, recommended as the first diagnostic test for all patients with suspected CVD (Gloviczki et al., 2011). The test includes three components: visibility, compressibility, venous flow, including duration of reflux and augmentation (Gloviczki et al., 2011). A colour duplex ultrasound is usually recommended using a high-frequency linear array transducer of 7.5 - 13MHz, for superficial veins (Coleridge-Smith et al., 2006) and 4 - 7 MHz, for deep veins (Gloviczki et al., 2011). In case of very edematous regions, a 3.5 - 5 MHz curvilinear array can be useful (Coleridge-Smith et al., 2006). At B-mode the image of lumen should be evaluated, at transversal or/and longitudinal view, and the scanning parameters should be adjusted for correct identification of acute or chronic thrombosis (Coleridge-Smith et al., 2006). Pulsed–waved spectral or colour Doppler to assess the velocity and direction of venous flow, should be undertaken with a recommended Doppler range of 5 - 10 cm/s with the wall filter at its lowest setting, and the angle of insonation should be at 45 - 60º (Coleridge-Smith et al., 2006). During the examination (reflux and diameter), patients are at upright position and several methods are used to elicit reflux: release after calf squeeze for proximal veins, and foot squeeze for calf veins (used during our studies), manual compression of vein cluster, calf pneumatic cuff deflation (the more reproducible method), active foot dorsiflexion and relaxation, and Valsalva maneuver, which is useful to demonstrate saphenofemoral incompetence (also used in our study when manual compression did not allowed to asses this outcome) (Coleridge-Smith et al., 2006). A reflux value of 0.5 seconds is

considered a reliable indication of presence of CVD (Coleridge-Smith et al., 2006) (employed in Study I using ultrasound equipment HI VISION 8500, Hitachi, with a L53 linear array-transducer, scanned at 9 MHz), but recent guidelines suggest s a 0.5 seconds cutoff values for saphenous, tibial, deep femoral, and perforating vein incompetence (also with vein diameter > 3.5 mm), whereas a value of 1 second is considered a cutoff value for femoral and popliteal veins incompetence (Gloviczki et al., 2011) (criteria employed in Study II-IV, using the ultrasound equipment ESAOTE mylab 30 cv, with 7 mm linear array-transducer, scanned at 6 - 12 MHz).

4.6 CEAP classification

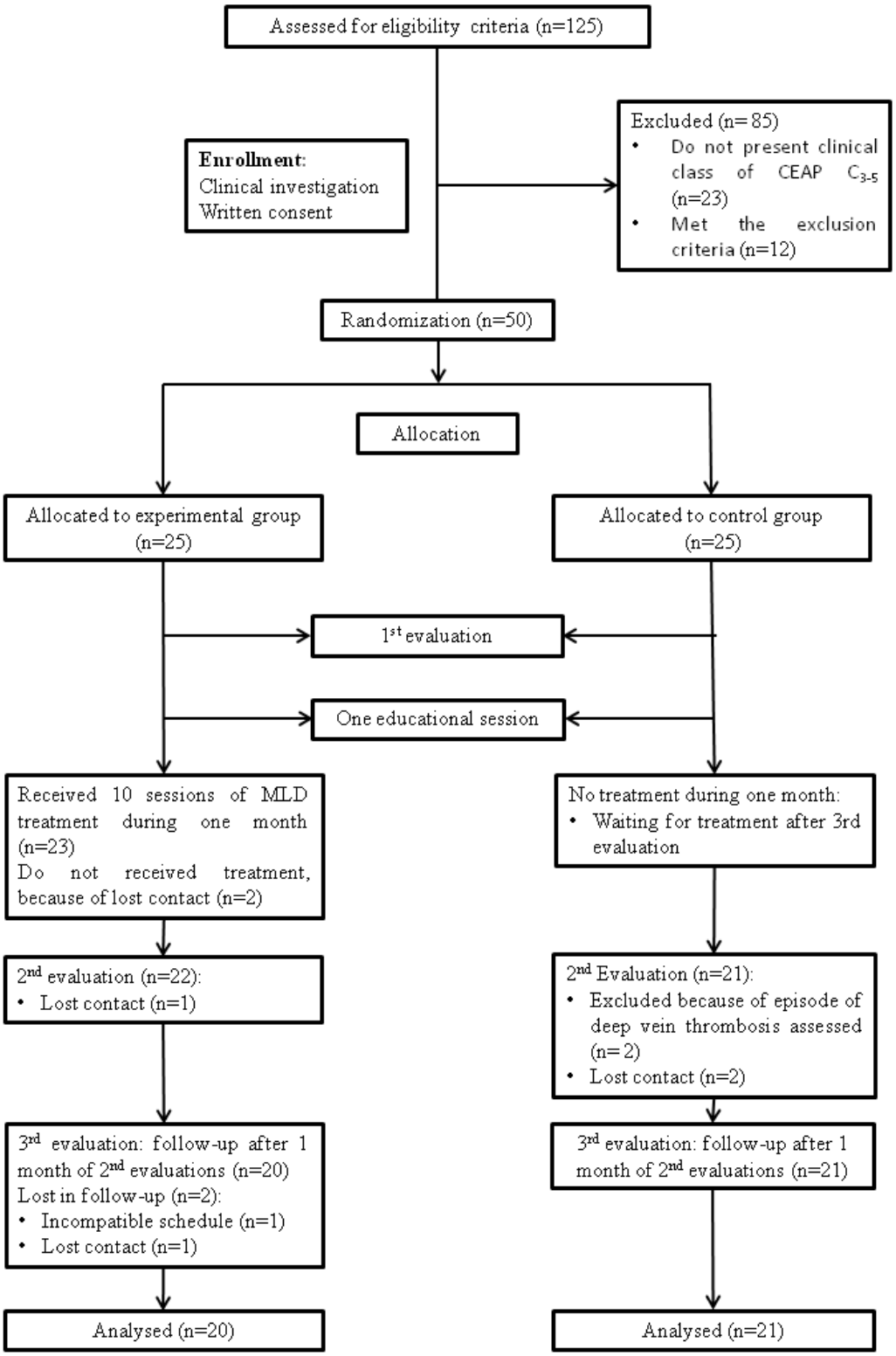
The CEAP classification was created to facilitate communication about CVD severity and for scientific research (Eklof et al., 2004). The CEAP classification was based on 1) clinical manifestations (C), 2) etiologic factors (E), described as congenital, primary, secondary (post-thrombotic), 3) anatomical distribution of disease (A), that can be located at superficial, perforator or deep veins, and 4) underlying pathophysiological findings (P), such as reflux, obstruction or both reflux and obstruction (Eklof et al., 2004). Subscripts are applied to designate S (symptomatic) from A (asymptomatic) limbs (Padberg, 2005). According to CEAP, there are six CVD categories that range from C_0 to C_6 (Eklof et al., 2004; Padberg, 2005). Also, the N subscript indicates no evidence of disease and is applicable to E, A, and/or P of CEAP (Eklof et al., 2004; Padberg, 2005).

The C_0 represents those individuals with objective evidence of venous disease (i.e., E, A, and/or P), but with no clinical manifestations. The C_1 is characterized by

the presence of telangiectasia or reticular veins (< 3mm in diameter). In the C_2 class, varicose veins (> 3 mm in diameter) are present. The C_3 distinguishes itself from the preceding categories by the presence of edema of venous etiology. In the C_4 class, there are now skin trophic changes, like C_{4a}, for pigmentation and/or eczema, and C_{4b}, for lipodermatosclerosis and/or white atrophy. Classes C_5 and C_6 are associated with the occurrence of venous ulcers: the C_5 corresponds to cases of prior ulceration that healed, and C_6 to cases with active venous ulcers (Eklof et al., 2004; Padberg, 2005).

The CEAP classification is the gold standard for classification of chronic venous disorders today and its use is recommended by the relevant guidelines (Gloviczki et al., 2011). Nevertheless, for proper use of CEAP some facts have to be taken into account: the CEAP classification is limited as a severity classification, C_2 summarizes all kinds of varicose veins, in C_3 it may be difficult to separate between venous and other reasons for edema, and corona phlebectatica is not included in the classification (Rabe & Pannier, 2012). Further revisions of the CEAP classification may help to overcome the still-existing deficits (Rabe et al., 2012). Complementary to this classification system, some concepts were defined to give consistency to the scientific terms, like the CVD concept that designates any venous disorder associated to every clinical class, and the concept of chronic venous insufficiency, which represents the more severe stages of the disease (C_{3-6}) (Eklof et al., 2004; Padberg, 2005). For the sake of consistency and simplicity, in this thesis we opted not to use the term chronic venous insufficiency even in the cases where its use would be correct, such as in Study IV, in which all CVD participants were in the severity range C_{3-5}. Hence, the term CVD was used at all times in this thesis.

Figure 1- Flow diagram for Study IV



4.7 Severity of disease: Venous Clinical Severity Score

The CEAP clinical score assesses the severity of CVD. The severity is given by a global score in the range 0 - 18 (best to worst) obtained by summing the items 1) pain, 2) edema, 3) venous claudication, 4) pigmentation, 5) lipodermatoesclerosis, 6) ulcer size, 7) ulcer duration, 8) ulcers number, and 9) ulcer recurrence, each item scored as 0-2 (Kakkos et al., 2003).

The procedure VCSS, that might be used in substitution of CEAP clinical score, was employed in all studies comprising this thesis. The VCSS was developed to supplement the CEAP classification and to give an additional weight to the more severe consequences of CVD (Rutherford et al., 2000). The VCSS score has shown good intra and inter-observer reliability and responsiveness to change (Kakkos et al., 2003; Ricci et al., 2003; Rutherford et al., 2000). This is a score that quantifies 10 items using the range: 0 (none), 1 (mild), 2 (moderate), and 3 (severe), with a total range score of 0-30 (best to worth) (Kakkos et al., 2003; Ricci et al., 2003; Rutherford et al., 2000; Vasquez et al., 2010). In CEAP classes C_0 to C_6, the VCSS score is reported to range between of 3-18 (Lozano Sanchez et al., 2012). A worthwhile clinical improvement for patients with CVD can be observed with a relative improvement of 70% in VCSS score (Kakkos et al., 2003) or with an absolute improvement of 4 points (Cesarone et al., 2010). Differences between clinical classes are 1-2 points of VCSS below C_3, and 2-5 points above C_3 (Lozano Sanchez et al., 2012). The items of the VCSS are:

- Pain or discomfort (i.e., aching, heaviness, fatigue, soreness, burning, with presumed venous origin), patients are asked to describe for each leg the category that best describes this item;
- Varicose veins (with diameter ≥ 3 mm in standing position);
- Venous edema (presumed venous origin, i.e., pitting edema present in every days and with significant effect of standing/limb elevation or evidence of venous etiology, like varicose veins or history of deep vein thrombosis) - clinical staff must exam both legs and should ask patients about the extent of edema experienced;
- Skin pigmentation (presumed of venous origin and not including focal pigmentation over varicose veins or pigmentation due to other chronic diseases) - clinical staff must exam each leg;
- Inflammation (more than just recent pigmentation, like erythema, cellulitis, venous eczema, dermatitis);
- Induration (presumed of venous origin with secondary skin and subcutaneous changes, such as chronic edema with fibrosis, hypodermitis, white atrophy, and lipodermatoesclerosis);
- Active ulcers number;
- Active ulcers duration (patients are referred to describe the duration of the longest unhealed ulcer);
- Active ulcers size (score according the size of the largest active ulcer);
- Use of compression therapy (patients should be asked about their compliance to compression therapy).

The assessment of the items of VCSS score should be carried out for both legs.

Demographic, CEAP and VCSS data were registered in one single characterization questionnaire (Appendix 5). The leg self-reported as presenting the worst symptoms and clinical signs and the dominant leg were chosen for subsequent measures in, respectively, the participants of CVD groups and the healthy-control groups.

4.8 Health-related quality of life and symptoms quantification

Health professionals-reported outcomes, such as VCSS and CEAP classification, are convenient, easily evaluated, and relevant. However, they remain biased by the observers' expectations. Patient-reported outcomes are recognized by medical authorities as the ultimate outcome for health-care interventions, and for chronic venous disorders self-reported assessment of symptoms and HRQL is recommended (Guex, 2012).

HRQL was assessed by the Chronic Venous Insufficiency Questionnaire (CIVIQ-20) (Annex 3), translated and culturally validated to Portuguese. Psychometric validations confer relevance, acceptability, reliability, construct validity and sensitivity to CIVIQ-20 questionnaire (Launois et al., 2013). The dimensions composing the CIVIQ-20 are physical (4 items), psychological (9 items), social (3 items), and pain (4 items) (Launois et al., 2010). In our studies, the scores for each dimension and for the global HRQL index were obtained using the scale 0-100 (best to worse). The global score and the score for each dimension is around 60-

70 points for patients with CVD of classes C_{0s-2}, and around 50-60 points for classes C_{3-4} (Launois et al., 2010), decreasing in C_{5-6} to a median score value of 52.4 points (Lozano Sanchez et al., 2012). An improvement higher than 8-10 points in CIVIQ-20 score represents a worthwhile clinical improvement for patients with CVD (Launois et al., 2010).

Authorization for using the Portuguese version of CIVIQ-20 was given and a memorandum of agreement was signed with authors Robert Lounois and *Les Laboratoires Seveier* (Neuilly-sur-Seine, France).

4.9 Symptoms

Additionally to HRQL, symptoms (fatigue and heaviness, very frequent symptoms in CVD) (Bobridge et al., 2010; Launois et al., 2010) were quantified by visual analogue scale (Launois et al., 2010). The visual analogue scale for fatigue and heaviness is completed by the respondent, which is asked to place a line perpendicular to the visual analogue scale line at the point that represents their symptom intensity. The quantification is made with a score of 0-10 (best to worse), using a ruler that measures the distance on the 10-cm line. The visual analogue scale presents good acceptability, validity, reliability, and sensitivity to detect changes in symptoms, such as pain in chronic conditions (Hawker et al., 2011), and is used to assess symptoms related to CVD (Bobridge et al., 2010; Launois et al., 2010). An improvement higher than 1.8-2.1 points in visual analogue scale score represents the minimum clinically important difference for patients with CVD (Launois et al., 2010).

4.10 Vascular ultrasound assessments for venous hemodynamic

Although previously employed in many studies (Breen et al., 2007; Hitos et al., 2007; Staubesand et al., 1995), ultrasound-measured venous blood peak velocity alone seems not to be an accurate measure of CMPF (Breen et al., 2007). However, calculating blood volume, or its variation, using vascular ultrasound looks otherwise to be a suitable procedure to assess CMPF (Breen et al., 2007). Measuring hemodynamic parameters, like volume flow (cross-sectional area X time average mean velocity) (Hitos et al., 2007) is also possible, and the measures are reproducible when the method is optimised by choosing a sampling volume that covers the entire venous lumen, using a probe incidence angle of 60 degrees, and measuring venous blood flow for a period of 40 seconds or longer (on pulse-wave imaging) (Ogawa et al., 2002). The vein's cross-sectional area can also be measured accurately by ultrasound (in B-mode imaging), after defining the vein's contour (Lurie et al., 2002).

Nevertheless several studies use venous blood peak flow velocity of lower limb veins to assess the effect of treatments on venous hemodynamics (Clarke Moloney et al., 2006; Izumi et al., 2010; Lurie et al., 2008).

4.10.1 Popliteal vein assessment for calf muscle pump function

After five minutes rest, the cross-sectional area of the popliteal vein was measured at the level just below the sapheno-popliteal junction (Figure 2) with subjects in prone position and using a linear array transducer (HI VISION 8500,

Hitachi, with a L53 linear array-transducer, scanned at 9MHz). Next, baseline mean and peak blood flow velocities in the popliteal vein were measured in standing position. Blood flow velocity was measured during a 10-seconds interval using an automatic time integral calculation. After completing the baseline measures, subjects performed a protocol of calf muscle contractions similar to that commonly used to assess the efficacy of CMPF by air-plethysmography and composed by three sets of ten repetitions of tip-toe movements (Figure 3) (Nicolaides, 2000; Padberg et al., 2004). A metronome set the timing of the tip-toe movement that took 2 seconds to rise to the tip-toe, 2 seconds to get down to the initial position and 1 second interval to the next repetition. The ultrasound probe was manually held at the lower margin of the popliteal fossa with adequate orientation to measure blood flow velocity in popliteal vein, while subjects performed the tip-toe movement. Between sets, subjects rested for five minutes in sitting position. Mean and peak blood volume flowing through the popliteal vein at baseline were calculated. Dynamic peak (at first and tenth contraction) and mean (at first contraction) blood flow velocity were calculated using the integral of the blood flow-time curve. To avoid the effect of the foot sole impact with the ground on venous blood flow, only the time interval corresponding to heel rising was selected to analysis. During tip-toe movements, flow velocity data was normalized against baseline popliteal vein flow velocity to calculate flow velocity augmentation using the following equation:

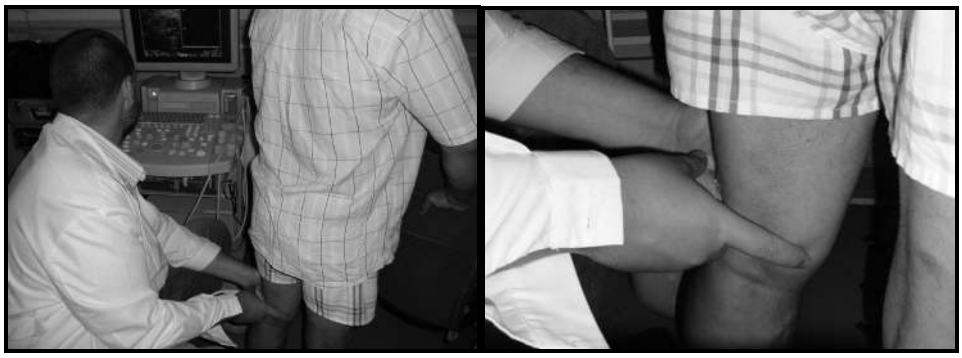
$$\text{Augmentation (\%)} = [(\text{flow velocity during contraction (cm/s)} - \text{flow velocity during baseline (cm/s)}) \div \text{flow velocity during baseline (cm/s)}] \times 100$$

The percentage augmentation in flow velocity was calculated using both the peak flow velocity registered during the first and tenth tip-toe movement in each set.

To assess reliability of ultrasound measures, 17 subjects (CVD group, N = 9; control group, N = 8) visited the laboratory for a second session. During this visit subjects repeated the tip-toe protocol in order to assess reproducibility of ultrasound measures of blood pumping by calf muscles, using test-retest reliability and calculating the absolute agreement intraclass correlation coefficient (ICC) (Schuck, 2004), and by agreement analysis using Bland and Altman plot analysis. For each participant, tests were carried out at the same time of day and at the same ambient temperature. Also to evaluate the bias eventually caused by probe unsteadiness during the movement repetitions, few subjects performed actual and simulated tip-toe movements. The simulated movements were done with subjects standing on a platform with the foot of the measured side hanging off the platform and performing active plantarfexions of the ankle joint by contracting the calf muscles. During this task, leg movement is minimal and the probe can be kept rather stationary.

This methodological procedure was used in Study I.

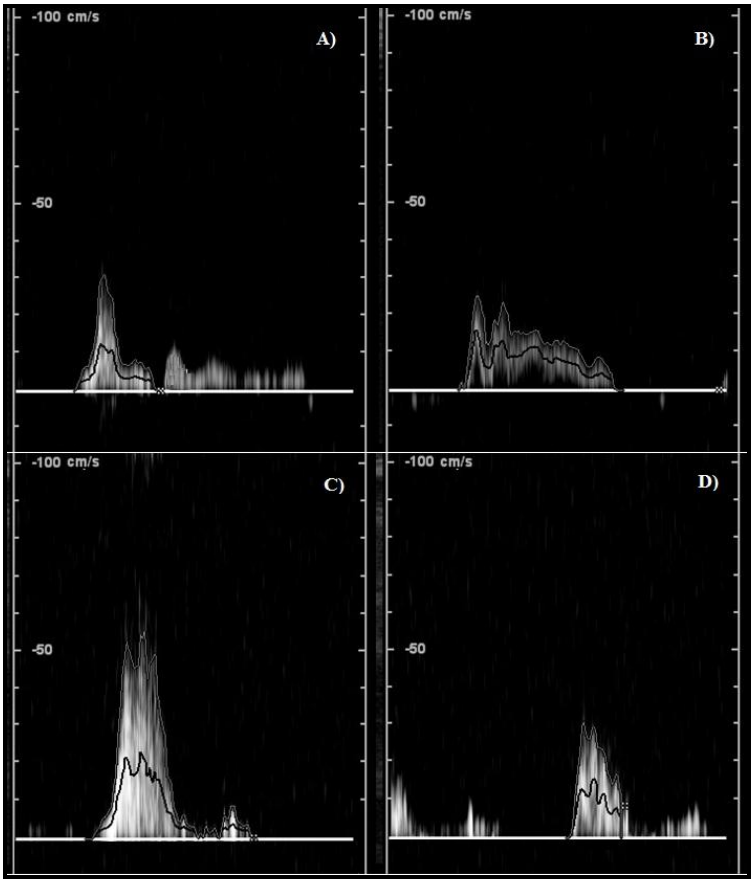
Figure 2 - Assessment of cross-sectional area and flow velocity at popliteal vein with vascular ultrasound



4.10.2 Venous hemodynamics during manual lymphatic drainage

Four veins were assessed using venous duplex ultrasound with ultrasound equipment ESAOTE mylab 30 cv, with 7 mm linear array-transducer, scanned at 6-12 MHz. These included two superficial veins, the GSV, insonated immediately below confluence of the superficial inguinal veins, and the SSV, insonated immediately below the saphenopopliteal junction, and two deep veins, the FV, insonated below confluence of the superficial inguinal veins, and the PV, insonated just below saphenopopliteal junction.

Figure 3 - Venous ultrasound assessment at popliteal vein during first and tenth calf contraction, during tip-toe movement for both control and CVD group



Popliteal vein blood flow velocity during: A) the first and B) the last (tenth) repetition of the tip-toe movement task in a CVD participant and popliteal vein blood flow velocity during C) the first and D) the last (tenth) repetitions of the tip-toe movement task in a healthy subject Also shown are the automatic tracings of the blood flow velocity curve envelope and of the calculated mean (dark trace).

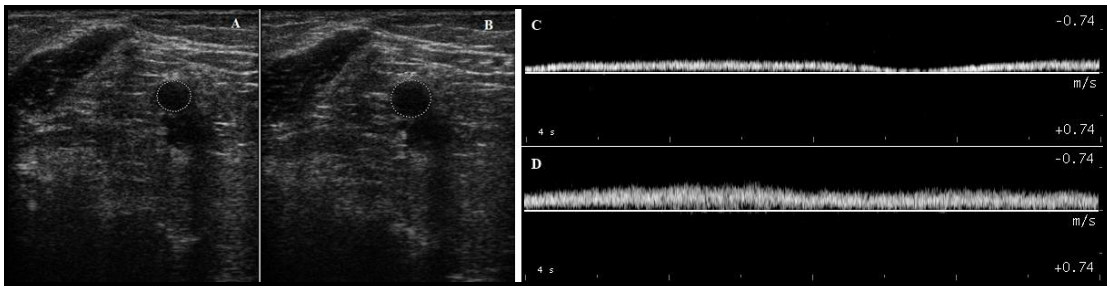
The veins were scanned in B-mode for 4 seconds to measure the vein cross-sectional area, using tracings of vein's contour in the ultrasound scan, and selecting the highest cross-sectional area corresponding to expiratory moment of respiratory cycle. Venous peak and mean flow velocities were measured during 4-second intervals (during approximately a respiratory cycle), using the time integral

calculation. Three measurements of cross-sectional area and of blood flow velocity were taken for veins and the average was computed for analysis (Figure 4).

Before measurements, the participants rested for 5 minutes in a supine position. Blood flow volume in the FV, GSV, PV, SSV was calculated based on measurements of cross-sectional area and blood flow velocity.

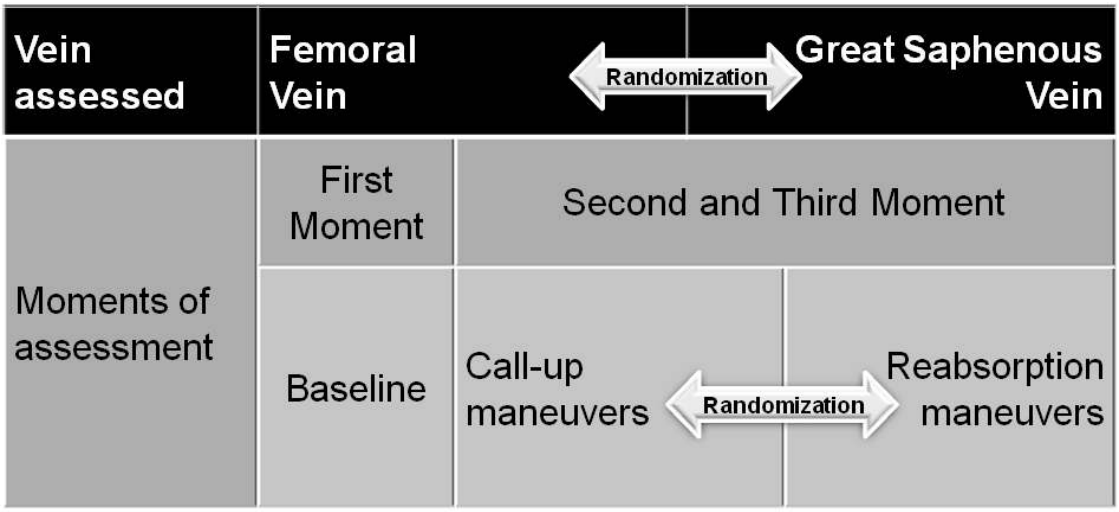
In Study II, to compare the effect of call-up and reabsorption maneuvers of MLD, ultrasound measurements were taken from FV and GSV first at baseline (with no maneuver) and thereafter during randomly applied call-up and reabsorption maneuvers to the medial aspect of the thigh. Measurements were taken with participants in a supine position (Figure 5 and Figure 6).

Figure 4 - Representative ultrasound images showing the effect of MLD



Cross-sectional area at A: baseline and B: with manual lymphatic drainage and blood flow velocity at C: baseline and D: with manual lymphatic drainage, for popliteal vein.

Figure 5 - Randomization for duplex ultrasound assessment



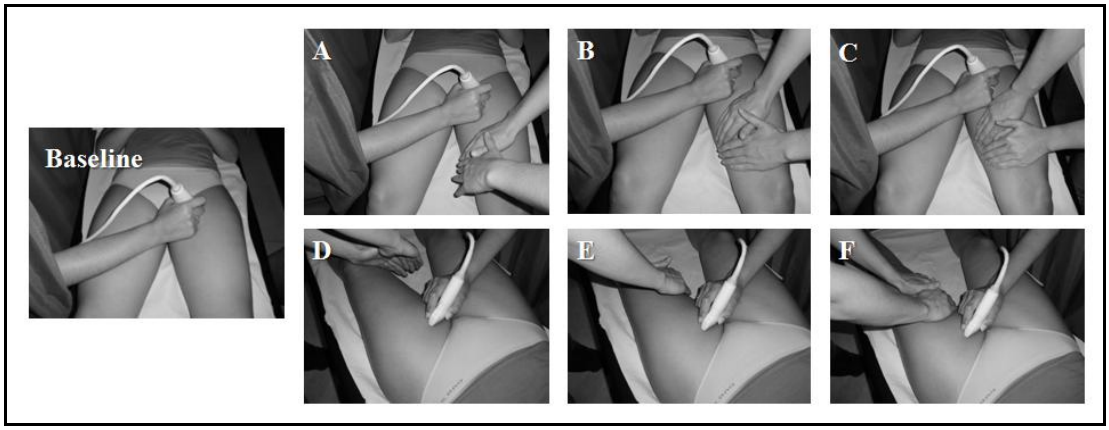
In Study III, to assess the hemodynamic effect of MLD in different regions of lower limb, ultrasound measurements were first taken at baseline (without MLD) and during the MLD applied to the medial and to the lateral aspect of the thigh for FV and GSV, and to the medial and to the lateral aspect of the leg for PV and SSV. To assess the flow velocity in FV and GSV, participants stayed in the supine position, whereas to assess PV and SSV participants were in prone position. The order of the blood flow measurements was randomized following a hierarchical procedure (see Figure 7 and Figure 8).

Cross-sectional area and venous hemodynamics (peak and mean flow velocity and flow volume) percent augmentations were calculated using the following relation:

$$\text{Augmentation (\%)} = (\text{Maneuver} - \text{Baseline}) \div \text{Baseline}) \times 100.$$

The operator collecting the ultrasound data was kept blind with regard to the application of MLD by staying behind an opaque screen. The MLD technique was executed with two hands placed onto the thigh or the leg and then by applying pressure just sufficient to stretch the skin. The stretching of the skin was maintained for at least 4 seconds. The MLD technique was started and ended by orders of the operator collecting the ultrasound data and therefore synchronized with ultrasound recording, and taking into account the respiratory cycle.

Figure 6 - Vascular ultrasound evaluation context with curtain separating sonographer, therapist and participant, during manual lymphatic drainage maneuvers



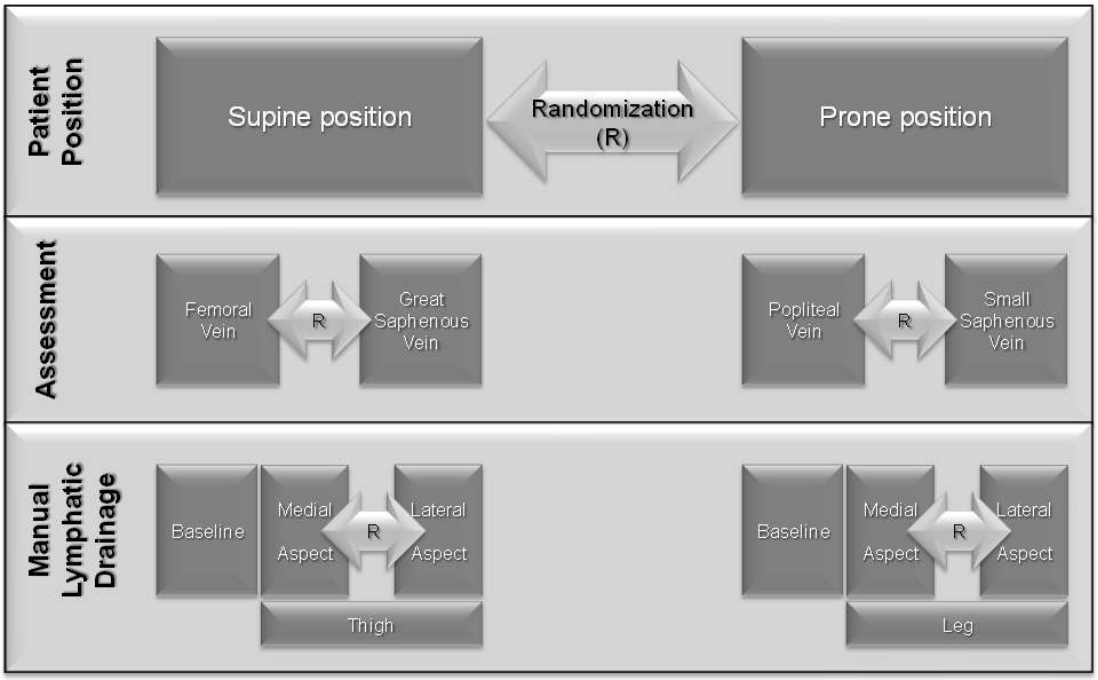
Assessment of the cross-sectional area and blood flow of femoral vein and great saphenous vein during baseline and during call-up maneuvers (A, B and C sequence) and reabsorption maneuvers (D, E and F sequence) in Study II.

The call-up maneuver initiates with the most proximal part of the proximal hand and ends with both hands touching the skin, while producing a skin-stretching (and was used in both studies II and III to assess hemodynamic effect of MLD). The reabsorption maneuver initiates with the most distal part of the distal hand and ends with both hands touching the skin while applying skin-stretching (this maneuver was

only used to compare the hemodynamic effect with that of the call-up maneuver, in Study II). Skin-stretching was applied in the two maneuvers in a proximal direction respecting the course of the lymph and venous flow.

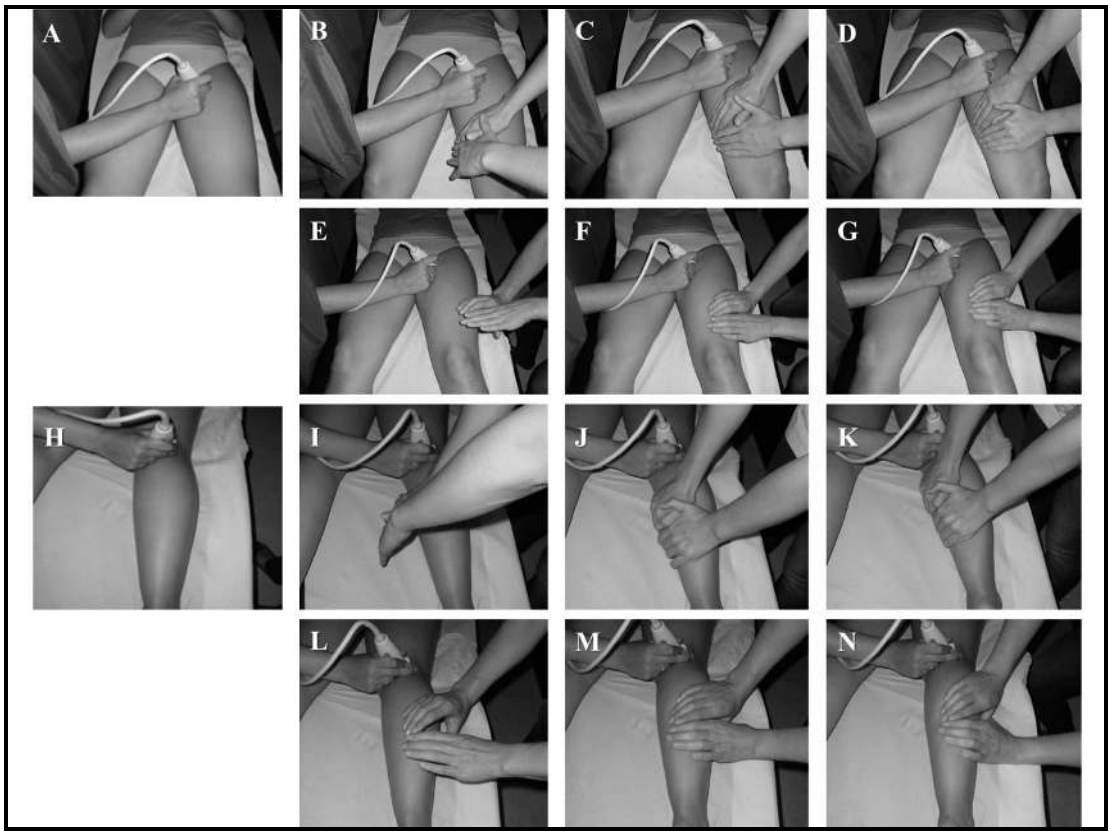
The veous blood flow parameters and percentage augmentation can easily be assessed with ultrasound assessment by venous duplex scanning and have been used in many studies which evaluate venous hemodynamics in the lower limb in response to treatments (Breen et al., 2007; Morris & Woodcock, 2004). Also, venous flow volume calculation based on cross-sectional area and flow velocities measurements is accurate (Lurie et al., 2002).

Figure 7 - Randomization for vascular ultrasound assessments during leg and thigh manual lymphatic drainage



To assess test-retest reliability of these evaluations, 9 healthy participants repeated the tests after a one week interval. The cross-sectional area, mean and peak flow velocities and flow volume of FV, GSV, PV, and SSV were assessed during baseline and during the application of MLD to the lower extremity with venous duplex ultrasound.

Figure 8 - Vein assessments during manual lymphatic drainage in lateral and medial aspect of thigh and leg



Femoral vein and great saphenous assessments at (A) baseline and during MLD (B-D) at medial and (E-G) lateral aspect of thigh. Popliteal and small saphenous vein assessments at (H) baseline and during MLD at (I-K) medial and (L-N) lateral aspect of leg.

4.11 Calf muscles architecture

The medial and lateral gastrocnemius muscles were imaged at 10 MHz and at a scan depth of 65 mm (HI VISION 8500, Hitachi, L53 linear array-transducer). The two muscle bellies were imaged at the upper third of the distance between the peroneal head and the lateral malleoulus with subjects lying prone with hip and knee at neutral position, and at three ankle joint positions: 1) foot at 90º (anatomical neutral position); 2) at maximal active plantarflexion; and 3) at maximal active dorsiflexion. The distance between the proximal and distal aponeurosis in both medial and lateral gastrocnemius muscles was traced to obtain the muscle thickness. In the medial gastrocnemius muscle, the direction of the muscle fascicles was also traced to derive the pennation angle for each ankle joint position. Pennation angle was defined as the angle between the load axis of the muscle and the axis of muscle fascicle. Muscle fascicle length, defined as the length of the fascicle between the deep and superficial aponeuroses, was directly measured for ankle plantarflexion position and using values of muscle thickness and pennation angle for ankle neutral and dorsiflexion positions (Figure 9), according to the following equation:

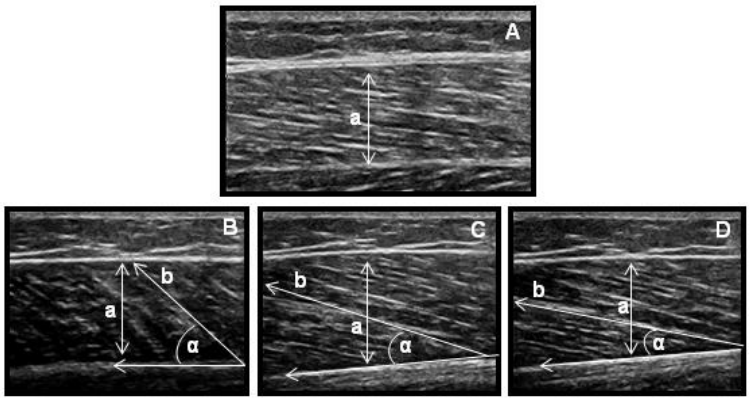
Fascicle length (mm) = muscle thickness (mm) ÷ sine of pennation angle

It has been suggested that B-mode ultrasonography can be used with confidence, revealing good reliability when investigating changes in muscle architecture in groups of older adults, but its use is limited in showing changes in individuals over time (Raj et al., 2012). The immediate (in the same day) test-retest

reliability between the three measures of lateral and medial gastrocnemius muscle thickness, pennation angle and fascicle length at neutral ankle position was assessed by calculating the absolute agreement ICC (Schuck, 2004).

This methodological procedure was performed in Study I.

Figure 9 - Calf muscle architecture assessment with ultrasound



Ultrasound assessment for muscle thickness (a), pennation angle (α) and fascicle length (b) for lateral gastrocnemius muscle at neutral position (A), and for medial gastrocnemius muscle at plantarflexion (B), neutral position (C), and dorsiflexion of the ankle (D).

4.12 Leg volume

Although water displacement is considered a more reliable method to asses volumetric changes for treatment of edema (Rabe et al., 2010), the perimeter can also be used to estimate a segment volume, with manual circumference-based volume measures comparing well with those determined by water displacement (Mayrovitz et al., 2007). Edema volume and its changes during the course of treatment (Mayrovitz et al., 2000) are often assessed clinically using limb circumferences, which are used to estimate limb volume changes employing suitable geometric

models of the limb (Mayrovitz et al., 2007; Mayrovitz et al., 2000). For the lower limb, circumferences measured from a set of 8-12 cm-long leg segments are appropriate to assess the leg volume (Mayrovitz et al., 2007; Mayrovitz et al., 2000). The segmental volume from two adjacent limb perimeter (P) values (P1 and P2 separately by length = 10 cm intervals) was calculated from the equation for a truncated cone model as:

$$\text{Segmental Volume (mL/s)} = \text{Length} \times (P1^2 + P1 \times P2 + P2^2) \div 12\pi$$

In our study, leg perimeter was measured in standing using a standard tape at the level of the malleolus (P0), and 10 cm (P1), 20 cm (P2) and 30 cm (P3) above the lateral malleolus. The leg volume was calculated according to the equation, which is the sum of three volume cones estimated between P0 and P1, P1 and P2, and P2 and P3:

$$\begin{aligned}\text{Leg Volume (mL/s)} = {} & 10 \times (P0^2 + P0 \times P1 + P1^2) \div 12\pi + \\ & 10 \times (P1^2 + P1 \times P2 + P2^2) \div 12\pi + \\ & 10 \times (P2^2 + P2 \times P3 + P3^2) \div 12\pi\end{aligned}$$

This procedure was performed in Study IV.

4.13 Ankle isokinetic dynamometer assessment

Ankle muscles strength and active range of motion was measured with an isokinetic dynamometer (Biodex system 3 pro, Biodex Medical Systems, Inc., New York, USA). This method has been described to be reliable to assess dorsiflexor and plantarflexor muscles strength and ankle range of motion (Karnofel et al., 1989; Padberg et al., 2004). Participants were seated on the dynamometer's chair with 70-85º seatback tilt, with the knee flexed at 20-30º and with the trunk and thigh restrained by fastened seatbelts and a strap placed around the thigh. The foot rested in neutral position on a footplate and the ankle joint axis was aligned with the rotational axis of the dynamometer. Muscle concentric contractions started from full dorsiflexion and were performed at slow (60 º/sec) and fast (120 º/sec) speeds. Each participant completed 2 sets of five repetitions at the slow speed, 60 seconds of rest, followed by 15 repetitions at the fast speed. Participants were instructed to exert near maximal effort during the first set followed by maximal exertion during the second set. A 5 to 10-min rest separated the two sets. Average peak torque (Nm), peak torque per unit body weight ($Nm.Kg^{-1}$), total work (J), average power (Watt), and ankle active range of movement [i.e., the maximal range of motion in which there is torque generation by ankle muscles] were obtained. Peak torque per body weight is a normalized form for reporting muscle strength that allows comparison between subjects. Total work is the area under the peak torque. Average power is the work divided by time, and it is influenced by both strength and speed, i.e., is the product of force and velocity. This procedure for assessing isokinetic strength of the ankle

muscles was used in Study IV and was found reliable, with ICC higher than 0.7 (Padberg et al., 2004).

4.14 Educational session

The participants in Study IV attended a single group (experimental and control participants together) educational session soon following T0 (baseline assessments). This session addressed the characterization of CVD, including general mechanisms of the disease, predisposing and aggravating factors, and the importance of general care, such as skin care, leg elevation, exercise, diet, as well as the importance of wearing compression stockings (Knapp et al., 2011). The session was organized in two parts: the first one was to present the information (approximately 40 minutes), and the second one for open discussion centered on the major concerns, questions, and experiences among participants (all CVD patients).

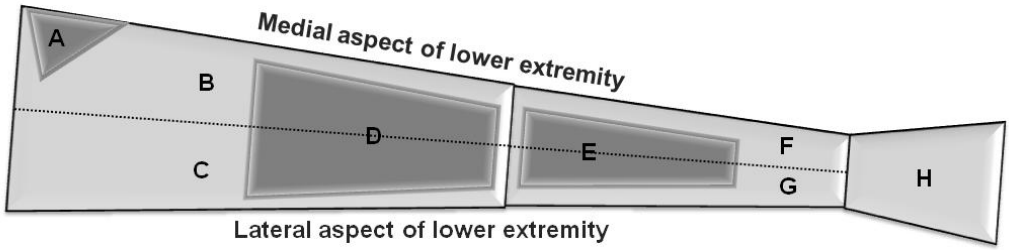
The objective of this session was providing all participants with basic information about the disease and care. However, it was not the purpose of the educational session to intervene on CVD prevention or treatment. So, no booklet compiling this information, or other matters regarding counseling about CVD care, was given to the participants. Also, no specific incentives for adopting prophylactic behaviors related to CVD were yielded during the educational session.

4.15 Manual lymphatic drainage sequence and method

The MLD technique was executed by two fully trained physical therapists in Study IV. The manual technique was performed placing the two hands side by side on the skin, like in the call-up maneuver (supposedly the best maneuver to improve venous flow) (Leduc et al., 2000), initiating with the most proximal part of the proximal hand and ending with both hands touching the skin while producing a skin-stretching. The pressure applied by the hands to the skin and underlying soft tissue was carefully adjusted to remain soft and just enough to stretch the skin for at least 4 seconds. Both legs were treated and the whole session took approximately 40-45 minutes. The duration and the number of sessions in the present study are similar to those used in previous studies assessing the role MLD in CVD patients (Molski et al., 2013; Molski et al., 2009). During four weeks, patients completed 10 sessions of MLD. The sequence of the maneuvers complied with that commonly used for lymphedema, firstly following from proximal to distal and secondly from distal to proximal (Leduc et al., 2011; Lee et al., 2011). The maneuvers were applied in the following sequence: inguinal region (10 MLD maneuvers), progressing downwards through the thigh (30 MLD maneuvers at medial and 30 at lateral aspect of thigh), the popliteal region (MLD maneuvers were applied 10 times immediately above and 10 times immediately below popliteal fossa), downwards to the leg (30 MLD maneuvers at medial and lateral aspect of leg), and finishing in the dorsal aspect of the foot (30 MLD maneuvers). Next, the maneuvers were carried out in the reverse order upwards: 15 maneuvers on the dorsal aspect of the foot, 15 maneuvers both on lateral and medial aspect of the leg, 5 maneuvers both below and above popliteal

fossa, 15 maneuvers above the lateral and above the medial aspect of the thigh, and 5 maneuvers on the inguinal region (Figure 10).

Figure 10 - Scheme of manual lymphatic drainage maneuvers sequence applied in lower extremity



With patient in supine position, manual lymphatic drainage (MLD) starts at the (A) inguinal region (10 MLD maneuvers); progressing downwards through the thigh, with 30 MLD maneuvers at medial (B) and 30 at lateral (C) aspect of thigh; after that MLD maneuvers were applied (gray in the scheme) 10 times immediately above (D) and 10 times immediately below (E) popliteal fossa; then 30 MLD maneuvers were applied in a downward direction at medial (F) and lateral (G) aspect of the leg and finishing the downward sequence with 30 MLD maneuvers on the dorsal aspect of the foot (H).Then the MLD maneuvers were initiated in reverse order: further 15 repetitions of MLD maneuvers applied to the dorsum of the foot (H), 15 repetitions of MLD maneuvers again on the lateral (G) and on the medial side of the leg (F) and 5 repetitions of MLD maneuvers both below (E) and above (D) the popliteal fossa. Further 15 repetitions of MLD maneuvers are applied both to the lateral (C) and medial (B) sides of the thigh. The sequence ends with 5 MLD maneuvers over the inguinal region (A).

Before initiating the study, the physical therapists received specific training in the MLD technique. This training focused on learning how to apply a strong pull to the skin yet without excessively pressing the underlying tissues, and to practice the maneuver sequence along the entire lower limb. Vascular ultrasound was employed to provide feedback to the training therapists about the immediate effect of their

manual technique on the rise of venous blood flow (the desired effect and a demonstration of a correctly performed technique) or vein collapse (undesired effect and a demonstration of an incorrectly applied technique).

When inflammation was present in the lower limb, the entire set of maneuvers was done up to the place of inflammation and reverse sequence was initiated in that moment. For example, if inflammation is present in the lower third of leg, the sequence is reversed immediately before touching the inflamed aspect of the leg, and maneuvers on the foot are not performed.

In Study IV, patients with CVD were randomly allocated to an experimental group and to a control group. The experimental group received a total of 10 MLD sessions, along four weeks. The control group received no MLD treatments but participants in this group were given the opportunity to receive MLD at the end of the study.

4.16 Statistical analysis

All statistical tests were performed with the help of the statistical software package SPSS Inc. v.17 (SPSS Inc., Chicago, USA).

In Study I, group differences were tested with Mann-Whitney U test. The differences between peak flow velocity (second session) at baseline, first and tenth contractions were calculated with Wilcoxon Signed-Rank test. The relationship between CVD severity and the gastrocnemius muscle and hemodynamic parameters was calculated using Spearman coefficient of correlation. Reproducibility was

assessed by test-retest reliability by calculating the absolute agreement ICC and by agreement analysis using Bland and Altman plot analysis.

In Study II, III and IV, the normal distribution was checked using Shapiro-Wilk Test.

In Study II, group differences were tested with two-tailed Student's T-test, while differences between maneuvers and between maneuvers and baseline were tested by repeated-measures ANOVA. The Bonferroni correction was used to correct for multiple comparisons. The relationship between the magnitude of the effect of the maneuvers, in terms of percentage augmentation from baseline, and CVD severity, in terms of CEAP and VCSS classification, was calculated using Spearman coefficient of correlation.

In Study III, a mixed factorial ANOVA with three factors: group (two levels: CVD groups vs. control group), body region (three levels: baseline vs medial side vs. lateral side), and type of vein (two levels: deep vein vs. superficial vein) was used to test the effects of the dependent variables and interactions. Statistical analysis using this model was carried out separately for the thigh and the leg. The Bonferroni correction was used to correct for multiple pairwise comparisons. The relationship between the magnitude of the effect of the MLD techniques, in terms of percentage augmentation from baseline, and CVD severity, in terms of VCSS classification was calculated using the Spearman coefficient of correlation. The test-retest reliability was assessed by calculating the absolute agreement using the ICC.

In Study IV, baseline variables were compared between groups using Independent Sample T-test. A mixed model ANOVA for repeated measures (2×2×3) with two between-subjects (Group; two levels: experimental and control; Stocking;

two levels compressive stockings use vs. non use) and one within-subjects variable (Time; three levels: T0, T1 and T2) was used to assess the effect of MLD on the outcome measures [CIVIQ-20, symptoms, leg volume, severity of disease (VCSS), and ankle muscles isokinetic strength]. The interactions are expressed with Group vs. Time, for interaction of both groups with the times (T0, T1 and T2) and Group vs. Stocking vs. Time to assess interaction of both group with use or not use of compressive stockings and time. Effect size was reported as partial eta squared (η^2_ρ) and was considered low when $\eta^2_\rho \leq 0.05$, medium with $\eta^2_\rho =]0.05; 0.25]$; high with $\eta^2_\rho =]0.25; 0.50]$; very high $\eta^2_\rho > 0.50$. The Bonferroni correction was used to correct for multiple pairwise comparisons. The results of pairwise comparisons are reported by *P-value* and the 95% interval of confidence (IC) = [min; max].

Descriptive data is presented as mean (standard deviation) unless otherwise stated. The significance level was set at $P < 0.05$.

5 RESULTS

The demographic and clinical data pertaining to the participants of Study I are presented in Table 3 and Table 4. All subjects in the CVD group presented pathologic venous reflux but were free of venous obstruction. No differences in age, height, weight and body mass index were found between the groups.

Regarding Study II, participants' demographic and clinical data are presented in Table 5. No differences in age, height, weight, and body mass index were found between the CVD and control group.

Demographic and clinical data pertaining to the participants of Study III are presented in Table 6 and Table 7. No differences in height, weight and body mass index were found between CVD and control groups. Nevertheless, the CVD group is slightly older than the control group [47.07 (12.22) yrs and 39.31 (13.76) yrs, CVD and control groups respectively, $P = 0.029$].

The demographic and clinical data of the participants of Study IV are presented in Table 8 and Table 9. No differences in height, weight and body mass index were found between the experimental and control groups. Participants in experimental group were slightly older than in control group [54.60 (11.30) years and 46.81 (11.10) years, experimental and control group respectively; $P = 0.032$, mean difference = 7.79 years, 95% IC = (0.72, 14.87)]. In addition, the experimental group was composed by a slightly higher number of C_5 patients, but the severity of disease (VCSS) was similar in both groups, [7.80 (3.49) and 8.86 (2.94), experimental and control group, respectively; $P = 0.354$, mean difference = 0.94, 95% IC = (1.09, 2.98)]. All participants using compression stockings reported

unchanged adherence to such use during the time of the study. At baseline, all outcomes were similar in experimental and control groups.

Table 3 - Demographic and clinical data of Study I

	CVD group		Control group		*P#*	*P##*	All Participants
	Entire Group	Subgroup with retest	Entire Group	Subgroup with retest			
N	16	9	15	8			31
Age (years)	44.1(12.3)	45.7(14.2)	36.1(10.0)	35.8(11.4)	0.086	0.139	40.3(11.8)
Height (cm)	162.8(7.3)	162.0(5.4)	166.3(9.6)	162.8(11.0)	0.281	0.114	164.5(8.5)
Weight (Kg)	71.2(13.4)	73.1(15.0)	66.1(15.2)	60.5(13.5)	0.247	0.936	68.8(14.3)
Body mass index (Kg/m^2)	27.4(6.0)	28.1(7.1)	23.6(3.0)	22.5(3.0)	0.281	0.114	25.4(5.0)
Gender							
Female	13(81.2)	7(77.8)	10(66.7)	6(75.0)	-	-	23(74.2)
Male	3(18.8)	2(22.2)	5(33.3)	2(25.0)	-	-	8(25.8)
Comorbidities							
Diabetes	1(6.25)	0(0.0)	0(0.0)	0(0.0)	-	-	1(3.2)
Treated thyroid dysfunction (controlled)	2(12.5)	1(11.1)	1(6.7)	1(12.5)	-	-	3(9.7)
Uterus malignant carcinoma (operated)	1(6.25)	1(11.1)	0(0.0)	0(0.0)	-	-	1(3.2)
Knee surgery (contralateral leg > 6 months ago)	2(12.5)	2(22.2)	0(0.0)	0(0.0)	-	-	2(6.5)
Lupus erythematosus,	1(6.25)	1(11.1)	0(0.0)	0(0.0)	-	-	1(3.2)
Surgical removal of greater saphenous with recurrence	1(6.25)	1(22.2)	0(0.0)	0(0.0)	-	-	1(3.2)

Quantitative variable: mean (standard deviation). Categorical variable: frequency (%). *P#*: differences between groups (entire group). *P##*: differences between subgroups (groups with retest with one week apart).

Table 4 - Clinical data of CVD group of Study I

	CVD group	
	Entire Group	Subgroup with retest
N	16	9
CEAP		
Clinical classification		
C_1	3(18.8)	2(22.2)
C_2	2(12.5)	1(11.1)
C_3	10(62.5)	5(55.6)
C_4	1(6.3)	1(11.1)
Anatomical reflux		
Superficial veins	9(56.3)	5(55.6)
Deep veins	1(6.3)	0(0.0)
Perforator veins	3(18.8)	3(33.3)
Superficial+Perforator	2(12.5)	0(0.0)
Superficial+Deep+Perforator	1(6.3)	1(11.1)
VCSS	3.8(3.2)	3.33(3.5)
Symptoms		
Fatigue	11(68.8)	6(66.7)
Cramps	8(50.0)	4(44.4)
Heavy legs	10(62.5)	5(55.6)
Pain	5(31.3)	3(33.3
Skin irritation	3(18.8)	2(22.2)
Itching	2(12.5)	1(1.1)
Without	4(25.0)	2(22.2)

Quantitative variable: mean (standard deviation). Categorical variable: frequency (%). VCSS: Venous Clinical Severity Score, total scale range 0-30 (best to worst) assessed in CVD (chronic venous disease) group.

Table 5 - Demographic and clinical data of Study II

	CVD Group	Control Group	*P*	All Participants
N	23	18		41
Age (years)	46.83(13.24)	38.72(15.96)	0.083	42.68(15.23)
Height (m)	1.67(0.91)	1.64(0.97)	0.419	70.62(15.03)
Weight (Kg)	74.04(14.63)	66.08(13.50)	0.082	1.66(0.10)
Body mass index (Kg/m^2)	26.53(4.01)	24.36(3.67)	0.081	25.58(4.11)
Gender				
Female	13(56.5)	10(55.6)	-	23(56.1)
Male	10(43.5)	8(44.4)	-	18(43.9)
CEAP clinical classification				
C_1	5(21.7)	-	-	-
C_2	3(13.0)	-	-	-
C_3	5(21.7)	-	-	-
C_4	6(26.1)	-	-	-
C_5	4(17.4)	-	-	-
VCSS	5.65(3.6)	-	-	-
Anatomical reflux				
Superficial veins	3(13.0)	-	-	-
Deep veins	1(4.3)	-	-	-
Superficial+Deep veins	7(30.4)	-	-	-
Superficial+Perforator veins	6(26.1)	-	-	-
Superficial+Deep+Perforator veins	4(17.4)	-	-	-
Comorbidities				
Surgical removal of great saphenous vein	3(13.0)	-	-	-
Lower limbs symptoms				
Fatigue	18(78.3)	-	-	-
Cramps	9(39.1)	-	-	-
Heavy legs	14(60.9)	-	-	-
Pain	14(60.9)	-	-	-
Skin irritation	8(34.8)	-	-	-
Itching	11(47.8)	-	-	-
Without symptoms	2(8.7)	-	-	-

Quantitative variable: mean (standard deviation). Categorical variable: frequency (%). VCSS: Venous Clinical Severity Score, total scale range 0-30 (best to worst). CEAP: Clinical Etiological Anatomical Pathological Classification.

Table 6 - Demographic and clinical data of Study III

	CVD group	Control group	*P*	All Participants
N	28	29		57
Age (years)	47.07(12.22)	39.31(13.76)	0.029*	43.12(13.49)
Height (cm)	163.89(9.57)	164.83(9.94)	0.719	164.37(9.68)
Weight (Kg)	69.30(13.59)	65.90(13.61)	0.349	67.57(13.59)
Body mass index (Kg/m^2)	25.73(3.81)	24.12(3.50)	0.101	24.90(3.71)
Gender				
Female	21(75.0)	17(58.6)		38(66.7)
Male	7(25.0)	12(41.4)		19(33.3)
Comorbidities				
Surgical removal of small and/or great saphenous vein	6(21.4)	0(0.0)		6(10.5)
Active ulcer (contralateral leg)	1(3.6)	0(0.0)		1(1.8)
History of deep venous thrombosis (>1year)	3(10.7)	0(0.0)		3(5.3)
Diabetes	0(0.0)	1(3.5)		1((1.8)
Arterial hypertension	0(0.0)	4(13.8)		4(7.0)
Cardio-respiratory disease (treated)	2(7.1)	4(13.8)		6(10.5)
Erysipelas (>1 year ago)	1(3.6)	0(0.0)		1(1.8)

Quantitative variable: mean (standard deviation). Categorical variable: frequency (%). *Significant difference between CVD (chronic venous disease) and control (healthy) groups.

Table 7 - Clinical data of CVD group in Study III

	CVD group
N	28
CEAP clinical classification	
C_3	13 (46.4)
C_4	11(39.3)
C_5	4 (14.3)
Anatomical reflux	
Superficial veins	5(17.9)
Superficial+Deep veins	6(21.4)
Superficial+Perforator veins	12(42.9)
Superficial+Deep+Perforator veins	5(17.9)
VCSS	7.07(3.6)
Symptoms	
Fatigue	24(85.7)
Cramps	14(50.0)
Heavy legs	21(75.0)
Pain	19(67.9)
Skin irritation	12(42.9)
Itching	17(60.7)

Quantitative variable: mean (standard deviation). Categorical variable: frequency (%). VCSS: Venous Clinical Severity Score, range 0-30 (best to worst). CEAP: Clinical Etiological Anatomical Pathological Classification for CVD (chronic venous disease) group.

Table 8 - Demographic data in Study IV

	Experimental Group	Control group	*P*
N	20	21	
Age (years)	54.60(11.30)	46.81(11.10)	0.032*
Height (cm)	164.90(8.78)	162.62(6.26)	0.342
Weight (Kg)	77.00(20.71)	71.53(13.42)	0.325
Body mass index (Kg/m^2)	28.14(6.13)	27.00(4.59)	0.506
Gender			
Female	15(75.0)	20(95.2)	-
Male	5(25.0)	1(4.8)	-

Quantitative variable: mean (standard deviation). Categorical variable: frequency (%). *Significant difference between CVD (chronic venous disease) and control (healthy) groups.

Table 9 - Clinical data of Study IV

	Experimental Group	Control group	*P*
N	20	21	
VCSS	7.80(3.49)	6.86(2.94)	0.354
CEAP classification			
Clinical Class			
C_3: edema	8(40.0)	17(81.0)	-
C_4: skin changes	7(35.0)	4 (19.0)	-
C_5: healed ulcer	5(25.0)	0(0.0)	-
Etiological Class			
Ep: primary	20(100)	21(100)	-
Anatomical Class			
As: superficial veins	6(30.0)	4(19.0)	-
Ad: deep veins	1(5.0)	0(0.0)	-
As,d: superficial+deep veins	7(35.0)	5(23.8	-
As,p: superficial+perforator veins	1(5.0)	5(23.8)	-
As,d,p: superficial+deep+perforator veins	5(25.0)	7(33.3)	-
Pathological Class			
Pr: reflux	20(100)	21(100)	-
Symptoms			
Fatigue	16(80.0)	18(85.7)	-
Cramps	11(55.0)	12(57.1)	-
Heavy legs	20(100)	17(81.0)	-
Pain	16(80.0)	18(85.7)	-
Skin irritation	10(50.0)	9(42.9)	-
Itching	9(45.0)	6(28.6)	-
Comorbidities			
Surgical removal of small and/or great saphenous vein	5(25.0)	3(14.3)	-
Sclerotherapy	2(10.0)	0(0.0)	-
History of deep venous thrombosis (>1year)	4(20.0)	0(0.0)	-
Diabetes	1(5.0)	3(14.3)	-
Arterial hypertension	6(30.0)	1(4.8)	-
CVD treatments			
Compression stocking	8(40.0)	10(47.6)	-
Medication to chronic venous disease	9(45.0)	1(4.8)	-

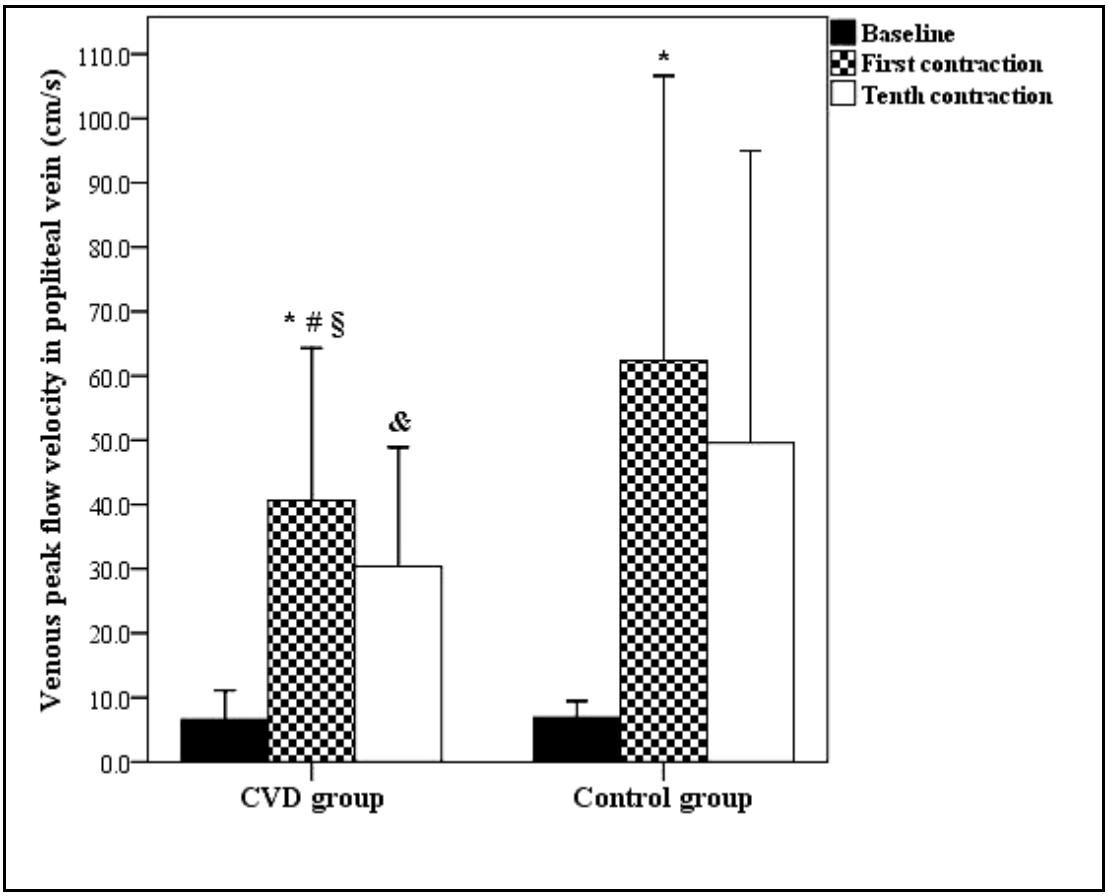
Quantitative variable: mean (standard deviation). Categorical variable: frequency (%). VCSS: Venous Clinical Severity Score total scale range 0-30 (best to worst). CEAP: Clinical Etiological Anatomical Pathological Classification.

5.1 The use of ultrasound in the evaluation of the efficacy of calf muscle pump function in primary chronic venous disease

5.1.1 Venous dynamic blood flow

During the first tip-toe movement, popliteal vein blood flow velocity increased significantly in both the CVD and control groups (P = 0.008 and P = 0.012, respectively for CVD group and control group). However, in the CVD group, but not in the control group, peak velocity decreased from the first to the tenth calf muscles contraction (first contraction vs. tenth contraction: P = 0.028 and P = 0.093, respectively for the CVD group and the control group). Dynamic blood flow velocity in popliteal vein was lower in the CVD group compared to the control group during both the first (P = 0.021) and the tenth contraction (P = 0.024) of the tip-toe movement set (Figure 3 and Figure 11). Peak velocity, given as percentage augmentation, was lower in the CVD group compared to the control group during the first contraction [530.6 (115.2)% vs. 831.5 (353.4)%, P = 0.027]. However, mean velocity augmentation during the first contraction and peak velocity augmentation in tenth contraction did not differ between groups. No differences in venous baseline hemodynamics were found between the two experimental groups (Table 10).

Figure 11 - Venous peak flow velocity in the popliteal vein during baseline, first and tenth tip-toe contractions



Mean and standard deviation of venous peak flow velocity in the popliteal vein during baseline, first and tenth tip-toe contractions in CVD (chronic venous disease) group and control (healthy) group obtained during retesting and showing the results from Wilcoxon Signed-Rank test: (*) significantly different from baseline, (#) significantly different from first contraction of control group; (§) significantly different from tenth contraction; and (&) significantly different from tenth contraction of control group. All significant differences at least at $P < 0.05$.

Table 10 - Popliteal vein hemodynamics in CVD and control groups

	Group	N	Mean(SD)	*P*
Baseline peak flow velocity (cm/s)	CVD	9	6.6(2.3)	0.370
	Control	8	6.9(1.3)	
Baseline mean flow velocity (cm/s)	CVD	9	2.7(1.7)	0.963
	Control	8	2.4(1.2)	
Baseline cross-sectional area of popliteal vein (cm^2)	CVD	9	0.6(0.2)	0.673
	Control	8	0.5(0.2)	
Baseline peak flow volume (mL/s/s)	CVD	9	3.5(1.4)	0.743
	Control	8	3.5(1.4)	
Baseline mean flow volume (mL/s/s)	CVD	9	1.3(0.6)	0.743
	Control	8	1.2(0.6)	
First contraction peak flow velocity (cm/s)	CVD	9	40.6(11.8)	0.021*
	Control	8	62.4(22.1)	
First contraction mean flow velocity (cm/s)	CVD	9	17.1(5.0)	0.083
	Control	8	26.7(12.5)	
Tenth contraction peak flow velocity (cm/s)	CVD	9	30.4(9.1)	0.024*
	Control	8	49.5(22.7)	
Peak flow velocity augmentation in first contraction (%)	CVD	9	530.6(115.2)	0.027*
	Control	8	831.5(353.4)	
Mean flow velocity augmentation in first contraction (%)	CVD	9	721.5(532.2)	0.124
	Control	8	1319.0(1234.7)	
Peak flow velocity augmentation in tenth contraction (%)	CVD	9	379.0(123.9)	0.068
	Control	8	623.5(302.4)	

The results were obtained during the retest trial performed one week after the first session for CVD (chronic venous disease) and control (healthy) groups. *Significant with $P < 0.05$ for Mann-Whitney U test.

5.1.2 Gastrocnemius muscle architecture

Table 11 presents the anatomical data for the gastrocnemius muscle in CVD and control groups. Muscle thickness, pennation angle and muscle fascicles' length were similar in both groups at the three different ankle joint positions.

No relationship could be noted between clinical severity (measured by CEAP and VCSS) and gastrocnemius architecture, as well as between clinical severity and dynamic popliteal vein blood flow. In CVD patients, a relationship could be found between peak flow velocity during the first contraction and medial gastrocnemius muscle fascicles length (r = 0.63; P = 0.041) and pennation angle (r = -0.68; P = 0.044) when both are measured with ankle dorsiflexion. Peak flow velocity during the last contraction of the tip-toe set also increased with medial gastrocnemius muscle pennation angle in neutral position (r = 0.73; P = 0.025) and with the range of change in pennation angle between ankle dorsiflexion and plantarflexion (r = 0.70; P = 0.025).

5.1.3 Test-retest reliability of calf muscle pump and gastrocnemius architecture ultrasound measures

Ultrasound measures of gastrocnemius muscle architecture were generally highly reproducible. High immediate test-retest reliability (ICC > 0.94) for ultrasound measures of muscle thickness was found for medial and for lateral gastrocnemius muscle, and for pennation angle and muscle fascicles' length of medial gastrocnemius muscle in ankle neutral position. The reliability of popliteal vein blood velocity in the first and tenth contractions was low, with an ICC < 0.5. However, peak and mean flow velocity in the first contraction presented good immediate test-retest reliability, with an ICC > 0.74. None of the hemodynamics measures showed high test-retest reliability (i.e., ICC > 0.5) (Table 12). The Bland-Altman plot analysis reveals a poor agreement for dynamic popliteal vein velocity measures (Figure 12).

For the estimation of probe movement bias, the peak velocity was 57.8(14.9) cm/s during the actual tip-toe movement and 52.8 (17.3) cm/s during the simulated tip-toe movement with an ICC = 0.85 [0.35; 0.98] and P = 0.005, and a bias = 4.9 cm/s (lower bound: -10.7 cm/s; upper bound: 20.7 cm/s).

5.2 Venous flow at superficial and deep venous system during manual lymphatic drainage

5.2.1 Baseline measures of venous blood flow

The cross-sectional area of the GSV was higher in the CVD group [0.14 (0.06) cm^2] than in the control group [0.09 (0.04) cm^2, P = 0.007]. Nevertheless, the cross-sectional area of FV and the mean flow velocity, the peak flow velocity, and the flow volume of both FV and GSV at baseline were similar in CVD and in the control group (Table 13 and Table 14).

Table 11 - Differences between CVD and control group in gastrocnemius muscle architecture

	Group	N	Mean(SD)	*P*
Medial gastrocnemius muscle thickness in neutral (mm)	CVD Control	16 15	18.0(2.5) 17.8(2.7)	0.890
Medial gastrocnemius muscle thickness in plantarflexion (mm)	CVD Control	16 15	17.0(2.6) 17.5(3.5)	0.968
Medial gastrocnemius muscle thickness dorsiflexion (mm)	CVD Control	16 15	19.1(2.2) 19.7(3.2)	0.654
Lateral gastrocnemius muscle thickness in neutral (mm)	CVD Control	16 15	12.2(3.3) 13.2(3.6)	0.384
Medial gastrocnemius muscle pennation angle in neutral (degrees)	CVD Control	16 15	19.5(2.3) 19.1(1.3)	0.664
Medial gastrocnemius muscle pennation angle in plantarflexion (degrees)	CVD Control	16 15	42.2(8.8) 46.3(5.4)	0.165
Medial gastrocnemius muscle pennation angle in dorsiflexion (degrees)	CVD Control	16 15	17.6(3.1) 17.3(2.0)	0.572
Δ Medial gastrocnemius muscle pennation angle (degrees)	CVD Control	16 15	24.6(8.0) 29.0(5.5)	0.096
Medial gastrocnemius muscle fascicle length in neutral (mm)	CVD Control	16 15	54.3(7.7) 54.8(8.7)	0.874
Medial gastrocnemius muscle fascicle length in plantarflexion (mm)	CVD Control	16 15	25.0(4.8) 24.7(6.4)	0.580
Medial gastrocnemius muscle fascicle length in dorsiflexion (mm)	CVD Control	16 15	65.3(15.8) 66.5(11.2)	0.477

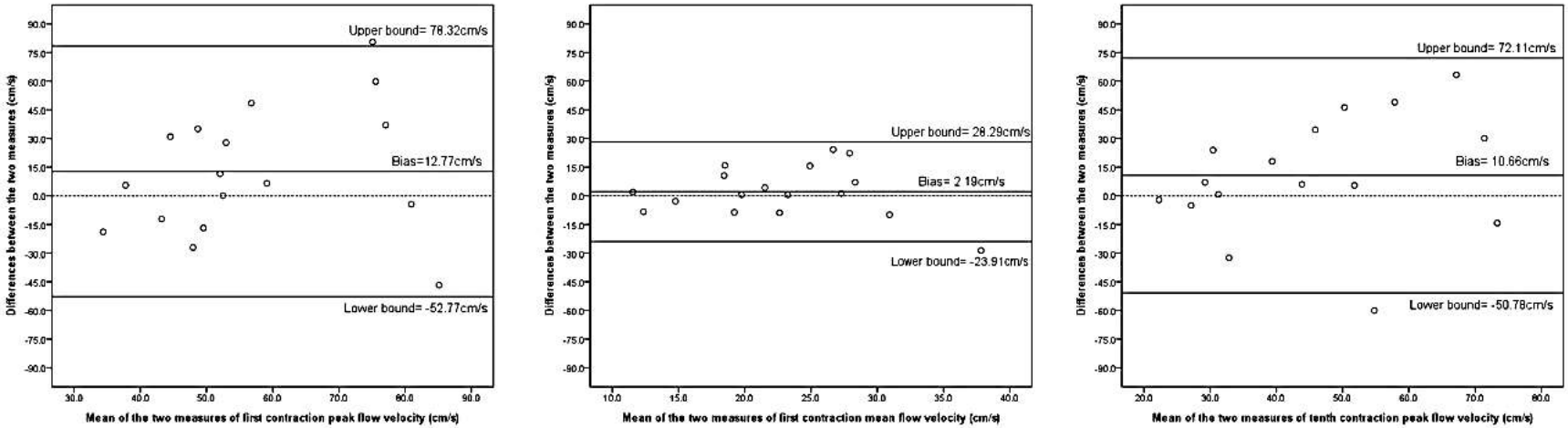
Mean (SD: standard deviation). Δ Medial gastrocnemius muscle (angle between maximal active dorsiflexion and plantarflexion in medial gastrocnemius). Differences between CVD (chronic venous disease) group and control (healthy) group are significant with $P < 0.05$ for Mann-Whitney U test.

Table 12 - Test-retest reliability of venous hemodynamics and muscle architecture measures

	N	Mean(SD)	ICC[range]	*P*
Immediate test-retest reliability				
First contraction peak flow velocity (cm/s)*	17	50.9(20.2)	0.74 [0.52─0.88]	0.000
First contraction mean flow velocity (cm/s)*	17	21.6(10.3)	0.78[0.58─0.90]	0.000
Tenth contraction peak flow velocity (cm/s)*	17	39.4(19.1)	0.49[0.20─0.75]	0.000
Medial Gastrocnemius thickness (mm)**	31	17.9(2.5)	0.98 [0.96─0.99]	0.000
Lateral Gastrocnemius thickness (mm)**	31	12.7(3.5)	0.98[0.97─0.99]	0.000
Fibre length of medial gastrocnemius muscle (ankle at 90 degrees) (mm)**	31	54.5(8.0)	0.94[0.87─0.97]	0.001
Pennation angle of medial gastrocnemius muscle fascicles (ankle at 90 degrees) (º)**	31	19.3(1.8)	0.94[0.89─0.97]	0.000
Delayed test-retest reliability				
First contraction peak flow velocity (cm/s)	17	53.7(7)	-0.06[-0.46─-0.40]	0.253
First contraction mean peak flow velocity (cm/s)	17	21.7(6.1)	0.28[-0.47─-0.50]	0.487
Tenth contraction peak flow velocity (cm/s)	17	48.1(33.1)	0.10[-0.39─-0.54]	0.722

* Calculated with subgroup with retest; ** calculated with all subjects. Intraclass Correlation Coefficient (ICC) significant with $P < 0.05$, with range [lower bound ─ upper bound] at 95% confidence interval. Mean (SD: standard deviation): results from the three measurements in the same day for immediate test-retest reliability and from the measures of first and second week for delayed test-retest reliability.

Figure 12 - Bland and Altman Plot of the two measures of first contraction peak flow velocity, first contraction mean flow velocity and tenth contraction peak flow velocity



Bounds and bias were set at 95% of confidence interval.

Table 13 - Venous blood flow in femoral vein

		CVD group (N=20)	Control group (N=18)	*P*	All Participants (N=38)
Cross-sectional area (cm^2)	Baseline	0.47(0.16)	0.46(0.21)	0.992	0.45(0.18)
	Call-up	0.52(0.20)*	0.49(0.19)	0.661	0.50(0.19)*
	Reabsorption	0.60(0.19)*	0.53(0.19)*‡†	0.229	0.57(0.19)*‡†
Peak flow Velocity (cm/s)	Baseline	19.38(8.77)	21.37(9.36)	0.504	20.32(8.99)
	Call-up	24.85(10.92)*	25.67(8.82)*	0.803	25.24(9.85)*
	Reabsorption	25.96(10.32)*	25.04(6.83)	0.754	25.51(8.69)*
Mean flow velocity (cm/s)	Baseline	12.14(5.67)	14.20(7.47)	0.342	13.11(6.57)
	Call-up	16.62(8.27)*	17.12(7.15)	0.843	16.86(7.66)*
	Reabsorption	15.88(7.30)*	15.37(4.76)	0.807	15.63(6.12)*
Flow volume (ml/s)	Baseline	5.47(3.40)	6.05(4.22)	0.644	20.32(8.99)
	Call-up	8.20(4.72)*	7.99(3.87)*	0.883	25.24(9.85)*
	Reabsorption	9.42(4.73)*	7.98(3.89)*	0.320	25.51(8.69)*

P-Value for differences between groups (*P*). * Significantly different from baseline ($P < 0.05$). †Significantly different from call-up maneuver ($P < 0.05$). ‡Significantly different from call-up maneuver for percentage augmentation from baseline [(maneuver-baseline)/baseline)*100, ($P < 0.05$). Results are presented as mean (standard deviation) for CVD (chronic venous disease) group and control (healthy) group, and all subjects.

Table 14 - Venous blood flow in great saphenous vein

		CVD group (N=20)	Control group (N=18)	P	All Participants (N=38)
Cross-sectional area (cm^2)	Baseline	0.14(0.06)	0.09(0.04)	0.007†	0.12(0.05)
	Call-up	0.16(0.07)	0.09(0.04)	0.001†	0.12(0.06)
	Reabsorption	0.16(0.06)	0.10(0.04)	0.001†	0.13(0.06)
Peak flow Velocity (cm/s)	Baseline	15.82(8.06)	21.09(17.52)	0.240	18.38(13.66)
	Call-up	26.22(13.65)*	33.31(28.96)*	0.313	29.45(22.61)*
	Reabsorption	28.58(15.66)*	35.19(29.05)*	0.393	31.84(23.10)*
Mean flow velocity (cm/s)	Baseline	10.05(4.63)	14.56(14.29)	0.194	12.22(10.72)
	Call-up	16.64(8.13)*	24.18(25.29)*	0.215	20.23(18.92)*
	Reabsorption	18.09(9.03)*	24.77(23.16)*	0.224	21.31(17.62)*
Flow volume (ml/s)	Baseline	1.35(0.82)	1.49(1.91)	0.784	1.42(1.45)
	Call-up	2.69(2.49)*	2.32(2.92)*	0.686	2.50(2.68)*
	Reabsorption	3.08(2.80)*	2.35(2.24)*	0.390	2.71(2.53)*

* Significantly different from baseline ($P < 0.05$). †Significant difference between CVD (chronic venous disease) group and control (healthy) group ($P < 0.05$). Results are presented as mean (standard deviation).

5.2.2 Call-up and Reabsorption maneuver

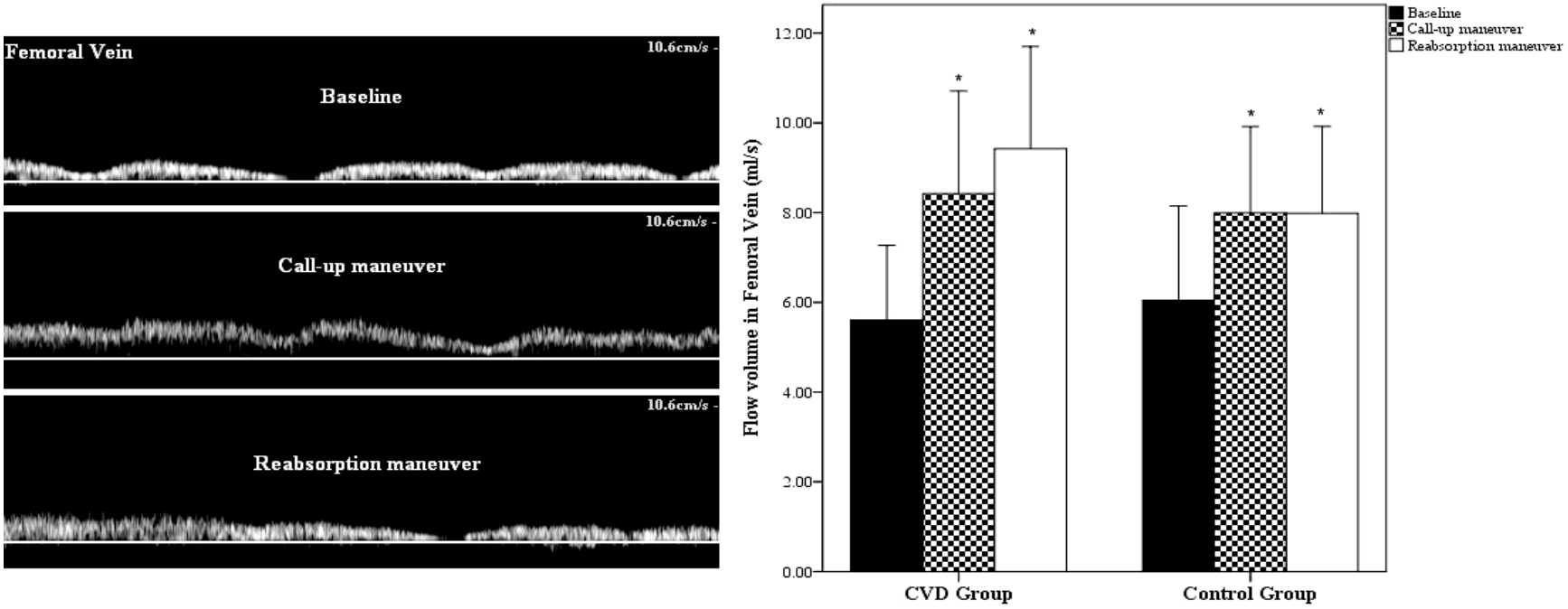
During the call-up maneuver, the FV blood flow volume increased significantly from baseline ($P < 0.05$; Figure 13). The FV peak flow velocity only increased during call-up maneuver in the control group ($P = 0.032$), and during reabsorption and call-up maneuvers in the CVD group ($P < 0.001$) and in both groups pooled together ($P < 0.01$). The FV mean flow velocity only increased during call-up and reabsorption maneuvers in the CVD group ($P < 0.001$ and $P < 0.001$, respectively) and in both groups pooled together ($P < 0.001$ and $P < 0.01$, respectively). The cross-sectional area of FV increased from baseline during the call-up maneuver only in the CVD group ($P < 0.01$) and with all groups pooled together

($P < 0.01$), and during reabsorption in both the CVD ($P < 0.01$) and the control group ($P < 0.01$) and with the two groups pooled together ($P < 0.001$).

The GSV blood flow (peak and mean flow velocity and flow volume) increased significantly ($P < 0.05$) from baseline during call-up and reabsorption maneuvers (Figure 14). The cross-sectional area of GSV, however, remained unchanged during the maneuvers.

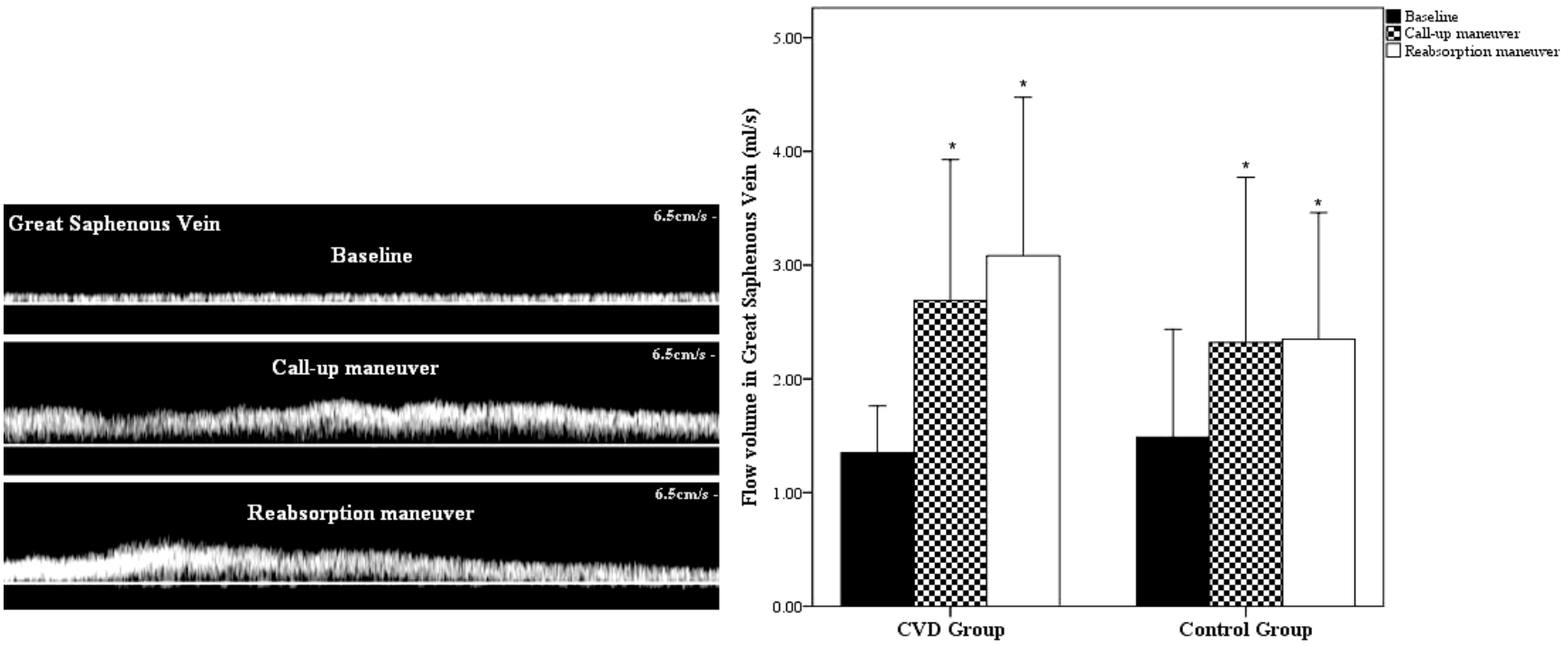
The venous blood flow (mean and peak flow velocities and flow volume) and cross-sectional area augmentation from baseline for both FV and GSV were similar in both call-up and reabsorption maneuvers, with the exception of the cross-sectional area augmentation of FV that was lower during call-up maneuver when compared with reabsorption in the control group [12.45 (30.00)% and 23.05 (32.89)%, respectively; $P < 0.01$]), and with the two groups pooled together [12.70 (23.85)% and 34.76 (58.40)%, respectively; $P < 0.05$]). The cross-sectional area (in absolute values) of FV during call-up was also lower than during reabsorption maneuver in the control group [(0.49 (0.19) cm^2, and 0.53 (0.19) cm^2 for call-up and reabsorption maneuvers, respectively; $P = 0.042$]), and with the two groups pooled together [0.50 (0.19) cm^2 and 0.57 (0.19) cm^2 for call-up and reabsorption maneuvers, respectively; $P < 0.05$]), but no differences were found between maneuvers in the CVD group. In addition, no differences in cross-sectional area for GSV, and in blood flow for FV and for GSV, were found were found between call-up and reabsorption maneuvers.

Figure 13 - Blood flow velocities and volume of femoral vein during baseline, call-up maneuvers and reabsorption maneuvers of MLD



Left: image of blood flow velocities of FV during baseline, call-up maneuver and reabsorption maneuver of MLD. Right: mean and error (95% of confidence interval) of flow volume in femoral vein in the CVD group (patients with chronic venous disease) and the control group (healthy subjects) during baseline and MLD maneuvers (call-up and reabsorption), showing the results of Bonferroni-adjusted pairwise comparisons. (*) Significantly different from the baseline.

Figure 14 - Blood flow velocities and volume of great saphenous vein during baseline, call-up maneuvers and reabsorption maneuvers of MLD



Left: image of blood flow velocities of great saphenous vein (GSV) during baseline, call-up maneuver and reabsorption maneuver of MLD. Right: mean and error (95% of confidence interval) of flow volume in the GSV in the CVD group (patients with chronic venous disease) and the control group (healthy subjects) during baseline and MLD maneuvers (call-up and reabsorption), showing the results from Bonferroni-adjusted pairwise comparisons. (*) Significantly different from the baseline.

5.2.3 Severity of CVD and MLD maneuvers

The CVD group presents a higher cross-sectional area of the GSV than the control group during call-up ($P < 0.001$) and reabsorption maneuvers ($P < 0.001$), but the cross-sectional area augmentations were similar. No other differences were found between the CVD and the control group during the application of each maneuver.

Blood flow (peak and mean flow velocity and the flow volume) augmentations in FV and as a result of applying the reabsorption maneuver decreased with the severity of CVD, as measured by VCSS ($r = -0.51$, $P < 0.05$ for peak flow velocity; $r = -0.51$, $P < 0.05$ for mean flow velocity; and $r = -0.49$, $P < 0.05$ for flow volume). Again in the FV, flow volume augmentation also decreased with the severity of CVD, measured by CEAP clinical classification ($r = -0.64$; $P < 0.05$). The GSV flow volume augmentation was not affected by the severity of the CVD disease.

5.3 Venous flow during manual lymphatic drainage applied to different regions of the lower extremity.

5.3.1 Manual lymphatic drainage at the thigh

The cross-sectional area of FV increased when MLD was applied over the medial side of the thigh ($P < 0.05$) for all groups, but not when applied to its lateral side in control group. The cross-sectional area of GSV remains unchanged with MLD applied to the thigh. Irrespective of changes in cross-sectional area, peak and

mean blood flow velocity and flow volume increased during medial and lateral thigh MLD both in FV and in GSV ($P < 0.001$ for all variables). However, the increase in blood flow velocity and in blood flow volume was higher with medial thigh MLD, when compared with the lateral thigh MLD, in both FV and GSV ($P < 0.001$ for all variables). In addition, the increase in venous blood flow velocity and flow volume as a result of medial thigh MLD was greater in GSV than in FV ($P < 0.05$). No differences could be found in the effect of MLD in blood flow and in the cross-sectional area in either veins between the CVD group and the control group (see Table 15).

5.3.2 Manual lymphatic drainage at the leg

The peak and mean flow velocity and flow volume of PV and SSV increased as a result of MLD applied to the leg ($P < 0.001$). The same procedure increased PV's cross-sectional area ($P < 0.001$), but not that of SSV. These increases, however, were similar ($P > 0.05$) when the MLD technique was performed over the lateral and the medial aspect of the leg (Table 16). The amount of increase in blood flow velocity and blood flow volume as a result of leg MLD was similar in PV and SSV. Again, the effect of leg MLD on peak flow velocity was similar in the two groups. A significant interaction effect between the vein assessed (PV and SSV) and the experimental groups (CVD group and control group) was found for the increase in mean blood flow velocity ($P < 0.01$), which was higher for SSV in the CVD group, and for PV in the control group.

Table 15 - Venous blood flow during manual lymphatic drainage at the thigh

Variables		CVD group		Control group		All Participants	
		N		N		N	
Femoral vein							
Cross-sectional area (cm^2)	Baseline	27	0.41(0.14)	29	0.43(0.20)	56	0.42(0.17)
	Medial aspect	27	0.45(0.17)*	29	0.48(0.20)*	56	0.46(0.19)*
	Lateral aspect	28	0.46(0.17)*	29	0.44(0.18)	57	0.44(0.18)
Peak flow velocity (cm/s)	Baseline	28	21.07(10.70)	29	20.14(8.11)	57	20.60(9.40)
	Medial aspect	28	25.39(15.14)*	29	24.59(8.03)*	57	24.98(11.95)*
	Lateral aspect	28	22.81(14.67)*†	29	23.19(7.80)*†	57	23.00(11.65)*†
Mean flow velocity (cm/s)	Baseline	28	14.04(8.60)	29	13.02(6.48)	57	13.52(7.53)
	Medial aspect	28	17.58(12.53)*	29	15.86(6.44)*	57	16.70(9.86)*
	Lateral aspect	28	15.70(12.19)*†	29	14.93(5.63)*†	57	15.31(9.36)*†
Flow volume (mL/s/s)	Baseline	27	5.09(2.91)	29	5.28(3.60)	56	5.19(3.25)
	Medial aspect	27	6.82(3.69)*	29	7.22(3.68)*	56	7.03(3.65)*
	Lateral aspect	28	6.09(3.44)*†	29	6.22(3.31)*†	57	6.16(3.35)*†
Great saphenous vein							
Cross-sectional area (cm^2)	Baseline	22	0.11(0.07)	29	0.09(0.04)	51	0.10(0.06)
	Medial aspect	22	0.11(0.08)	29	0.10(0.05)	51	0.10(0.06)
	Lateral aspect	22	0.10(0.07)	29	0.10(0.05)	51	0.10(0.06)
Peak flow velocity (cm/s)	Baseline	22	19.19(12.25)	29	20.71(14.43)	51	20.06(13.42)
	Medial aspect	22	28.82(19.97)*	29	32.81(24.77)*	51	24.98(11.95)*
	Lateral aspect	22	22.00(14.70)*†	29	25.68(19.78)*†	51	23.00(11.65)*†
Mean flow velocity (cm/s)	Baseline	22	13.41(9.18)	29	14.02(11.60)	51	13.76(10.53)
	Medial aspect	22	20.31(15.62)*	29	22.92(21.00)*	51	21.80(18.74)*
	Lateral aspect	22	15.66(11.01)*†	29	18.07(16.31)*†	51	17.03(14.20)*† †
Flow volume (mL/s/s)	Baseline	22	1.63(1.96)	29	1.34(1.56)	51	1.47(1.73)
	Medial aspect	22	2.41(2.66)*	29	2.26(2.47)*	51	2.33(2.53)*
	Lateral aspect	22	1.87(2.28)*†	29	1.72(1.80)*†	51	1.78(2.00)*†

Manual lymphatic drainage technique at medial and lateral aspect of thigh. * Significantly different from baseline ($P < 0.05$). † Significantly different from technique applied at medial aspect of thigh ($P < 0.05$). Results are presented as mean (standard deviation) for CVD (chronic venous disease) group and control (healthy) group.

Table 16 - Venous blood flow during manual lymphatic drainage on the leg

Variables		CVD group		Control group		All Participants	
		N		N		N	
Popliteal vein							
Cross-sectional area (cm^2)	Baseline	28	0.21(0.15)	29	0.19(0.12)	57	0.20(0.13)
	Medial aspect	28	0.27(0.19)*	29	0.23(0.15)*	57	0.25(0.17)*
	Lateral aspect	28	0.25(0.17)*	29	0.23(0.13)*	57	0.24(0.15)*
Peak flow velocity (cm/s)	Baseline	27	18.17(8.53)	29	19.69(9.78)	56	18.96(9.15)
	Medial aspect	28	37.12(20.38)*	29	42.00(20.48)*	57	39.60(20.40)*
	Lateral aspect	28	33.90(16.00)*	29	42.78(21.27)*	57	38.42(19.23)*
Mean flow velocity (cm/s)	Baseline	27	11.95(6.28)	29	12.30(7.89)	56	12.14(7.10)
	Medial aspect	28	23.45(14.21)*	29	27.90(16.73)*	57	25.71(15.57)*
	Lateral aspect	28	21.43(11.33)*	29	28.07(17.14)*	57	24.80(14.83)*
Flow volume (mL/s/s)	Baseline	27	2.31(2.74)	29	2.26(2.16)	56	2.28(2.44)
	Medial aspect	28	5.76(5.64)*	29	6.36(5.41)*	57	6.06(5.48)*
	Lateral aspect	28	4.99(4.38)*	29	6.88(6.15)*	57	5.95(5.39)*
Small saphenous vein							
Cross-sectional area (cm^2)	Baseline	23	0.05(0.03)	29	0.05(0.03)	52	0.05(0.03)
	Medial aspect	22	0.06(0.04)	29	0.06(0.03)	51	0.06(0.03)
	Lateral aspect	23	0.05(0.03)	29	0.06(0.04)	52	0.05(0.03)
Peak flow velocity (cm/s)	Baseline	23	10.10(2.67)	29	11.86(5.25)	52	11.08(4.36)
	Medial aspect	23	12.74(4.66)*	29	14.59(5.98)*	52	13.77(6.89)*
	Lateral aspect	23	14.31(10.82)*	29	13.99(7.82)*	52	14.13(9.17)*
Mean flow velocity (cm/s)	Baseline	23	5.95(2.26)	29	7.20(3.94)	52	6.64(3.33)
	Medial aspect	23	7.88(3.57)*	29	9.28(5.98)*	52	8.66(5.06)*
	Lateral aspect	23	9.09(8.06)*	29	8.61(5.76)*	52	8.82(6.80)*
Flow volume (ml/s)	Baseline	23	0.30(0.28)	29	0.40(0.38)	52	0.35(0.34)
	Medial aspect	22	0.46(0.49)*	29	0.55(0.52)*	51	0.51(0.50)*
	Lateral aspect	23	0.55(0.95)*	29	0.52(0.52)*	52	0.53(0.73)*

Manual lymphatic drainage techniques at the medial and lateral aspect of leg. * Significantly different from baseline ($P < 0.05$). Results are presented as mean (standard deviation) for CVD (chronic venous disease) group and control (healthy) group.

5.3.3 Clinical severity of chronic venous disease and response to MLD

No correlation could be found between percent increase in blood flow (i.e., peak and mean flow velocity and the flow volume) or cross-sectional area, and the degree of severity of CVD for any of the veins or MLD techniques investigated in this study.

5.3.4 Reliability

High to moderate test-retest reliability for vein cross-sectional area and venous blood volume measurements, taken at the baseline and during MLD, was confirmed in this study, excepting for measures of blood flow velocity during MLD, for which ICC values were below 0.50 (Table 17).

Table 17 - Test-retest reliability for duplex ultrasound assessments

Variables	Baseline (n=9)	MLD (n=9)
	ICC (range)	ICC (range)
Cross-sectional area (cm^2)	0.97(0.84;0.96)	0.95(0.84;0.99)
Peak flow velocity (cm/s)	0.47(-0.14;0.87)	0.14(0.15;0.54)
Mean flow velocity (cm/s)	0.50(-0.13;0.89)	0.15(-0.13;0.54)
Flow volume (mL/s/s)	0.90(0.58;0.98)	0.57(0.00;0.85)

Intraclass correlation coefficient (ICC), with range (lower bound; upper bound) at 95% confidence interval.

5.4 Efficacy of manual lymphatic drainage in chronic venous disease patients

The effect of 4-week MLD treatment on signs and symptoms of CVD was assessed in Study IV, a single blind randomized controlled trial.

5.4.1 Health-related quality of life

A significant interaction effect of Group versus Time was found for pain dimension of CIVIQ-20, with medium effect size [F (2,70) = 3.417; $P < 0,05$; $\eta^2_\rho = 0.089$]. In the experimental group, but not in the control group, pain scores improved between T0 [55.75 (14.17)] and T1 [(47.11 (14.17)] ($P < 0.05$; 95% IC = [0.617; 17.537]) and between T0 and T2 [(46.00 (15.27)] ($P < 0.05$; 95% IC = [0.181; 17.546]). No differences between experimental and control were found for physical, psychological and social dimensions or in the global score of HRQL assessed with CIVIQ-20.

No interaction effect between Group versus Stocking versus Time could be found for any of the CIVIQ-20 scores (Table 18).

Table 18 - Severity of chronic venous disease, symptoms and leg volume

Variables		Experimental Group	Control group
Severity of disease			
VCSS	T0	7.80(3.49)	6.86(2.94)
	T1	4.85(3.10)*	6.24(2.32)
	T2	5.25(3.21)*	6.43(3.20)
Symptoms			
Fatigue	T0	5.21(2.43)	4.71(2.86)
	T1	2.19(2.18)*	3.89(2.89)
	T2	2.40(2.53)*	4.53(3.18)
Heaviness	T0	5.25(2.40)	4.71(2.86)
	T1	2.12(2.15)*	4.31(2.92)
	T2	2.24(2.53)*	4.20(3.31)
Leg volume (mL/s)	T0	2556.83(389.59)	2476.24(459.41)
	T1	2485.57(399.74)	2502.10(451.23)
	T2	2552.98(331.24)	2473.08(456.08)
Health-related quality of life			
CIVIQ- Global score	T0	48.05(12.53)	48.67(16.03)
	T1	44.84(14.89)	50.23(17.63)
	T2	44.50(12.88)	49.85(19.06)
CIVIQ – Pain dimension	T0	55.75(14.17)	55.71(16.22)
	T1	47.11(14.17)*	55.00(17.61)
	T2	46.00(15.27)*	56.75(19.28)
CIVIQ – Physical dimension	T0	54.00(16.27)	50.24(21.23)
	T1	48.68(19.85)	50.71(21.23)
	T2	48.25(17.41)	52.50(21.91)
CIVIQ – Psychological dimension	T0	37.22(11.07)	39.89(15.48)
	T1	42.22(41.43)	48.25(18.53)
	T2	41.43(11.25)	46.11(22.27)
CIVIQ – Social dimension	T0	52.00(19.77)	47.94(18.57)
	T1	44.56(18.20)	49.21(19.49)
	T2	45.65(17.98)	48.33(20.73)

* Significantly different from T0 ($P < 0.05$). CIVIQ-20: Chronic Venous Insufficiency Questionnaire, range 0-100 (best to worth). VCSS: Venous Clinical Severity Score total scale range 0-30 (best to worst). Symptoms: assessed with visual analogue scale, range 0-10(best to worth). Results are presented as mean (standard deviation).

5.4.2 Severity of the disease, symptoms and leg volume

A significant Group versus Time interaction was found for clinical severity, with medium effect size [F (2; 74) = 3.025, $P < 0.05$, $\eta^2\rho = 0.117$]. In the experimental group, clinical severity improved between T0 [7.80 (3.49)] and T1 [4.85 (3.10)]; $P < 0.001$, 95% IC = [1.426; 4.324]) and between T0 and T2 [5.25 (3.21)]; $P < 0.001$; 95% IC = [1.312; 3730]). In the control group, no changes in clinical severity were noticed during the study. For VCSS item, a significant Group versus Time interaction was found for the item "Venous Edema" [F (2; 72) = 3.308, $P < 0.05$, $\eta^2\rho = 0.084$]. In the experimental group, edema improved between T0 [1.97 (0.20)] and T1 [1.30 (0.21)] ($P < 0.05$, 95% IC = [0.012; 1.334]) but not from T0 and T1 to T2 [1.54 (0.17)].

A significant Group versus Time interaction effect could be found for symptom "fatigue", with medium effect size [F (2; 74) = 4.390, $P < 0.05$, $\eta^2_\rho = 0.106$], improving only in the experimental group between T0 [5.21 (2.43)] and T1 [2.19 (2.18)] ($P < 0.01$; 95% IC = [1.148; 4.994]), and between T0 and T2 [2.40 (2.53)] ($P < 0.01$; 95% IC = [0.677; 4.773]).

A significant Group versus Time interaction effect was also observed for the symptom "heaviness", with medium effect size [F (1.56; 57.72) = 9.710, $P < 0.001$, $\eta^2_\rho = 0.208$], improving only in the experimental group between T0 [5.25 (2.40)] and T1 [2.12 (2.15); $P < 0.01$; IC 95% IC = [1.334; 5.154]) and between T0 and T2 [2.24 (2.53)], ($P < 0.01$; 95% IC = [0.791; 5.372]). Heaviness symptoms in the control group remained unaltered throughout the study.

No Group versus Time interaction effect was found for leg volume. Also, no Group versus Stocking versus Time interaction could be found for severity of disease, fatigue, heaviness and leg volume (Table 18).

5.4.3 Ankle muscles strength

Ankle muscles isokinetic strength and ankle range of motion were similar in experimental and control groups and did not vary across T0, T1 and T2 (Table 19 and Table 20).

Table 19 - Calf muscle dynamometer isokinetic performance for ankle dorsiflexion

		Experimental Group	Control group
Dorsiflexion			
Peak torque 60º/sec (Nm)	T0	30.87(17.90)	25.23(11.12)
	T1	33.26(22.34)	24.37(8.94)
	T2	35.68(20.04)	25.63(13.80)
Peak torque 120º/sec (Nm)	T0	26.27(13.57)	21.21(7.21)
	T1	29.17(16.97)	21.35(5.88)
	T2	30.29(15.84)	22.79(9.31)
Peak torque/body weight 60º/sec	T0	0.40(0.21)	0.37(0.20)
(Nm/Kg)	T1	0.43(0.28)	0.36(0.17)
	T2	0.47(0.22)	0.38(0.22)
Peak torque/body weight 120º/sec	T0	0.35(0.11)	0.31(0.14)
(Nm/Kg)	T1	0.39(0.21)	0.31(0.12)
	T2	0.40(0.18)	0.34(0.16)
Total work 60º/sec (J)	T0	67.35(33.47)	73.00(46.87)
	T1	73.82(49.83)	58.57(21.84)
	T2	66.21(40.92)	63.01(33.37)
Total work 120º/sec (J)	T0	177.99(113.03)	152.91(69.46)
	T1	177.24(117.80)	148.69(69.28)
	T2	171.64(89.60)	141.96(74.76)
Average power 60º/sec (Watt)	T0	15.52(7.83)	13.93(6.03)
	T1	17.29(10.09)	14.45(5.08)
	T2	18.12(9.33)	14.83(7.41)
Average power 120º/sec (Watt)	T0	19.29(11.74)	17.10(7.62)
	T1	20.63(13.56)	17.19(7.78)
	T2	20.72(10.66)	17.44(8.65)

T0 (Baseline); T1 (4 weeks of MLD treatment for experimental group, and without MLD treatement for control group); and T2 (follow-up of 4 weeks after T1).

Table 20 - Calf muscle dynamometer isokinetic performance for ankle plantarflexion

		Experimental Group	Control group
Plantarflexion			
Peak torque 60º/sec (Nm)	T0	42.44(24.35)	52.82(24.23)
	T1	50.70(25.63)	65.22(25.20)
	T2	51.87(25.48)	57.06(22.59)
Peak torque 120º/sec (Nm)	T0	34.30(20.30)	41.90(18.52)
	T1	45.67(24.42)	52.96(21.46)
	T2	40.88(21.28)	46.37(23.95)
Peak torque/body weight 60º/sec	T0	0.61(0.39)	0.78(0.43)
(Nm/Kg)	T1	0.71(0.42)	0.94(0.38)
	T2	0.74(0.44)	0.83(0.35)
Peak torque/body weight 120º/sec	T0	0.49(0.34)	0.62(0.32)
(Nm/Kg)	T1	0.63(0.34)	0.76(0.32)
	T2	0.58(0.33)	0.67(0.32)
Total work 60º/sec (J)	T0	100.57(69.47)	130.82(82.54)
	T1	105.00(53.40)	140.97(66.08)
	T2	100.78(54.32)	139.71(109.75)
Total work 120º/sec (J)	T0	222.89(193.03)	294.49(164.70)
	T1	254.02(133.76)	354.78(174.30)
	T2	238.97(154.50)	280.41(146.17)
Average power 60º/sec (Watt)	T0	19.37(10.41)	25.77(13.71)
	T1	25.26(13.35)	31.30(13.90)
	T2	25.99(13.44)	29.51(15.71)
Average power 120º/sec (Watt)	T0	23.81(18.81)	30.08(16.06)
	T1	29.32(15.98)	38.41(20.41)
	T2	27.16(16.76)	33.43(19.39
Active range of motion 60º/sec (degrees)	T0	57.86(13.64)	64.53(11.03)
	T1	61.56(10.59)	59.43(7.84)
	T2	53.19(6.82)	56.91(5.55)
Active range of motion 120º/sec (degrees)	T0	59.13(10.55)	64.93(10.17)
	T1	61.02(9.71)	60.12(7.90)
	T2	54.73(5.66)	56.31(6.57)

T0 (Baseline); T1 (4 weeks of MLD treatment for experimental group, and without MLD treatement for control group); and T2 (follow-up of 4 weeks after T1).

6 DISCUSSION

6.1 Ultrasound assessment of calf muscle pump function

CMPF is impaired in CVD (Araki et al., 1994; O'Brien et al., 2012; Panny et al., 2009; Recek, 2013; Shiman et al., 2009; Simka, 2007). In addition to normal venous functioning, CMPF function relies on the contraction ability of the calf muscles and in a good ankle range of motion (Cavalheri et al., 2008; de Moura et al., 2012; Panny et al., 2009; Shiman et al., 2009). Despite its importance in preventing venous stasis and hypertension, calf muscle pump is not commonly assessed in dynamic conditions. Likewise, the relationship between muscle pump efficacy and measures of muscle size and architecture has not been explored before. Hence, we hypothesized that calf muscles architecture is affected in CVD subjects and that this is related with the efficacy of the blood pumping function of this musculature, which could be assessed dynamically by means of duplex ultrasound. Despite that air-plethysmography provides a non-invasive and accurate assessment of CMP, it does not allow examining individual veins (deep and superficial) during muscle contraction or ankle motion. These measures can be gathered using duplex scanning and might be important for understanding CVD physiopathology for guiding the treatment and perhaps used as a strategy to assess efficacy of CVD treatments for CMPF.

Our results show a clear increase in venous blood flow caused by calf muscle contractions in individuals with CVD and control participants. Popliteal peak flow volume was maximal during the first contraction of the tip-toe set when the venous

reservoir is full, which has also been seen in other studies (Staubesand et al., 1995). In the CVD patients, but not in the healthy subjects, venous flow augmentation diminished during the muscle contraction set. Such apparent calf pump dysfunction might be related to weak calf muscles in CVD patients (Panny et al., 2009; Qiao et al., 2005) and is compatible with a lower ejection volume, such as has been measured before in this population with air-plethysmography (Nicolaides, 2000). In addition, abnormal venous blood reflux from deep to superficial venous system through incompetent perforator veins may blunt blood flow through the popliteal vein.

As a result of CVD, distal leg muscles may exhibit reductions in strength and power, which then might contribute to aggravate the condition (Webber et al., 2010). In fact, patients with CVD present decreased ankle muscle strength (Panny et al., 2009) with a decreased peak torque per kilogram of body weight, as well as diminished power ability (de Moura et al., 2012) and muscle resistance (van Uden et al., 2005). Accompanying muscle weakness, CVD patients are also characterized by decreased ankle range of motion (de Moura et al., 2012; Dix et al., 2003; van Uden et al., 2005), decreased gait speed (de Moura et al., 2012; van Uden et al., 2005), and impaired functional capacity and mobility (de Moura et al., 2012).

Nonetheless, it seems that calf muscle size is not a strong indicator of the efficacy of muscles to pump venous blood during contractions, at least in patients with venous ulcer (Moloney et al., 2007). In our study, gastrocnemius thickness and some other muscle architectural features were similar in patients with low to moderate severity of CVD and healthy participants and were unrelated to the severity of CVD. Despite this fact, for the medial gastrocnemius, few morphological parameters were associated with the degree of increase in peak flow velocity in the

popliteal vein during tip-toe movement. Higher muscle fascicle length and a lower pennation angle in the medial gastrocnemius, and with the ankle in dorsiflexion, were associated with larger increase in peak flow volume in the popliteal vein during tip-toe. Likewise, higher gastrocnemius muscle fascicles pennation angle with ankle in neutral position, and a larger change in pennation angle between maximal active dorsiflexion and plantarflexion are accompanied by increased venous flow volume at the end of a whole set of calf muscle contractions. The exact meaning of these findings is unclear but lower pennation angle and longer muscle fascicles at ankle dorsiflexion may indicate higher ankle joint range of motion and improved blood pumping function by the calf muscles (Duclay et al., 2009; Manal et al., 2008; Morse et al., 2007; Rassier & Herzog, 2004). Improved ankle range of motion is likely important for muscle pump function during gait and patients with CVD show notable gait alterations (de Moura et al., 2012). In addition, increased tissue hydrostatic pressure and tissue edema in the lower leg likely restricts ankle dorsiflexion and gastrocnemius muscle lengthening and can be related to less efficient calf pump function (Back et al., 1995; Cavalheri et al., 2008; Meissner et al., 2007b; Panny et al., 2009; Shiman et al., 2009).

In this study, the ultrasound measures of gastrocnemius muscle architecture were highly reproducible, which is in agreement with previous studies (Duclay et al., 2009; Narici et al., 1996). In contrast, those of popliteal venous blood flow showed quite large variation when measured within a week interval (Breen et al., 2007; Lurie et al., 2002). Such somewhat low reproducibility might be related with probe unsteadiness, combined with short sampling time of venous velocity (Breen et al., 2007; Lurie et al., 2002). We attempted to determine the error introduced by leg

movement on probe steadiness and on the measures of blood flow volume by contrasting such measures with those collected with subjects performing similar foot movement while standing supported on the opposite limb. In this case, the measures of venous blood flow collected from the popliteal vein were highly reproducible and showed good agreement with measures collected during actual tip-toe movement. This suggests that probe movement relative to the underlying vein is not a strong limitation to the use of continuous-wave Doppler ultrasound in evaluating venous hemodynamics in the lower extremity during dynamical weight-bearing conditions.

The poor test-retest reliability found for popliteal vein measures during tip-toe movements points to the need for strict standardization regarding probe positioning and movement task conditions if the use of ultrasound techniques to assess CMPF is warranted. However, the high bias of this procedure might preclude its usefulness in assessing the response to treatment interventions. Hence, this methodology was not used in our study to assess therapeutic efficacy of MLD on treatment of the impaired calf muscle pump function in this patients.

6.2 Hemodynamic effects of manual lymphatic drainage

The hypothesis that MLD increases blood flow in superficial veins has already been proposed (Leduc et al., 2000), especially by means of the call-up maneuver (Leduc et al., 2000), but the evidence supporting such contention was not strong. In fact, data collected from patients with heart failure and lower limb edema point to small or even insignificant effects of MLD on venous hemodynamics (after applying sequentially call-up and reabsorption maneuvers) (Leduc et al., 2011) but

the direct effect of MLD in venous blood flow across the lower limbs was not evaluated. Despite the lack of strong evidence demonstrating its efficacy, MLD is commonly prescribed as a treatment for patients with CVD, especially when the lymphatic system is affected and edema is present (Raju et al., 2012). Before CVD surgery, MLD also appears to have an important role in improving the severity of the disease and the HRQL of these patients, as well as in improving hemodynamics parameters, such as the reflux volume index, an important marker of CVD (Molski et al., 2009).

Based on this evidence we hypothesized that call-up and reabsorption maneuvers would enhance venous blood flow and that this would be apparent by an elevated blood flow in both the superficial and the deep veins of the lower extremity, a possibility based on the anatomical features of the venous system in the lower extremity. We also hypothesized that venous blood flow enhancement would be higher when the MLD technique (call-up maneuver) is applied to the medial aspect of the thigh and of the leg, thus in coincidence with the course of GSV. Such effects of MLD will then be translated into elevated blood flow in both the superficial and the deep veins of the lower extremity.

6.2.1 Call-up versus Reabsorption maneuver

The results we have obtained demonstrate that indeed MLD techniques, which are based on manual stretching of the skin and underlying soft tissues, increase venous blood flow along the superficial veins, as has already been suggested

(Leduc et al., 2000), but also along the deep venous system, which course beneath the deep fascia. That MLD enhances blood flow in deep veins is a novel observation.

Current concepts regarding MLD indicate that each maneuver should take around 4 seconds from beginning to end (Lee et al., 2011). We strictly followed this recommendation when applying MLD techniques during the different studies comprising this thesis. Also, the two MLD techniques, the call-up and reabsorption maneuvers, presumably affect venous circulation differently. As commonly taught, the call-up maneuver is applied in a proximal to distal direction and enhances venous blood flow. The reabsorption maneuver instead begins distally and then moves proximally, stimulating lymph flow and fluid reabsorption (Lee et al., 2011). Our findings do not substantiate such differential effects between the two maneuvers. In fact, the two maneuvers enhanced venous blood flow to a similar extent and in both FV and GSV. The only difference between the two MLD maneuvers that could be pointed regarded changes in the cross-sectional area of the FV and only in non-CVD participants. In this case, the reabsorption maneuver produced a higher increase in vein's size.

The amount of strain applied to the skin and deeper tissues by MLD is not known. Although studies using radiolabelled tracers demonstrate the efficacy of MLD in stimulating lymph flow, the exact mechanisms by which these techniques work are not fully elucidated (Leduc et al., 1988; Tan et al., 2011). Skin and deep fasciae are connected by ligamentous structures at the level of the thigh, knee, popliteal fossa and leg, which give stability to the skin and act like an anchor during lower limb movements (Nash et al., 2004). The skin-stretching applied during MLD, such as that employed along this thesis, might produce enough increase in pressure

upon underlying structures to enhance venous flow in superficial and deep veins, as occurs during physiological skin-stretching accompanying movement (Benjamin, 2009).

At baseline, venous blood flow was found to be similar in CVD and healthy control participants, which is a common observation. Nevertheless, the cross-sectional area of the GSV was found to be higher in the CVD group, which is in accordance with previous observations (Mendoza et al., 2013) and reveals that the diameter of GSV can be a good predictor of the presence and severity of CVD.

6.2.2 The effect of manual lymphatic drainage when applied to different regions of the lower limb

When applied to the medial aspect of the thigh, MLD results in a greater enhancement of venous blood flow in FV and GSV. In our study, the MLD technique was applied exactly along the course of the GSV (Meissner et al., 2007b) and over the medial compartment of the thigh, following the path of the FV (Toomayan et al., 2005). This finding confirms that MLD should be applied over the trajectory of the GSV as is normally recommended (Felty & Rooke, 2005; Molski et al., 2009; Peyre et al., 2000).

In turn, the effect of MLD in increasing the blood flow at the level of the PV and SSV was similar whether the MLD technique was applied over the medial or over the lateral aspect of the leg. Anatomically, the SSV lies posterior and laterally in the leg (Meissner et al., 2007b), while the PV is deeply and centrally placed within the posterior muscle compartment of the leg (Toomayan et al., 2005). Therefore, both the PV and the SSV are placed relatively equidistant from the medial and the

lateral aspects of the leg where the MLD maneuvers were applied. Also, the smaller size of the leg, at least compared with that of the thigh, makes it difficult to restrict the effect of the MLD technique to just one of the sides of this segment. These findings suggest that MLD must take into account venous anatomy and venous blood flow direction just like the lymphatic anatomy and the lymph flow directions, particularly when applied to larger body segments, such as the thigh (Leduc et al., 2011; Lee et al., 2011; Martin et al., 2011).

As previously said, the mechanisms explaining the increases in venous flow during MLD are still unknown. A likely mechanism would be that skin traction increases the pressure over superficial vessels reducing their caliber and leading to an increase in blood flow velocity. The increased blood flow in the superficial veins would result in higher blood flow across perforating veins and into the deep veins, thereby raising blood flow in the deep venous system as well. In addition, the pressure applied to the skin, as said before, would probably reach the muscles underneath, and pressure would also increase in deep seated structures including veins, further stimulating blood flow. Also, muscle tone might increase during the time MLD techniques are being applied. Although participants were instructed to remain as relaxed as they possibly could during the MLD maneuvers, unnoticed muscle contraction could have occurred either induced by the manual stimulation, or in response to the movement of the lower extremity, which could have contributed to the observed increase in venous blood flow.

This results suggest that MLD should be applied as a low pressure, manual skin-stretching form of massage applied from distal to proximal throughout the lower limb, with the two hands of the physical therapist placed side by side and respecting

the anatomy and flow of venous vessels, in order to increase venous return from the lower limb in subjects with or without CVD (C_{1-5}). The sequence of MLD maneuvers should be applied proximal to distal, followed by a sequence in reverse direction (i.e., from distal to proximal) such as recommended for lymphedema (Leduc et al., 2011; Leduc et al., 1998). Because inflammation may be present in these patients, direct manual skin-stretching should be avoided in that specific anatomical place, as for local inflammation in lymphedema.

6.2.3 The effect of chronic venous disease severity on manual lymphatic drainage efficacy in venous return

CVD causes significant damage to the skin and underlying tissues. Persistent inflammation of the skin leads to disease complications such as lipodermatosclerosis, characterized by fibrosis and microcirculatory changes that together increase the risk of ulceration (Smith, 2006). Leg edema (Meissner et al., 2007b) and limited ankle range of motion (Panny et al., 2009) also restrain the normal movement of the skin and underlying soft tissues, thus contributing to blood stasis and CVD disease complications. Furthermore, the endothelium and the smooth muscle of vein walls also undergo structural changes as a result of chronic inflammation and venous hypertension, causing vein dilatation (Lim et al., 2009). During MLD (call-up maneuvers), the cross-sectional area and hemodynamic augmentations in superficial and deep veins were not associated with the severity of CVD. Nevertheless, more severe CVD was accompanied by diminished flow volume augmentations in FV during reabsorption. Also, MLD-related GSV's cross-sectional area increase was correlated with CVD severity ranging between C_1 and C_5.

The severity of the disease is related with the difficulty of the peripheral venous system to evacuate the venous blood from the periphery in the direction of the heart (Gloviczki et al., 2011), resulting in venous stasis (Gloviczki et al., 2011; Lurie et al., 2000). Furthermore, it is assumed that there is a strict relation between blood flow velocity and secondary deep vein thrombosis (Morris et al., 2004). The prevention of stasis is a main goal in CVD treatment and decisive in preventing venous complications, and is frequently done through conservative approaches. Conservative CVD treatment might include intermittent pneumatic compression (Lurie et al., 2008), compression stockings and bandages (Clarke Moloney et al., 2006; Downie et al., 2008; Partsch et al., 2002), and muscle pump activation using electrical muscle stimulation (Clarke Moloney et al., 2006; Izumi et al., 2010), transcutaneous electrical nerve stimulation (Izumi et al., 2010), or active and passive movements (Izumi et al., 2010; Staubesand et al., 1995). In this regard, MLD maneuvers may be an alternative treatment to enhance venous flow. Nevertheless, this intervention needs specialized professionals and could be an expensive health care treatment. In addition, middle and long-term effects of MLD in venous flow are unknown. Teaching caregivers or patients simple lymphatic drainage, despite the lower efficacy showed in the treatment of lymphedema, when compared with MLD applied by professionals (Huang et al., 2013), could be an alternative.

High pressures (> 40mmHg) reached by intermittent pneumatic compression or compression bandages and stockings, increase venous blood flow velocity (Downie et al., 2008; Lurie et al., 2000) but veins suffer compression and therefore their cross-sectional area decreases (Lurie et al., 2008; Partsch et al., 2002). Despite increased venous flow velocity in deep veins, pressures applied to the lower

extremity, within the range of 80-100 mmHg or higher, force the collapse of the superficial veins (Lurie et al., 2000; Morris et al., 2004). In contrast, and as we have demonstrated, the MLD maneuvers increase the cross-sectional area of both the superficial and the deep veins, most likely because the amount of pressure applied was adequate (Leduc et al., 1998). During the application of the MLD maneuver, vein enlargement was accompanied by the acceleration of the blood flow and therefore the volume of venous blood flowing through the scanned cross section of the deep and superficial veins increased substantially.

The mean and peak flow velocity augmentations in FV during active and passive movements of the ankle (20 - 40%) (Staubesand et al., 1995) and in GSV (34.00 - 46.58 cm/s in healthy participants, and 21.02 - 43.12 cm/s in CVD participants) (Sochart et al., 1999) were very similar to the augmentations produced by MLD in our studies. Calf muscle electrical stimulation, and compression bandaging (Clarke Moloney et al., 2006) are all procedures that increase venous flow in PV. Compared to these procedures, which enhance venous blood flow mainly in the deep vein system, the MLD technique is able to increase blood flow in both superficial and deep veins. Moreover, the increase of venous blood flow during the tip-toe movements seems to be lower in patients with CVD, when compared to age-matched healthy controls, as the results of our Study I suggest, whereas MLD efficacy seems to be the same in CVD patients and in subjects without vascular disease.

6.3 Therapeutic efficacy of manual lymphatic drainage for treatment of patients with chronic venous disease

Since we have observed increased venous blood flow on deep and superficial veins of lower limb during MLD, and because previous studies reveal that this technique can improve HRQL, symptoms, severity of disease, edema following vascular surgery (Molski et al., 2013; Molski et al., 2009), we hypothesized that MLD alone (10 sessions during 4 weeks), would improve HRQL, clinical and functional status of patients with CVD and that this effect would still be noticed following a short-term follow-up (4 weeks). We also anticipated that the effect of MLD on CVD severity and symptoms could be enhanced by compliance to compression stockings.

Our data reveals that a 4-weeks period of MLD treatment, comprising ten 40 to 45 min-duration sessions, improves CVD clinical severity (mostly related to venous edema), symptoms (leg heaviness, fatigue), and pain-HRQL. Furthermore, this study shows that the positive effects of MLD on CVD can still be observed after 4 weeks of follow-up. Nonetheless, leg volume, ankle muscles isokinetic performance, and ankle active range of motion were not affected by the MLD treatment.

Previous studies show that MLD (10 sessions in 2 weeks) used in CVD patients who were referred to vascular surgery is effective in diminishing pain and edema and in improving HRQL (Molski et al., 2013). When employed for a longer period of time (14 sessions in 5 weeks), MLD also seems to effectively contribute, together with surgery, to improve CVD severity (Molski et al., 2009). However, such

effect of MLD could be explained by faster recovery during the post-operative time. Our results extend this evidence by showing that MLD has a real effect in improving CVD-related symptoms, pain-HRQL and clinical severity (mostly related to venous edema), independently from vascular surgery.

When measured by VCSS, the severity of CVD has been shown to improve with surgery alone (relative improvement: 70%) (Kakkos et al., 2003), drug treatment (absolute improvement: 4 points) (Cesarone et al., 2010), and with conservative treatments, like kinesio taping (1.8 points) (Aguilar-Ferrandiz et al., 2013a). In our study the improvement in clinical severity reached 37.8% or 3.05 points at T1, and 32.7% or 2.55 points at T2, comparatively to baseline (T0). Therefore, in terms of clinical severity in CVD, our MLD intervention seems to provide a clinical effect that lies well within the range of effects offered by other conservative treatment modalities (Aguilar-Ferrandiz et al., 2013a). On the other hand, the improved overall VCSS score observed in the experimental group was the result of an effect of the MLD treatment on the item "venous edema", thus cannot be ascribed to changes in self-reported measures. The venous edema item is defined as an augmented tissue volume of presumed venous origin, i.e., with significant changed magnitude due to standing or limb elevation, or with evidence of venous etiology, like varicose veins or history of deep vein thrombosis, and that is present in most if not every days (Passman et al., 2011; Vasquez et al., 2010).

Our MLD treatment also improved pain-HRQL, heaviness and fatigue. An improvement of an 8-10 points and of 20-24 in CIVIQ scores represents a worthwhile improvement in signs and symptoms, respectively for drug (Launois et al., 2010) and compression (Andreozzi et al., 2005) therapy. In our study, pain-

HRQL improved by approximately 8.6 points in T1, and 9.8 in T2; therefore such improvements might be regarded as clinically significant. Nevertheless, MLD was ineffective in changing the physical, social or psychological components of HRQL. For visual analogue scale, clinical meaningful changes require a minimum variation of 2.1-5 cm in the ratings (Aguilar-Ferrandiz et al., 2013a; Launois et al., 2010). Our observed improvement in fatigue and heaviness symptoms matched this range (2.84-3.55), revealing an important improvement in patients with CVD after MLD treatment.

The reason which explains pain and discomfort that accompanies CVD is not completely clear. Leg symptoms in CVD patients may not have an exclusive venous origin and may otherwise be related with adhesion of leucocytes to the endothelium (Boisseau, 2007). Leucocytes are believed to be the source of large amounts of inflammatory mediators that are released into the vein walls and the interstitial space (responsible for trophic skin changes), because of hypoxia and blunted venous flow (Boisseau, 2007). Such inflammatory mediators are presumed responsible for stimulating nociceptive nerve endings in the skin and other tissues and to cause pain and discomfort (Boisseau, 2007). It is possible that MLD, by improving venous flow, and also by stimulating lymphatic reabsorption (Leduc et al., 1998) and decreasing tissue edema, could blunt the leucocyte-endothelial inflammatory reaction (Boisseau, 2007). Also, there is the possibility that stimulation of cutaneous, subcutaneous, and even muscle and other deep tissues afferents by MLD maneuvers may interfere with the transmission of discomfort and pain sensation, like other techniques, such as Kinesio taping that also stimulates the skin through straining (Aguilar-Ferrandiz et al., 2013a). In addition, a placebo effect, not measured in our study, may have an

important effect in reducing activity in central neural pathways associated with pain perception (Dobrila-Dintinjana & Nacinovic-Duletic, 2011). Nevertheless, changes in the severity of disease, specially decreased venous edema item (a sign evaluated by a blind evaluator) from T0 to T1, support a real effect of MLD in reducing venous stasis and improving the CVD condition conservatively.

Nevertheless previous studies were unable to provide distinct demonstration of MLD efficacy in treating lymphedema (Huang et al., 2013) or sports injury-associated edema (Vairo et al., 2009), as well as in improving functional status (Ebert et al., 2013). Nevertheless, MLD continues to be suggested as an adjuvant rehabilitative intervention following orthopedic surgery (Ebert et al., 2013), and it is efficacious in the treatment of lymphedema if applied in combination with other interventions, generally known as the complex decongestive therapy (Kim et al., 2012).

The important role played by the ankle range of motion and calf muscle strength in the efficacy of CMPF is now widely recognized (de Moura et al., 2012; Padberg et al., 2004; Panny et al., 2009; Shiman et al., 2009; van Uden et al., 2005). Likewise, altered CMPF seems to play a key role in the physiopathology of CVD (de Moura et al., 2012; Panny et al., 2009; Shiman et al., 2009; van Uden et al., 2005). However, we were unable to find any significant change in ankle muscles performance (either plantarflexors or dorsiflexiors) and ankle range of motion after MLD treatment. Physical exercise is nowadays widely recommended for CVD management (Kahn et al., 2011; Padberg et al., 2004). In previously conducted randomized controlled trials, exercise training in patients with CVD (Padberg et al., 2004) or with post-thrombotic syndrome (Kahn et al., 2011) was shown to improve

calf muscles' peak torque at slow (60º/s) and fast (120º/s) speeds (Padberg et al., 2004), maximal heel rise repetitions (Kahn et al., 2011), CMPF (Padberg et al., 2004), and HRQL (Kahn et al., 2011). However, the role of physical exercise in ameliorating the measures of clinical severity of CVD or in improving few performance features, such as joint range of motion or work and power ability of ankle plantarflexors could not be clearly demonstrated (Kahn et al., 2011; Padberg et al., 2004). However, we cannot rule out the possibility that MLD might improve ankle function during more natural activities, such as gait. Future work might assess whether MLD improves ankle function (improved ankle muscles recruitment and ankle range of motion) during gait in CVD patients.

Finally, we also hypothesized that the effect of MLD would rely on using compression stockings. The rationale for this hypothesis was that MLD would decrease venous stasis, would diminish tissue edema and that the use of compressive stockings would prolong these effects of MLD. Despite the information and the advice given about the importance of wearing compression stockings to manage symptoms and complications of CVD, our study participants did not change their habitual behavior regarding such use. The adherence to compressive stockings treatment is usually decisive (Ziaja et al., 2010). In this our study, 23 out of the 41 patients participants that completed the study were not wearing compression stockings and only four participants, two in each group, (2 participants from experimental group and 2 participants from the control group) fully adhere to this treatment. However, in our study wearing or not compression stockings had no effect on MLD efficacy.

7 CONCLUSION

Assessing CMPF in an easy and assessable way is needed. By using ultrasound (vascular and muscular), hemodynamics in the popliteal vein and gastrocnemius muscle architecture, the two major components of calf muscle, can be measured. Such measures might then be employed to dynamically evaluate CMPF during tip-toe movements. Duplex ultrasound scanning demonstrated lower efficiency of calf muscles in patients with mild CVD when compared to a group of controls, which extends previous findings obtained with air-plethysmography. However, no differences in gastrocnemius muscle architecture and blood flow velocity of popliteal vein at baseline existed between the CVD group and the control group. Few associations were found between gastrocnemius muscle parameters and muscle pump efficacy but no relationship between these measures and CVD severity were founded. Ultrasound evaluation of CMPF shows good reliability if measures are in collected in the same testing session. However, reliability and agreement of this technique was poor when tests are repeated in separate days. Decidedly, further research is needed in order to improve ultrasound-based assessment of CMPF in CVD patients.

Because the effect of MLD in venous hemodynamics is still unclear, we compared the two major MLD maneuvers (call-up and reabsorption). When these maneuvers were applied to the medial aspect of the thigh, both improved in a similar amount the venous blood flow in FV and GSV. When applied along the entire lower limb, MLD increases deep (FV and PV) and superficial (GSV and SSV) venous blood flow. The amount of blood flow augmentation within deep and superficial

veins is significantly higher when MLD maneuvers are applied along the course of veins, like on the medial aspect of the thigh, than when it is applied on the lateral side of this segment. In the leg, MLD shows equal efficacy in increasing venous blood flow when applied to the medial or the lateral side of this body segment, as a result of more central position of the leg veins (PV and SSV). These increases in venous blood flows occur to a similar extent in CVD patients (C_{1-5}) and healthy participants. However, the efficacy of the reabsorption maneuvers in increasing venous blood velocity may decrease with CVD increased severity.

To verify if the MLD techniques investigated in our more mechanistic studies would improve CVD (C_{3-5}) severity, we undertook a single blind randomized controlled trial involving 10 sessions of MLD over 4 weeks. Patients with CVD improved their clinical severity, most related to venous edema, symptoms, like fatigue and heaviness, and pain-HRQL, after the treatment, and much of these effects were still observed after 4 weeks of follow-up. However, MLD seems unable to modify the majority of the components of HRQL, leg volume or ankle muscles isokinetic strength and ankle range of motion. Also, it seems that these effects of MLD on the treatment of CVD patients were not related with compliance to compression stockings usage.

7.1 Clinical notes

For treating patients with CVD, MLD should be applied as a low pressure, manual skin-stretching form of massage applied from distal to proximal, with both hands of physical therapist side by side, respecting the anatomy and flow of venous

vessels, in order to increase venous return of the lower limb in subjects with or without CVD (C_{1-5}). Applying four weeks of MLD may be used as a strategy to relieve fatigue and heaviness symptoms related do CVD, improve pain dimension of HRQL, decrease clinical severity of disease and related edema with at least a short term effect (four weeks after treatment).

The reliability of ultrasound assessment of muscular and hemodynamics components of calf muscle pump show only moderate reliability and are not sensitive to distinguish between different severity levels of CVD.

7.2 Limitations

The major limitation found in our studies was the poor test-retest reliability found for venous hemodynamics measures during tip-toe movements. This points to the need for strict standardization regarding probe positioning and movement task conditions if use of ultrasound techniques is warranted to assess calf muscle pump function. Also, no trigger was used to synchronize MLD techniques and ultrasound assessments, and some effect of respiratory cycle or other stimulant effects (not controlled) might have influenced venous flow. Because of this limitation, we use augmentations to reduce error. Also, the limitation due to the lack of information about placebo effect of MLD must be acknowledged and addressed in future studies.

7.3 Future directions

In the future it will be important to address the effect of MLD in known physiopathology mechanisms of CVD. In particular, the effect of MLD on markers of tissue inflammation and microcirculation should be evaluated. From the technical side, there are also questions that should deserve attention. One of these questions regards the minimal amount of technique repetitions that are needed to exert an effect. This is particular important since the time cost of this type of treatment is considered high. Related with this question, is to know how efficacious self-drainage is in managing CVD. Because MLD still relies heavily on specialized health professionals, and because the costs to treat patients with venous ulcer are very high, future studies should evaluate the impact of MLD in this specific group of patients, as well as its cost-effectiveness.

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9 APPENDIXES

9.1 Appendix 1 -Informed Consent of Study I

CARTA DE EXPLICAÇÃO DO ESTUDO

Doutoranda/investigadora: Rute Sofia dos Santos Crisóstomo (crisóstomo.rute@gmail.com; tm: 968584992)

Orientador: Professor Doutor Paulo Armada da Silva (parmada@fmh.utl.pt)

Este estudo, intitulado "Estudo piloto – avaliação da bomba muscular venosa da perna por ultrassonografia", tem como objectivo avaliar a fiabilidade e validade de construção da avaliação por eco-doppler da velocidade do fluxo, durante a contracção da bomba muscular venosa da perna.

Enquadra-se no programa de Doutoramento em Fisioterapia, da Faculdade de Motricidade Humana da Universidade Técnica de Lisboa e Centro Interdisciplinar para o Estudo da Performance Humana (CIPER).

A cada elemento de estudo será feita entrevista para que possam ser recolhidos dados da história clínica e outros complementares e garantir que cada um deles está de acordo com os critérios de inclusão e exclusão (ficha de avaliação e ficha de caracterização).

Será necessário deslocar-se às instalações da Faculdade de Motricidade Humana de Lisboa uma ou duas vez, onde serão avaliados: o refluxo venoso nos membros inferiores e os valores hemodinâmicos (por um Técnico de

Cardiopneumologia) durante a realização de exercício (elevação dos calcanhares), que terá a duração aproximada de uma hora. Após estes procedimentos existe a possibilidade de algum desconforto ou fadiga.

Toda a informação recolhida pelo médico e doutoranda, será tratada de forma confidencial e conservada à responsabilidade da doutoranda. Os participantes terão acesso aos resultados do estudo, que por sua vez serão apresentados, sem que nunca sejam divulgados de forma individual.

A escolha de participação no estudo é voluntária e caso seja esse o seu desejo poderá abandonar o mesmo em qualquer momento abandonar o estudo sem risco de represália ou de exigência de compensação.

Com os melhores cumprimentos,

A doutoranda,

Com os melhores cumprimentos,

A doutoranda,

(Rute Sofia dos Santos Crisóstomo)

CONSENTIMENTO INFORMADO

Eu, _____(nome), declaro que os procedimentos de investigação descritos na carta anexa me foram explicados e que todas as minhas questões foram esclarecidas.

Autorizo que o médico e doutoranda recolham a informação constante nas fichas de avaliação e caracterização; e que sejam recolhidos os dados no Laboratório da Escola Superior de Saúde Dr. Lopes Dias constantes na carta de explicação do estudo. Autorizo igualmente, que todos estes dados fiquem com a doutoranda, para que possam constar no estudo.

Compreendo os riscos de participação no estudo, e que não serei prejudicado ou beneficiado pela minha participação.

Entendo que tenho o direito de colocar agora e durante o desenvolvimento do estudo, qualquer questão relacionada com o mesmo. Foi-me garantido que os meus dados pessoais, incluindo a minha identidade, serão guardados de forma confidencial.

Sei que sou livre de, a qualquer momento, abandonar o estudo sem risco de represália ou de exigência de compensação.

Pelo presente documento, eu consinto a minha participação no estudo em questão.

Lisboa, ____ de_____ de 20___

Assinatura do Participante: _____

Assinatura do Investigador: _____

(Rute Sofia dos Santos Crisóstomo)

9.2 Appendix 2 -Informed Consent of Study II

CARTA DE EXPLICAÇÃO DO ESTUDO

Doutoranda/investigadora: Rute Sofia dos Santos Crisóstomo (crisóstomo.rute@gmail.com; tm: 968584992)

Orientador: Professor Doutor Paulo Armada da Silva (parmada@fmh.utl.pt)

Este estudo, intitulado "Drenagem linfática manual na doença venosa crónica: um estudo por ultrassonografia vascular", tem como objetivo avaliar o fluxo venoso nas veias grande safena e femoral durante a aplicação das manobras de reabsorção e de chamada da técnica de drenagem linfática manual, em pessoas com e sem doença venosa crónica. Enquadra-se no programa de Doutoramento Motricidade Humana na especialidade de Fisioterapia, da Faculdade de Motricidade Humana da Universidade Técnica de Lisboa e Centro Interdisciplinar para o Estudo da Performance Humana (CIPER).

Será necessário deslocar-se às instalações da Escola Superior de Saúde Dr. Lopes Dias uma única vez, onde será realizado uma entrevista para que possam ser recolhidos dados sociodemográficos, a história clínica e outros dados complementares (médico, doutoranda e técnico de cardiopneumologia) e garantir que cada um deles está de acordo com os critérios de inclusão e exclusão do estudo. Será ainda realizado um eco Doppler venoso aos membros inferiores para diagnóstico da

ausência/presença de Doença Venosa Crónica. Posteriormente será também aplicada as manobras de reabsorção e chamada (técnicas de drenagem linfática manual) ao nível da coxa e em simultâneo avaliado a hemodinâmica venosa das veias grande safena e femoral com o ecógrafo. Estes procedimentos terão a duração máxima de 1hora e 30 minutos e poderão causar alguma fadiga e desconforto.

Toda a informação recolhida pelo médico, técnico de cardiopnemologia e doutoranda será tratada de forma confidencial e conservada à responsabilidade da doutoranda. Os participantes terão acesso aos resultados do estudo, que por sua vez serão apresentados, sem que nunca sejam divulgados de forma individual.

A escolha de participação no estudo é voluntária e caso seja esse o seu desejo poderá abandonar o mesmo em qualquer momento, sem risco de represália ou de exigência de compensação.

Com os melhores cumprimentos,

A doutoranda,

_____

(Rute Sofia dos Santos Crisóstomo)

CONSENTIMENTO INFORMADO

Eu, _____(nome), declaro que os procedimentos de investigação descritos na carta anexa me foram explicados e que todas as minhas questões foram esclarecidas.

Autorizo que o médico e doutoranda recolham a informação sociodemográfica e clinica relevante para o estudo, assim como ser avaliada por eco Doppler venoso aos membros inferiores, para diagnóstico e avaliação dos efeitos das manobras de reabsorção e chamada, e que sejam recolhidos os dados nos Laboratórios da Escola Superior de Saúde Dr. Lopes Dias constantes na carta de explicação do estudo. Autorizo igualmente, que todos estes dados fiquem com a doutoranda, para que possam constar no estudo.

Compreendo os riscos de participação no estudo, e que não serei prejudicado ou beneficiado pela minha participação.

Entendo que tenho o direito de colocar agora e durante o desenvolvimento do estudo, qualquer questão relacionada com o mesmo. Foi-me garantido que os meus dados pessoais, incluindo a minha identidade, serão guardados de forma confidencial. Sei que sou livre de, a qualquer momento, abandonar o estudo sem risco de represália ou de exigência de compensação.

Pelo presente documento, eu consinto a minha participação no estudo em questão.

Castelo Branco, ____ de_____ de 20___

Assinatura do Participante: _____

Assinatura do Investigador: _____

(Rute Sofia dos Santos Crisóstomo)

9.3 Appendix 3 -Informed Consent of Study III

CARTA DE EXPLICAÇÃO DO ESTUDO

Doutoranda/investigadora: Rute Sofia dos Santos Crisóstomo (crisóstomo.rute@gmail.com; tm: 968584992)

Orientador: Professor Doutor Paulo Armada da Silva (parmada@fmh.utl.pt)

Este estudo, intitulado "Efeito da drenagem linfática manual no fluxo venoso na insuficiência venosa crónica", tem como objetivo avaliar o fluxo venoso nas veias grande safena e pequena safena (superficiais) e veias femoral e poplítea (profundas) durante a aplicação de drenagem linfática manual, em pessoas com e sem insuficiência venosa crónica.

Enquadra-se no programa de Doutoramento em Motricidade Humana, na especialidade de Fisioterapia, da Faculdade de Motricidade Humana da Universidade Técnica de Lisboa e Centro Interdisciplinar para o Estudo da Performance Humana (CIPER).

Será necessário deslocar-se às instalações da Escola Superior de Saúde Dr. Lopes Dias uma única vez, onde será realizado uma entrevista para que possam ser recolhidos dados sociodemográficos, a história clínica e outros dados

complementares (médico, doutoranda e técnico de cardiopneumologia) e garantir que cada um deles está de acordo com os critérios de inclusão e exclusão do estudo. Será ainda realizado um eco Doppler venoso aos membros inferiores para diagnóstico da ausência/presença de Insuficiência Venosa Crónica. Posteriormente será também aplicada drenagem linfática manual (massagem suave) nos membros inferiores e em simultâneo avaliado a hemodinâmica venosa das veias por eco Doppler. Estes procedimentos terão a duração máxima de duas horas e poderão causar alguma fadiga e desconforto.

Toda a informação recolhida pelo médico, técnico de cardiopnemologia e doutoranda será tratada de forma confidencial e conservada à responsabilidade da doutoranda. Os participantes terão acesso aos resultados do estudo, que por sua vez serão apresentados, sem que nunca sejam divulgados de forma individual.

A escolha de participação no estudo é voluntária e caso seja esse o seu desejo poderá abandonar o mesmo em qualquer momento, sem risco de represália ou de exigência de compensação.

Com os melhores cumprimentos,

A doutoranda,

_____

(Rute Sofia dos Santos Crisóstomo)

CONSENTIMENTO INFORMADO

Eu, _____(nome), declaro que os procedimentos de investigação descritos na carta anexa me foram explicados e que todas as minhas questões foram esclarecidas.

Autorizo que o médico e doutoranda recolham a informação sociodemográfica e clinica relevante para o estudo, assim como ser avaliada por eco Doppler venoso aos membros inferiores, para diagnóstico e avaliação da drenagem linfática manual, e que sejam recolhidos os dados nos Laboratórios da Escola Superior de Saúde Dr. Lopes Dias constantes na carta de explicação do estudo. Autorizo igualmente, que todos estes dados fiquem com a doutoranda, para que possam constar no estudo.

Compreendo os riscos de participação no estudo, e que não serei prejudicado ou beneficiado pela minha participação.

Entendo que tenho o direito de colocar agora e durante o desenvolvimento do estudo, qualquer questão relacionada com o mesmo. Foi-me garantido que os meus dados pessoais, incluindo a minha identidade, serão guardados de forma confidencial. Sei que sou livre de, a qualquer momento, abandonar o estudo sem risco de represália ou de exigência de compensação.

Pelo presente documento, eu consinto a minha participação no estudo em questão.

Castelo Branco, ____ de_____ de 20___

Assinatura do Participante: _____

Assinatura do Investigador: _____

(Rute Sofia dos Santos Crisóstomo)

9.4 Appendix 4 -Informed Consent of Study IV

CARTA DE EXPLICAÇÃO DO ESTUDO

Doutoranda/investigadora: Rute Sofia dos Santos Crisóstomo (crisóstomo.rute@gmail.com; tm: 968584992)

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Este estudo, intitulado "Avaliação da eficácia terapêutica da drenagem linfática manual na insuficiência venosa crónica", tem como objetivo avaliar a eficácia terapêutica drenagem linfática manual em doentes com Insuficiência Venosa Crónica e encontra-se a ser realizado na Escola Superior de Saúde Dr. Lopes Dias.

Enquadra-se no programa de Doutoramento em Fisioterapia, da Faculdade de Motricidade Humana da Universidade Técnica de Lisboa e Centro Interdisciplinar para o Estudo da Performance Humana (CIPER).

A cada elemento de estudo será feita entrevista para que possam ser recolhidos dados da história clínica e outros complementares (médico, doutoranda e técnica de cardiopneumologia) e garantir que cada um deles está de acordo com os critérios de inclusão e exclusão (ficha de avaliação).

Será necessário deslocar-se às instalações da Escola Superior de Saúde Dr. Lopes Dias 14 vezes: (I) três vezes, a fim de ser avaliado (com o intervalo de um mês entre cada), onde serão recolhidos dados quanto à qualidade de vida relacionada com

a saúde, à funcionalidade, aos sintomas, à força muscular, à perimetria da perna e caracterização clínica e anatómica da doença, e terá a duração aproximada de uma hora; (II) uma outra vez de modo a participar numa sessão de educação para a saúde (durante uma hora); (III) e dez vezes a fim de ser aplicada a técnica de tratamento durante uma hora, por fisioterapeutas treinadas. Estes procedimentos poderão causar alguma fadiga e desconforto.

Toda a informação recolhida pelo médico e doutoranda, será tratada de forma confidencial e conservada à responsabilidade da doutoranda. Os participantes terão acesso aos resultados do estudo, que por sua vez serão apresentados, sem que nunca sejam divulgados de forma individual.

A escolha de participação no estudo é voluntária e caso seja esse o seu desejo poderá abandonar o mesmo em qualquer momento, sem risco de represália ou de exigência de compensação.

Com os melhores cumprimentos,

A doutoranda,

(Rute Sofia dos Santos Crisóstomo)

CONSENTIMENTO INFORMADO

Eu, _____(nome), declaro que os procedimentos de investigação descritos na carta anexa me foram explicados e que todas as minhas questões foram esclarecidas.

Autorizo que o médico e doutoranda recolham a informação constante na ficha de avaliação; assim como ser tratada com drenagem linfática manual, e que sejam recolhidos os dados nos Laboratórios da Escola Superior de Saúde Dr. Lopes Dias constantes na carta de explicação do estudo. Autorizo igualmente, que todos estes dados fiquem com a doutoranda, para que possam constar no estudo.

Compreendo os riscos de participação no estudo, e que não serei prejudicado ou beneficiado pela minha participação. Entendo que tenho o direito de colocar agora e durante o desenvolvimento do estudo, qualquer questão relacionada com o mesmo. Foi-me garantido que os meus dados pessoais, incluindo a minha identidade, serão guardados de forma confidencial.

Sei que sou livre de, a qualquer momento, abandonar o estudo sem risco de represália ou de exigência de compensação.

Pelo presente documento, eu consinto a minha participação no estudo em questão.

Castelo Branco, ____ de_____ de 20___

Assinatura do Participante: _____

Assinatura do Investigador: _____

(Rute Sofia dos Santos Crisóstomo)

9.5 Appendix 5 - Characterization Questionnaire

FICHA DE CARACTERIZAÇÃO		**Nº**
Nome:	Contato telefónico:	
Morada		
Peso:	Género: Masculino ☐ Feminino ☐	
Altura:	Profissão:	Escolaridade:

SINTOMAS	
Fadiga:	
Sensação de peso:	
Prurido:	
Irritação da pele:	
Cãibras:	

CLASSE CLINICA CEAP	
C_1	
C_2	
C_3	
C_4	
C5	

COMORBILIDADES:

CLASSIFICAÇÃO ANATÓMICA CEAP		
Insuficiência:	**MI. Direito**	**M. Esquerda**
Veias Superficiais		
Veias Profundas		
Veias Perfurantes		

VCSS							
	Dor	**Veias varicosas**	**Edema venoso**	**Pigmentação da pele**	**Inflamação**	**Endurecimento**	**Terapia compressiva**
Ausente (0)							
Suave (1)							
Moderado (2)							
Severo (3)							

10 ANNEXES

10.1 Annex 1 - Article: The use of ultrasound in the evaluation of the efficacy of calf muscle pump function in primary chronic venous disease

Crisóstomo, R. S., Candeias, M. S., & Armada-da-Silva, P. A. (2014). The use of ultrasound in the evaluation of the efficacy of calf muscle pump function in primary chronic venous disease. Phlebology, 29(4), 247-256. doi: 10.1177/0268355512471757

Original article

The use of ultrasound in the evaluation of the efficacy of calf muscle pump function in primary chronic venous disease

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Abstract

***Objectives*:** To evaluate popliteal vein blood flow during calf muscle contraction in chronic venous disease (CVD) patients and healthy controls using ultrasound imaging and to investigate the relationship between venous blood flow and gastrocnemius muscle (GM) morphology.

***Methods*:** Thirty-one subjects participated in this study (mean age: 40.3 [11.8] years), 15 healthy controls and 16 with CVD (clinical classification: C_{1-4}). Popliteal vein cross-sectional area and venous blood flow velocity (FV) were evaluated by Doppler ultrasound at baseline and during three sets of 10 tip-toe movement repetitions. Muscle thickness, muscle fascicle length and pennation angle of both medial and lateral GM were measured by ultrasound. Measures were repeated a week later in 17 participants in order to assess reproducibility with intraclass correlation coefficient (ICC) and Bland–Altman analysis.

***Results*:** Peak FV was lower in CDV group compared with Control group for both first (40.6 [11.8] versus 62.4 (22.1) cm^2/second; $P = 0.021$) and last (30.4 [9.1] versus 49.5 (22.7) cm^2/second; $P = 0.024$) contraction. In CVD group, peak FV during first contraction increased with GM's muscle fascicle length ($r = 0.63$; $P = 0.041$). Popliteal FV also increased with rising range of muscle fascicles pennation change between ankle dorsiflexion and plantar flexion ($r = 0.70$; $P = 0.025$). No associations were found between haemodynamics and medial or lateral GM thickness. Calf muscular architecture was similar in both CVD and control participants. Test–retest reliability of FV measured in the same session was high (ICC$\approx$0.70) for measures taken in the first contraction of the set but lowered when using the last contraction (ICC$<$0.50). Reproducibility of ultrasound evaluation of calf pump is acceptable within the same session but is unsatisfactory when testing in separate days.

***Conclusion*:** Patients with moderate CVD have lower FV during calf muscles contraction but similar muscle anatomical characteristics compared with healthy controls. Changes in calf muscles flexibility and fatigue resistance may be investigated as possible causes of calf pump dysfunction.

Keywords: calf muscle pump function; chronic venous disease; Doppler ultrasound; venous flow

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Introduction

Chronic venous disease (CVD) is an underestimated pathology affecting around 2.5 million people in the USA[1] that decrease patients' ability to engage in normal daily activities[1] and has a negative impact on the quality of life.[2] In advanced

Table 1 Demographic and clinical data

	CVD group		Control group				
	Entire group	Subgroup with retest	Entire group	Subgroup with retest	P^*	$P^\dagger$	All Subjects
N	16	9	15	8			31
Age (years)	44.1 (12.3)	45.7 (14.2)	36.1 (10.0)	35.8 (11.4)	0.086	0.139	40.3 (11.8)
Height (cm)	162.8 (7.3)	162.0 (5.4)	166.3 (9.6)	162.8 (11.0)	0.281	0.114	164.5 (8.5)
Weight (kg)	71.2 (13.4)	73.1 (15.0)	66.1 (15.2)	60.5 (13.5)	0.247	0.936	68.8 (14.3)
BMI (kg/m^2)	27.4 (6.0)	28.1 (7.1)	23.6 (3.0)	22.5 (3.0)	0.281	0.114	25.4 (5.0)
Gender							
Female	13 (81.2)	7 (77.8)	10 (66.7)	6 (75.0)	–	–	23 (74.2)
Male	3 (18.8)	2 (22.2)	5 (33.3)	2 (25.0)	–	–	8 (25.8)
Co-morbidities							
Diabetes	1 (6.25)	0 (0.0)	0 (0.0)	0 (0.0)	–	–	1 (3.2)
Treated thyroid dysfunction (controlled)	2 (12.5)	1 (11.1)	1 (6.7)	1 (12.5)	–	–	3 (9.7)
Uterus malignant carcinoma (operated)	1 (6.25)	1 (11.1)	0 (0.0)	0 (0.0)	–	–	1 (3.2)
Knee surgery (contralateral leg >6 months ago)	2 (12.5)	2 (22.2)	0 (0.0)	0 (0.0)	–	–	2 (6.5)
Lupus erythematosus	1 (6.25)	1 (11.1)	0 (0.0)	0 (0.0)	–	–	1 (3.2)
Surgical removal of greater saphenous with recurrence	1 (6.25)	1 (22.2)	0 (0.0)	0 (0.0)	–	–	1 (3.2)

CVD, chronic venous disease; BMI, body mass index
Quantitative variable: mean (SD); categorical variable: frequency (%)
P^*: Differences between groups (entire group)
$P^\dagger$: Differences between subgroups (groups with retest with one week apart)
Differences are significant with $P < 0.05$ for Mann-Whitney *U* test

stages, CVD is characterized by venous insufficiency, tissue oedema and trophic changes of the skin, including skin ulcerations in severe cases.[3] However, venous insufficiency alone does not seem to explain venous skin ulceration and it has been hypothesized that deficient calf muscle pump function (CMPF) might be a primary factor in CVD-associated skin and soft tissue damage[4] and a cause of inflammation, reduced blood perfusion and scattered muscle cell necrosis.[5,6]

Muscle pump impairment is likely caused by diminished calf muscle strength combined with decreased ankle joint motion, particularly during walking.[7–10] In this sense, CMPF might be related to changes in skeletal muscle architecture. These can be readily evaluated using ultrasound imaging, particularly the muscle thickness, which is a major determinant of muscle strength. Ultrasound imaging can also measure muscle fascicle length and pennation angle, morphological characteristics also associated with the contractile force produced by skeletal muscles.[11]

The efficacy of CMPF is usually evaluated using air plethysmography.[12] However, this method requires equipment not readily available in most clinical settings and the development of alternative method to accurately assess CMPF could stimulate further investigation about the role of muscle pump function in CVD.[9,12–15] Doppler ultrasound is commonly employed as a diagnostic tool in CVD. This technique provides measures of blood flow velocity (FV) in given veins or arteries and can potentially be adapted to be used during dynamic conditions and to assess the blood pumping function of the calf muscles.[12] Therefore, in this study we evaluated the potential of ultrasound to measure the efficacy of CMPF in a group of CVD patients and healthy controls. Ultrasound imaging was further employed to assess the architecture of the two heads of the gastrocnemius muscle (GM) and the relationship between these measures and the blood pumping function of the calf contractions was investigated. We hypothesize that calf muscles architecture is affected in CVD subjects and that this is related with the efficacy of the blood pumping function of this musculature.

Methods

Fifteen healthy control subjects (10 women and 5 men) and 16 subjects (13 women and 3 men) with a diagnosis of CVD (CVD group) were enrolled in this study (Table 1). All CVD subjects presented venous blood reflux in at least one lower extremity vein with a minimum duration of

Table 2 Clinical characteristics of CVD group

	CVD group (all subjects)	Subgroup with retest
N	16 (100)	9 (56.3)
CEAP clinical classification		
C_1	3 (18.8)	2 (22.2)
C_2	2 (12.5)	1 (11.1)
C_3	10 (62.5)	5 (55.6)
C_4	1 (6.3)	1 (11.1)
Anatomical reflux		
Superficial veins	9 (56.3)	5 (55.6)
Deep veins	1 (6.3)	0 (0.0)
Perforator veins	3 (18.8)	3 (33.3)
Superficial+Perforator	2 (12.5)	0 (0.0)
Superficial+Deep+Perforator	1 (6.3)	1 (11.1)
VCSS	3.8 (3.2)	3.33 (3.5)
CSCEAP	2.9 (2.9)	2.7 (3.4)
Symptoms		
Fatigue	11 (68.8)	6 (66.7)
Cramps	8 (50.0)	4 (44.4)
Heavy legs	10 (62.5)	5 (55.6)
Pain	5 (31.3)	3 (33.3
Skin irritation	3 (18.8)	2 (22.2)
Itching	2 (12.5)	1 (1.1)
Without symptoms	4 (25.0)	2 (22.2)

Quantitative variable: mean (SD); categorical variable: frequency (%) VCSS (Venous Clinical Severity Score) total scale range 0–30 (best to worst); CSCEAP (clinical score of clinical, etiological, anatomical, and pathological) total scale range 0–18 (best to worst); CVD, chronic venous disease

0.5 seconds and CEAP (clinical, etiological, anatomical, and pathological) clinical classification in the range C_{1-4}. Before study enrolment, subjects were informed about the purpose and procedures of the study and signed an informed consent. The study received ethical approval by the review board of the scientific council of Faculty of Human Kinetics, Technical University of Lisbon. The exclusion criteria in this study included the presence of severe cardiac insufficiency, acute venous or arterial obstruction, arterial insufficiency, renal insufficiency, uncompensated thyroid dysfunction, pregnancy, neoplasic pathology, systemic or limb infection, recent musculoskeletal injury of the lower limb and peripheral neuropathy of the lower limb. The clinical history, the symptoms (fatigue, heavy sensation, hitching, cramps and skin irritation), the severity of the disease according to the clinical score of CEAP (CSCEAP) and the venous clinical severity score (VCSS) were collected.[16]

The anatomical location (i.e. superficial, perforating and/or deep vein system) and severity of venous blood reflux were determined during the first visit to the laboratory. The control subjects also went through a thorough vascular ultrasound of both lower extremities to confirm that they were free from CVD. All ultrasound tests were performed by a certified vascular sonographist and with the same ultrasound equipment (HI VISION 8500, Hitachi, with a L53 linear array-transducer, scanned at 9 MHz). The leg self-reported as presenting the worst symptoms and clinical signs and the dominant leg were chosen for subsequent measures in, respectively, the CVD group and the Control group.

Baseline and dynamic popliteal vein blood flow

After five-minute rest, the cross-sectional area (CSA) of the popliteal vein was measured at the level just below the saphenopopliteal junction with subjects in prone position. Next, baseline mean and peak blood flow velocities in the popliteal vein were measured in standing position. Blood FV was measured during a 10-second interval using an automatic time integral calculation. After completing the baseline measures, subjects performed a calf muscle contractions protocol similar to that commonly used to assess the efficacy of CMPF by air-plethysmography and composed of three sets of 10 repetitions of tip-toe movements.[12,14] A metronome set the timing of the tip-toe movement that took two seconds to rise to the tip toe, two seconds to get down to the initial position and one second interval to the next repetition. The ultrasound probe was manually held at the lower margin of the popliteal fossa with adequate orientation to measure blood FV in popliteal vein while subjects performed the tip-toe movement. In between the sets, subjects rested for five minutes in sitting position. Mean and peak popliteal vein blood volume at baseline were calculated by the following relation:[17] Flow volume (mean or peak) (cm^3/second) = popliteal CSA (cm^2) × flow velocity (mean or peak) (cm/second).

Dynamic peak and mean blood FV were calculated using the integral of the blood flow–time curve. To avoid the effect of the foot sole impact with the ground on venous blood flow, only the time interval corresponding to heel rising was selected to analysis. To evaluate the bias eventually caused by probe unsteadiness during the movement repetitions, few subjects performed actual and simulated tip-toe movements. The simulated movements were done with subjects standing on a platform with the foot of the measured side hanging off the platform and performing active extension of the ankle joint by contracting the

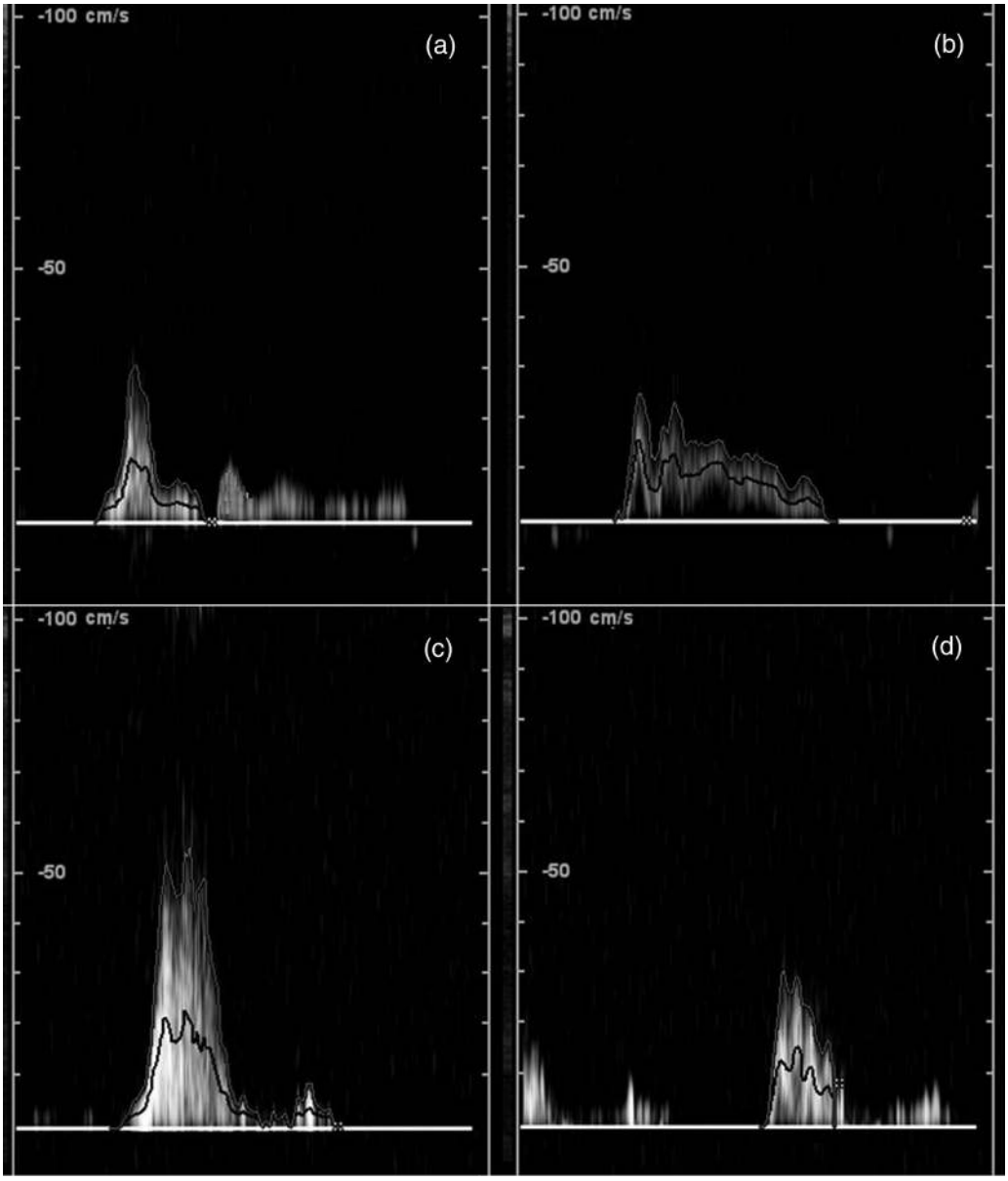

Figure 1 Popliteal vein blood flow velocity during: (a) the first and (b) the last (10th) repetition of the tip-toe movement task in a chronic venous disease (CVD) participant and popliteal vein blood flow velocity during (c) the first and (d) the last (10th) repetitions of the tip-toe movement task in a healthy subject. Also shown are the automatic tracings of the blood flow velocity curve envelope and of the calculated mean (dark trace)

calf muscles. During this task, leg movement is minimal and the probe can be kept rather stationary.

During tip-toe movements, FV data were normalized against baseline popliteal vein FV to calculate FV augmentation using the following equation: augmentation (%) = ([FV during contraction (cm^2/second) – FV during baseline (cm^2/second)]/FV during baseline (cm^2/second))100.

The percentage augmentation in FV was calculated using both the mean FV and the peak FV registered during the first and 10th tip-toe movement in each set.

Muscle architecture

The medial gastrocnemius (MG) and the lateral gastrocnemius (LG) were imaged at 10 MHz and at a scan depth of 65 mm. The two muscle bellies were imaged at the upper third of the distance between the peroneal head and the lateral malleolus with subjects lying prone and at three ankle joint positions: (1) foot and leg at 90° (anatomical neutral position), (2) at maximal active plantar flexion and (3) at maximal active dorsiflexion. The distance between the proximal and distal aponeurosis in

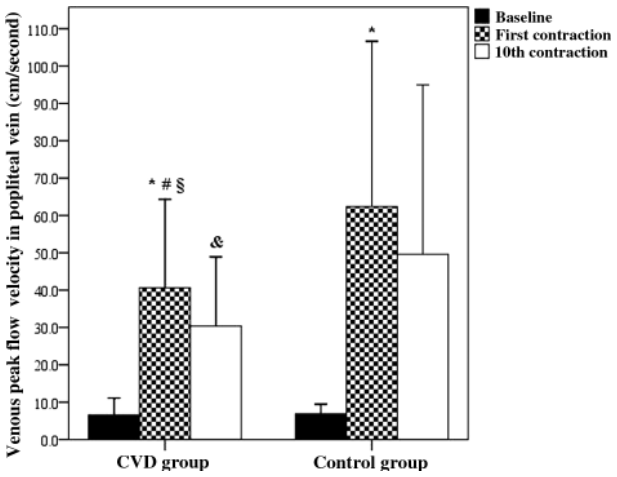

Figure 2 Mean and standard deviation of venous peak flow velocity in the popliteal vein during baseline, first and 10th tip-toe contractions in chronic venous disease and control groups obtained during retesting and showing the results from Wilcoxon signed-rank test: (*) significantly different from baseline, (#) significantly different from first contraction of control group; (§) significantly different from tenth contraction; and (&) significantly different from 10th contraction of control group

both LG an MG was traced to obtain the muscle thickness. In the MG, the direction of the muscle fascicles was also traced to derive the pennation angle for each ankle joint position. Pennation angle was defined as the angle between the load axis of the muscle and the axis of muscle fascicle. Muscle fascicle length, defined as the length of the fascicle between the deep and superficial aponeuroses, was directly measured for the ankle plantar flexion position and using the values of muscle thickness and pennation angle for the ankle neutral and dorsiflexion positions, according to the following equation: Muscle fascicle length (mm) = muscle thickness (mm)/sin (pennation angle).

One week later, 17 subjects (CVD group, $n = 9$; Control group, $n = 8$) visited the laboratory for a second session. During this visit subjects repeated the tip-toe protocol in order to assess the test–retest reliability of ultrasound measures of blood pumping by calf muscles. For each participant, tests were carried out at the same time of day and at the same ambient temperature.

Statistical analysis

All statistical tests were performed with the help of the statistical software package SPSS Inc. v.17 (SPSS Inc., Chicago, IL, USA). Group differences were tested with Mann-Whitney *U* test. The differences between peak FV at baseline, first and 10th contractions were calculated with Wilcoxon signed-rank test. The relationship between CVD severity and the GM and haemodynamic parameters was calculated using Spearman coefficient of correlation. Reproducibility was assessed by test–retest reliability by calculating the absolute agreement intraclass correlation coefficient (ICC)[18] and by agreement analysis using Bland and Altman plot analysis. The significance level was set at $P < 0.05$.

Results

Demographic and clinical data pertaining to the participants are presented in Tables 1 and 2. All subjects in the CVD group presented pathological venous reflux but were free of venous obstruction. No differences in age, height, weight and body mass index (BMI) were found between the groups.

Venous dynamic blood flow

Popliteal vein blood FV increased during calf contractions in both CVD and Control groups with a $P = 0.008$ and 0.012, respectively, for first contraction. But peak FV decreased from first to 10th contraction ($P = 0.028$) in CVD group but in Control group no significant differences ($P = 0.093$) were found between first and 10th contraction. Dynamic blood FV in popliteal vein was lower in the CVD group compared with the Control group during both the first ($P = 0.021$) and the 10th contraction ($P = 0.024$) of the tip-toe movement set (Figures 1 and 2). Peak FV, expressed as percentage augmentation, was lower in the CVD group compared with the Control group during the first contraction (530.6 [115.2]% versus 831.5 [353.4]%, $P = 0.027$). Nevertheless, the mean FV augmentation during the first contraction and peak FV augmentation in 10th contraction did not differ between groups. No differences in venous baseline haemodynamics were found between the two experimental groups (Table 3).

GM architecture

Table 4 presents the anatomical data of GM in CVD and Control groups. Muscle thickness, pennation angle and muscle fascicles length were similar in both groups for all the three ankle joint positions.

No relationship could be noted between clinical severity (measured by CSCEAP and VCSS) in CVD patients and gastrocnemius architecture as well as between clinical severity and dynamic popliteal vein blood flow. In CVD patients, peak FV in the first contraction increased with MG

Table 4 Differences between CVD and control group in gastrocnemius muscle architecture

	Group	*N*	Mean (SD)	*P*
MG thickness in neutral (mm)	CVD	16	18.0 (2.5)	0.890
	Control	15	17.8 (2.7)	
MG thickness in plantar flexion (mm)	CVD	16	17.0 (2.6)	0.968
	Control	15	17.5 (3.5)	
MG thickness dorsiflexion (mm)	CVD	16	19.1 (2.2)	0.654
	Control	15	19.7 (3.2)	
LG thickness in neutral (mm)	CVD	16	12.2 (3.3)	0.384
	Control	15	13.2 (3.6)	
MG pennation angle in neutral (degrees)	CVD	16	19.5 (2.3)	0.664
	Control	15	19.1 (1.3)	
MG pennation angle in plantar flexion (degrees)	CVD	16	42.2 (8.8)	0.165
	Control	15	46.3 (5.4)	
MG pennation angle in dorsiflexion (degrees)	CVD	16	17.6 (3.1)	0.572
	Control	15	17.3 (2.0)	
ΔMG pennation angle (degrees)	CVD	16	24.6 (8.0)	0.096
	Control	15	29.0 (5.5)	
MG fascicle length in neutral (mm)	CVD	16	54.3 (7.7)	0.874
	Control	15	54.8 (8.7)	
MG fascicle length in plantar flexion (mm)	CVD	16	25.0 (4.8)	0.580
	Control	15	24.7 (6.4)	
MG fascicle length in dorsiflexion (mm)	CVD	16	65.3 (15.8)	0.477
	Control	15	66.5 (11.2)	

MG, medial gastrocnemius muscle; LG, lateral gastrocnemius muscle; ΔMG, angle between maximal active dorsiflexion and plantar flexion in medial gastrocnemius; CVD, chronic venous disease
Differences are significant with $P < 0.05$ for Mann-Whitney *U* test

Table 3 Differences between CVD and Control group of the venous haemodynamics in popliteal vein

	Group	*N*	Mean (SD)	*P*
Baseline peak flow velocity (cm/second)	CVD	9	6.6 (2.3)	0.370
	Control	8	6.9 (1.3)	
Baseline mean flow velocity (cm/second)	CVD	9	2.7 (1.7)	0.963
	Control	8	2.4 (1.2)	
Baseline cross-sectional area of popliteal vein (cm^2)	CVD	9	0.6 (0.2)	0.673
	Control	8	0.5 (0.2)	
Baseline peak flow volume (cm^3/second)	CVD	9	3.5 (1.4)	0.743
	Control	8	3.5 (1.4)	
Baseline mean flow volume (cm^3/second)	CVD	9	1.3 (0.6)	0.743
	Control	8	1.2 (0.6)	
First contraction peak flow velocity (cm/second)	CVD	9	40.6 (11.8)	0.021*
	Control	8	62.4 (22.1)	
First contraction mean flow velocity (cm/second)	CVD	9	17.1 (5.0)	0.083
	Control	8	26.7 (12.5)	
Tenth contraction peak flow velocity (cm/second)	CVD	9	30.4 (9.1)	0.024*
	Control	8	49.5 (22.7)	
Peak flow velocity augmentation in first contraction (%)	CVD	9	530.6 (115.2)	0.027*
	Control	8	831.5 (353.4)	
Mean flow velocity augmentation in first contraction (%)	CVD	9	721.5 (532.2)	0.124
	Control	8	1319.0 (1234.7)	
Peak flow velocity augmentation in 10th contraction (%)	CVD	9	379.0 (123.9)	0.068
	Control	8	623.5 (302.4)	

CVD, chronic venous disease
The results were assessed in second week
*Significant with $P < 0.05$ for Mann-Whitney *U* test

muscle fascicle length in dorsiflexion ($r = 0.63$; $P = 0.041$) and decreased with this muscle's pennation angle in dorsiflexion ($r = -0.68$; $P = 0.044$). Peak FV during the last contraction of the tip-toe set also increased with MG pennation angle in neutral position ($r = 0.73$; $P = 0.025$) and with

Table 5 Test–retest reliability of venous haemodynamics and muscle architecture measures

	N	Mean (SD)	ICC (range)	*P*
Immediate test–retest reliability				
First contraction peak flow velocity (cm/second)*	17	50.9 (20.2)	0.74 (0.52–0.88)	0.000
First contraction mean flow velocity (cm/second)*	17	21.6 (10.3)	0.78 (0.58–0.90)	0.000
Tenth contraction peak flow velocity (cm/second)*	17	39.4 (19.1)	0.49 (0.20–0.75)	0.000
Medial gastrocnemius thickness (mm)†	31	17.9 (2.5)	0.98 (0.96–0.99)	0.000
Lateral gastrocnemius thickness (mm)†	31	12.7 (3.5)	0.98 (0.97–0.99)	0.000
Fibre length of medial gastrocnemius (ankle at 90°) (mm)†	31	54.5 (8.0)	0.94 (0.87–0.97)	0.001
Pennation angle of medial gastrocnemius muscle fascicles (ankle at 90°) (degrees)†	31	19.3 (1.8)	0.94 (0.89–0.97)	0.000
Delayed test–retest reliability				
First contraction peak flow velocity (cm/second)	17	53.7 (7.0)	−0.06 (−0.46 to −0.40)	0.253
First contraction mean peak flow velocity (cm/second)	17	21.7 (6.1)	0.28 (−0.47 to −0.50)	0.487
Tenth contraction peak flow velocity (cm/second)	17	48.1 (33.1)	0.10 (−0.39 to −0.54)	0.722

Mean (SD): results from the tree measures in the same day for immediate test–retest reliability and from the measures of first and second week for delayed test–retest reliability
Intraclass correlation coefficient (ICC) significant with $P < 0.05$, with range (lower bound – upper bound) at 95% confidence interval
*Calculated with subgroup with retest
†calculated with all subjects

the range of change in pennation angle between ankle dorsiflexion and plantar flexion ($r = 0.70$; $P = 0.025$).

Test–retest reliability

Ultrasound measures of GM architecture were generally highly reproducible. High immediate test–retest reliability (ICC>0.94) for ultrasound measures of muscle thickness was found for MG and for LG, and for pennation angle and muscle fascicles length of MG in ankle neutral position. The reliability in popliteal vein blood velocity in first and 10th contractions was low, with an ICC<0.5. However, peak FV and mean FV in the first contraction presented good immediate test–retest reliability, with an ICC>0.74. None of the haemodynamic measures showed acceptable test–retest reliability (i.e. ICC>0.5) (Table 5). The Bland–Altman plot analysis reveals a poor agreement for dynamic popliteal vein FV measures (Figure 3).

For the estimation of probe movement bias, the peak FV was 57.8 (14.9) cm/second during the actual tip-toe movement and 52.8 (17.3) cm/second during the simulated tip-toe movement with an ICC = 0.85[0.35;0.98] and $P = 0.005$, and a bias = 4.9 cm/second (lower bound: −10.7 cm/second; upper bound: 20.7 cm/second).

Discussion

Patients with primary CVD have venous reflux that is commonly accompanied by calf muscle pump dysfunction.[7–9] The efficacy of calf muscle pump relies on the integrity of venous valves and in the contraction ability of the calf muscles, together with an adequate range of motion at the ankle joint.[7–10] Any alteration in these factors potentially affects venous blood flow in the lower limb and predisposes to the development of venous hypertension.[10] Qiao *et al*.[5] reported that CVD patients present muscle atrophy affecting the calf muscles, accumulation of blood lactate during mild exercise and elevated venous pressure that persists after ceasing the physical effort. Reduced blood perfusion to leg muscles also characterizes CVD, which in severe situations may cause tissue necrosis.[5,6] However, the size of the calf muscles *per se* seems not to be a key determinant of muscle pump efficacy and of the amount of venous blood flow including in cases with venous ulceration.[19] In fact, in our study the patients had mild CVD (only one subject was C_4) with no differences in GM size compared with the control participants. In addition, no relationship existed between clinical severity of CVD and GM architecture or with venous blood FV.

In contrast, we found a number of correlations between venous FV and few GM architecture features. In our CVD group, a higher peak FV at the end of the tip-toe movement repetition set was associated with higher MG pennation angle in ankle neutral position, and a higher change of this angle occurring between maximum active dorsiflexion and maximum active plantar flexion. Higher peak FV in the first contraction in the CVD group (that was lower than in the Control group)

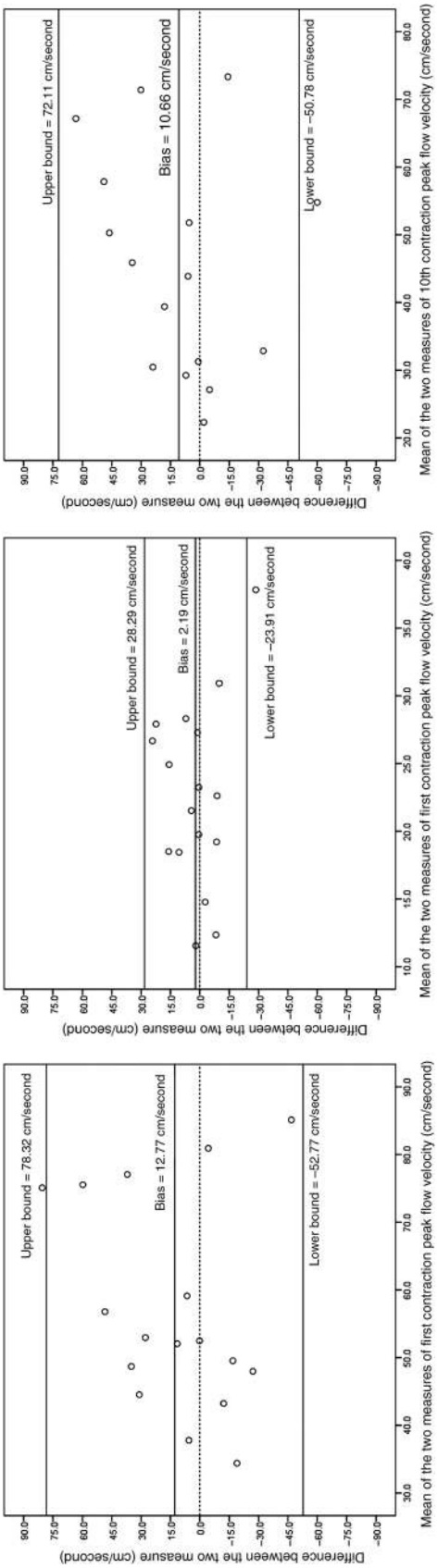

Figure 3 Bland and Altman Plot of the two measures of first contraction peak flow velocity, first contraction mean flow velocity and 10th contraction peak flow velocity, with bounds and bias at 95% of confidence interval

was related to lower pennation angle and higher muscle fascicle length of MG in dorsiflexion. The exact meaning of these findings is unclear but lower pennation angle and longer muscle fascicle may indicate higher ankle-joint range of motion and higher muscle stiffness. These two factors likely affect blood pumping function of calf muscles, through increased muscle activity[20] and strength[21–23] of the GM. Also, increased tissue hydrostatic pressure and tissue oedema in the lower leg likely restricts ankle dorsiflexion and GM lengthening and can be related to less efficient CMPF.[7–10,24]

The results of this study show a clear increase in venous blood flow caused by calf contractions in individuals with CVD and control participants. Previous reports, which examined flow velocity at the popliteal vein employing duplex scanning, show an increase in FV in response to voluntary calf contraction in upright position to a mean value of 70 cm/second,[25] which is comparable to our findings. Moreover, such increase in venous flow velocity is considerably higher than that resulting from calf muscle electrical stimulation or leg compression at the same leg position.[25,26]

Popliteal peak FV was maximal during the first contraction of the tip-toe set. A highest venous flow enhancement, consistently observed at the beginning of calf contractions in the two groups, likely results from a full venous reservoir at this stage. In the CVD group, but not in the Control group, venous flow augmentation diminished during the contractions set. This suggests lower ability of the calf muscles to pump venous blood in the CVD participants compared with the control participants. Such apparent calf pump dysfunction might be related to weak calf muscles in CVD patients.[5,9] In addition, abnormal venous blood reflux from deep to superficial venous system through incompetent perforator veins may blunt blood flow through the popliteal vein. Previous reports, which examined flow velocity at the popliteal vein employing duplex ultrasonography, also found progressive decrease in blood flow acceleration with repetitions of calf dynamic contractions.[27] Furthermore, in this study the healthy participants were slightly younger and with lower BMI compared with the CVD participants. Younger age and less weight are usually associated with stronger muscles,[28,29] which also may contribute to improve the venous flow velocity during contractions.

The present finding of blunted popliteal blood velocity enhancement by calf contractions in the CVD group is compatible with lower ejection

volume as measured with air-plethysmography in this population.[12] Although air-plethysmography provides a non-invasive and accurate assessment of calf pump function, it does not allow examining individual veins. Assessing blood flow in a given vein and during dynamic conditions, such as muscle contraction or ankle motion, might be important for understanding the role of these factors on venous insufficiency and for therapeutic decisions. For instance, a better understanding of calf pump dysfunction might depend on the ability to assess the blood flow through both the deep and superficial venous system, which can be achieved using duplex scanning but not air-plethysmography.

In this study, the ultrasound measures of GM architecture were highly reproducible, which is in agreement with previous studies.[21,30] In contrast, those of popliteal venous blood flow are highly variable when measured within a week interval.[31,32] In the present study, the reliability of peak FV during the first contraction of the tip-toe task was high within the same testing session but low reproducibility (i.e. reliability and agreement) when measured within one-week interval. The poor reliability of ultrasound measures taken in dynamic conditions are likely due to probe unsteadiness, combined with short sampling time of venous velocity.[31,32] We attempted to determine the effect of the leg movement on probe steadiness and blood FV measures by contrasting such measures with those collected with subjects performing similar foot movement while standing supported on the opposite limb. The results showed very high reproducibility and agreement of popliteal vein FV in the two conditions. This suggests that probe movement relative to the underlying vein is not a strong limitation to the use of continuous-wave Doppler ultrasound in evaluating venous haemodynamics in the lower extremity during dynamical weight-bearing conditions. Notwithstanding, the poor test–retest reliability found for popliteal vein FV measures during tip-toe movements points to the need for strict standardization regarding probe positioning and movement task conditions if use of ultrasound techniques is warranted to assess CMPF.

In summary, duplex ultrasound demonstrated lower efficiency of calf muscles in patients with mild CVD when compared with a group of controls. However, no differences in GM architecture and baseline popliteal venous FV existed between the CVD group and the Control group. Few associations were found between GM parameters and muscle pump efficacy but no relationship between these measures and CVD severity existed. Ultrasound evaluation of CMPF shows good reliability if measures are in the same testing session. However, reliability and agreement of this technique was poor when tests are repeated in separate days. Future studies should evaluate the role of calf muscles fatigue in calf pump function and assess antegrade and retrograde flow in deep and superficial veins during movements.

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Conflict of interest: None.

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10.2 Annex 2 - Article: Manual lymphatic drainage in chronic venous disease: A duplex ultrasound study

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Manual lymphatic drainage in chronic venous disease: A duplex ultrasound study

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Abstract

Objectives: To compare the effect of call-up and reabsorption maneuvers of manual lymphatic drainage on blood flow in femoral vein and great saphenous vein in patients with chronic venous disease and healthy controls.

Methods: Forty-one subjects participated in this study (mean age: 42.68(15.23)), 23 with chronic venous disease (chronic venous disease group) with clinical classification C_{1-5} of clinical-etiological-anatomical-pathological (CEAP) and 18 healthy subjects (control group). Call-up and reabsorption maneuvers were randomly applied in the medial aspect of the thigh. The cross-sectional areas, as well as the peak and the mean blood flow velocity at femoral vein and great saphenous vein, were assessed by Duplex ultrasound at the baseline and during maneuvers. The venous flow volume changes were calculated.

Results: The venous flow volume in femoral vein and great saphenous vein increased during both manual lymphatic drainage maneuvers and in both groups ($P < 0.05$). The two maneuvers had a similar effect on femoral vein and great saphenous vein hemodynamics, and in both the chronic venous disease and control groups. As a result of the call-up maneuver, the flow volume augmentations, as a result of call-up maneuver, decreased with the severity of chronic venous disease in those patients measured by the clinical classification of CEAP ($r = -0.64$; $P = 0.03$).

Conclusions: Manual lymphatic drainage increases the venous blood flow in the lower extremity with a magnitude that is independent from the specific maneuver employed or the presence of chronic venous disease. Therefore, manual lymphatic drainage may be an alternative strategy for the treatment and prevention of venous stasis complications in chronic venous disease.

Keywords

Manual lymphatic drainage, chronic venous disease, duplex ultrasound, venous flow

Introduction

Manual lymphatic drainage (MLD) is a specialized manual technique that stimulates superficial lymphatic vessels to remove excessive interstitial fluid[1,2] and increase lymph flow.[3,4] There are four recognized techniques of MLD: the Földi,[3] Vodder,[5] Casley-Smith[6] and Leduc,[7] and these consist of a skin massage that includes substantial skin-stretching[8] but which applies very low pressure to the underlying tissues.[9,10] The evidence of MLD for the treatment, for example, of breast cancer-related lymphedema[9] and in sports medicine and rehabilitation[11] is unclear, and it is suggested as being minor, despite some clinical and statistical inconsistencies in the studies. Nevertheless, MLD when combined with other treatments, like low-stretch bandaging, exercise and skin care (called decongestive lymphatic therapy)[4,8,12] may reveal itself as effective in

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the treatment of lymphedema of the lower and upper limb related to cancer.[13,14]

MLD has also been used as a conservative treatment for chronic venous disease (CVD),[15] often applied along the course of the great saphenous vein.[10,16,17] Molski et al.[17] studied the effect of MLD before surgery in patients with CVD and found significant improvements in the clinical class of clinical-etiological-anatomical-pathological (CEAP) classification, quality of life, depression, anxiety and in reflux volume index after surgery when compared with a control group not receiving MLD treatment.

There is also the assumption that MLD has an effect on blood flow in superficial veins, especially through the call-up maneuver.[10] Nevertheless, the real impact of MLD in hemodynamics is unclear, although it has been suggested as being insignificant.[18] In the lower extremity, the call-up maneuver, a technique belonging to the so-called Leduc method, initiates with inciting (or call-up) maneuvers in the inguinal region and then progresses distally along the lower extremity down to the edematous region, in order to stimulate lymph flow.[18,19] The reabsorption maneuver, another Leduc technique is then applied over the edematous region to drain the interstitial fluid and soluble macromolecules through the lymphatic circulation.[2,18,19] The whole procedure finishes with a second round of call-up maneuvers that are then applied in the reverse direction, ending at the groin region.[18,19]

As for the lymphatic circulation, these MLD maneuvers may have an impact on venous blood flow both on superficial and deep veins of the lower extremity. Thus, the present study was designed to assess the effect of the two low-pressure manual skin-stretching maneuvers: the call-up and the reabsorption on both deep and superficial venous blood flow in patients with CVD. For this purpose, vascular ultrasonography was used to measure the cross sectional area (CSA) and blood velocity at the femoral vein (FV) and great saphenous vein (GSV), when each of the MLD maneuvers was applied to the medial aspect of the thigh in CVD and healthy participants. We hypothesize that both techniques will enhance venous blood flow and that this will be apparent by an elevated blood flow in both the superficial and the deep veins of the lower extremity.

Methods

Subjects

Twenty-three participants (13 women and 10 men) with a diagnosis of CVD (CVD group), most of whom were outpatients in a local health unit, and 18 healthy control participants (10 women and 8 men), participated in this study. All CVD participants presented venous blood reflux of at least 0.5 s duration in the lower extremity and CEAP clinical classification in the range C_{1-5}. Before study enrolment, subjects were informed about the purpose and procedures of the study and signed an informed consent. The study received ethical approval by the review board of the scientific council of the Faculty of Human Kinetics, Technical University of Lisbon. The exclusion criteria in this study included the presence of severe cardiac insufficiency, acute venous or arterial obstruction, arterial insufficiency, renal insufficiency, uncompensated thyroid dysfunction, pregnancy, neoplastic pathology, systemic or limb infection, recent musculoskeletal injury in the lower limb, and peripheral neuropathy in the lower limb. Seven subjects were excluded: two participants presented active ulcer (C_6); two participants were diagnosed with cardiac insufficiency; and three participants had severe arterial insufficiency. Three participants from the CVD group had not had their GSV evaluated due to previous surgery on this vein. In three other participants from this group, the FV was not evaluated due to time constraints.

Clinical evaluations

The clinical history, the symptoms (fatigue, heavy sensation, itching, cramps, and skin irritation), the severity of the disease according to clinical classification of CEAP and the venous clinical severity scores (VCSS)[20] were collected.

The anatomical location (i.e. superficial, perforating and/or deep vein system) and severity of the venous blood reflux were determined. The control participants also went through a thorough vascular ultrasound of both lower extremities to confirm that they were free from CVD. All ultrasound tests were performed by a certified vascular sonographer and with the same ultrasound equipment (ESAOTE *mylab* 30cv, with 7 mm linear array-transducer, scanned at 6–12 MHz). The leg self-reported as presenting the worst symptoms and clinical signs and the dominant leg were chosen for subsequent measurements, respectively in the CVD group and the control group.

Venous hemodynamics during baseline and during call-up and reabsorption maneuvers

Doppler vascular ultrasound was employed to evaluate venous blood hemodynamics in FV and GSV. The FV and GSV were scanned below confluence of the superficial inguinal veins and the superficial vein, and immediately below confluence of the superficial inguinal veins, respectively. Both veins were scanned in B-mode for 4 s and the image presenting the larger CSA was selected. The vein's CSA was measured by

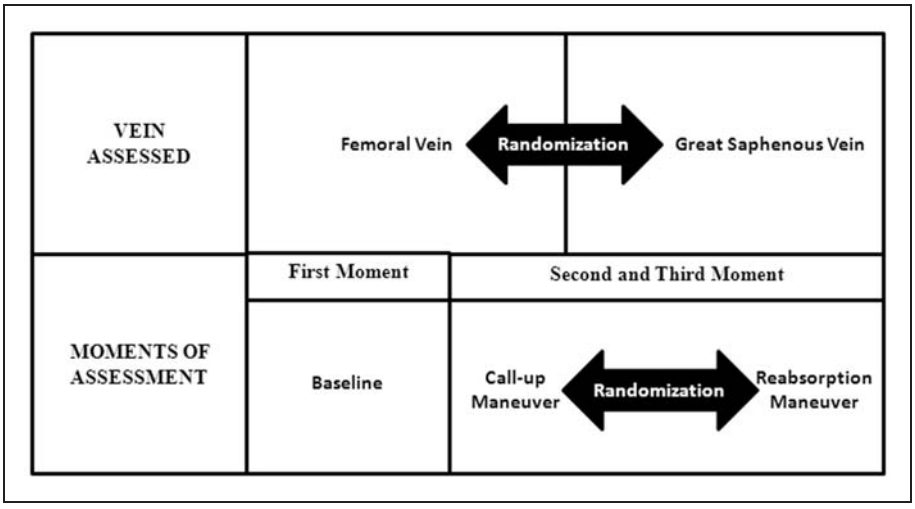

Figure 1. Randomization for duplex ultrasound assessment.

tracing its contour in the ultrasound scan. Venous flow velocity (mean and peak) was measured during 4-s intervals, using the time integral calculation. Three measurements of CSA and of blood flow velocity were taken for both FV and GSV, and the average was computed for analysis.

Ultrasound measurements were taken first at baseline (with no maneuver) and thereafter during randomly applied call-up and reabsorption maneuvers to the medial aspect of the thigh (Figure 1). Before the measurements were taken, the participants rested for 5 min in a supine position and then remained in this position during the whole testing procedure. Blood flow volume in the FV and GSV was calculated based on measurements of CSA and blood flow velocity using the following relation:[21] Flow volume (ml/s) = vein CSA (cm^2) × mean flow velocity (cm/s).

The augmentation of CSA and venous hemodynamics (peak and mean flow velocity and flow volume) percent augmentations were calculated using the following relation: Augmentation (%) = (Maneuver − Baseline)/(Baseline) × 100.

The venous blood flow parameters and percentage augmentation can easily be assessed with Doppler ultrasound and have been used in many studies which evaluate venous hemodynamic in the lower limb in response to treatments.[22,23] Also, venous flow volume calculation based on CSA and Doppler ultrasound measurements are accurate.[24]

Ultrasound data were collected by a certified sonographer kept in the blind regarding the application of manual techniques. The MLD maneuvers, based on the Leduc technique, were performed with two hands placed onto the medial aspect of the thigh and applying a pressure just sufficient to stretch the skin during a minimum time interval of 4 s. The maneuver started and ended on command of the sonographer and in synchronization with ultrasound recording. The call-up maneuver initiates with the most proximal part of the proximal hand and ends with both hands touching the skin while producing a skin stretching. This MLD maneuver is applied to promote the increase of lymph flow by enhancing the contractility of lymphagions of lymph collectors.[18,19] The reabsorption maneuver initiates with the most distal part of the distal hand and ends with both hands touching the skin while applying skin stretching. This maneuver is applied to stimulate the reabsorption of macromolecules and the excess of venous filtration from interstitial space to lymphatic capillaries by stretching leak filaments (connections between connective tissue to endothelial cell of lymphatic capillaries) when the skin is mobilized.[2,18,19] Skin-stretching is applied in the two maneuvers in a proximal direction respecting the course of the lymph and venous flow (Figure 2).

Statistical analysis

All statistical tests were performed with the help of the statistical software package SPSS Inc. v.17 (SPSS Inc., Chicago, USA). Normal distribution was checked using Shapiro–Wilk-Test. Group differences were tested with two-tailed Student's *t*-test, while differences between maneuvers and between maneuvers and baseline were tested by repeated-measures ANOVA. The Bonferroni correction was used to correct for multiple comparisons. The relationship between the magnitude of the effect of the maneuvers, in terms of percentage augmentation from baseline, and CVD severity, in terms of CEAP and VCSS classification was calculated using Spearman coefficient of correlation. The significance level was set at $P < 0.05$.

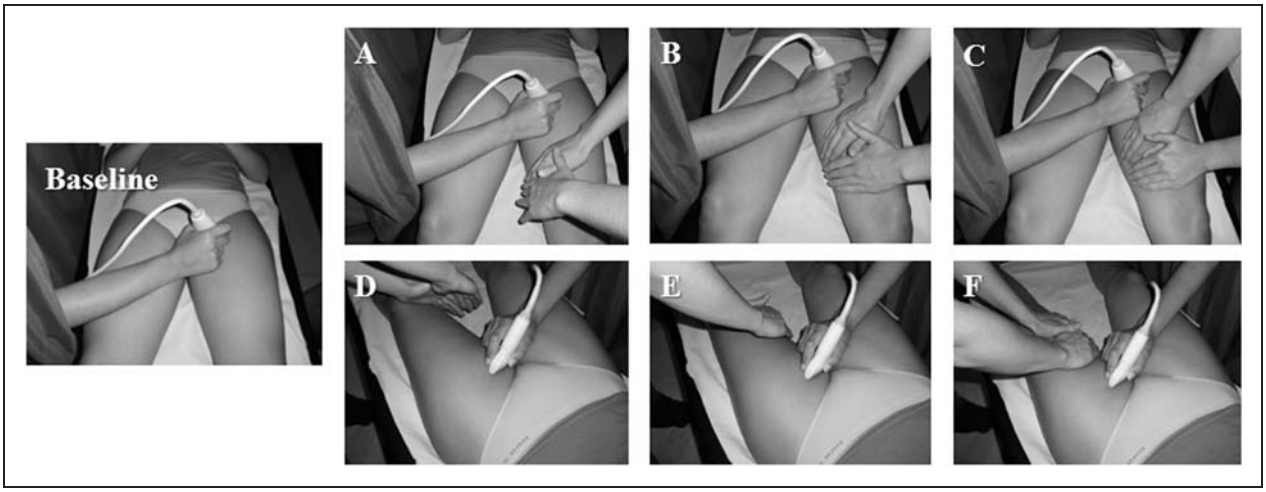

Figure 2. Evaluation context with curtain separating sonographer, therapist and participant, during the assessment of the cross sectional area and blood flow of FV and GSV during baseline and during call-up maneuvers (A, B and C sequence) and reabsorption maneuvers (D, E and F sequence).

Results

The demographic and clinical data pertaining to the participants are presented in Table 1. No differences in age, height, weight, and body mass index were found between the CVD and control group.

Baseline

The CSA of the GSV was higher in CVD group (0.14(0.06) cm^2) than in the control group (0.09(0.04) cm^2), with $P = 0.007$. Nevertheless, the CSA of FV and the mean flow velocity, the peak flow velocity, and the flow volume of both FV and GSV at baseline were similar in CVD and in the control group (Tables 2 and 3).

Call-up and reabsorption maneuver

During the call-up maneuver the FV blood flow volume increased significantly ($P < 0.05$) from baseline (Figure 3). The FV peak flow velocity only increased during call-up maneuver in the control group ($P = 0.032$), and during reabsorption maneuver in the CVD group ($P = 0.000$) and in both groups pooled together ($P = 0.008$). The FV mean flow velocity only increased during call-up and reabsorption maneuvers in the CVD group ($P = 0.000$ and $P = 0.000$, respectively) and in both groups pooled together ($P = 0.000$ and $P = 0.008$, respectively). The CSA of FV increase from the baseline during the call-up maneuver only in the CVD group ($P = 0.006$) and with all groups pooled together ($P = 0.002$), and during reabsorption in both the CVD ($P = 0.003$) and the control group ($P = 0.007$) and with the two groups pooled together ($P = 0.000$).

The GSV blood flow (peak and mean flow velocity and flow volume) increased significantly ($P < 0.05$) from the baseline during call-up and reabsorption maneuvers (Figure 4). The CSA of GSV, however, remained unchanged during the maneuvers.

The venous blood flow (mean, peak flow velocity and flow volume) and CSA augmentations from the baseline of FV and GSV were similar in both call-up and reabsorption maneuvers, with the exception of the CSA augmentation of FV that was lower during call-up maneuver when compared with reabsorption in the control group (12.45(30.00)% and 23.05(32.89)%, respectively; $P = 0.004$) and with the two groups pooled together (12.70(23.85)% and 34.76(58.40)%, respectively; $P = 0.021$). The CSA (in absolute values) of FV during call-up was also lower than during reabsorption maneuver in the control group (0.49(0.19) cm^2 and 0.53(0.19) cm^2 for call-up and reabsorption maneuvers respectively; $P = 0.042$) and with the two groups pooled together (0.50(0.19) cm^2 and 0.57(0.19) cm^2 for call-up and reabsorption maneuvers respectively; $P = 0.042$), but no differences were found between maneuvers in the CVD group. In addition, no differences were found between call-up and reabsorption maneuvers in CSA for GSV and in blood flow for FV and for GSV.

Severity of chronic venous disease and maneuvers

The CVD group presents a higher CSA of the GSV than the control group during call-up ($P = 0.001$) and reabsorption maneuvers ($P = 0.001$), but the CSA augmentations were similar. No other differences were

Table 1. Demographic and clinical data.

	CVD group ($n=23$)	Control group ($n=18$)	P	All subjects ($n=41$)
Age (years)	46.83 (13.24)	38.72 (15.96)	0.083	42.68 (15.23)
Height (m)	1.67 (0.91)	1.64 (0.97)	0.419	70.62 (15.03)
Weight (kg)	74.04 (14.63)	66.08 (13.50)	0.082	1.66 (0.10)
BMI (kg/m^2)	26.53 (4.01)	24.36 (3.67)	0.081	25.58 (4.11)
Gender				
Female	13 (56.52)	10 (55.56)	–	22 (57.89)
Male	10 (43.48)	8 (44.44)	–	16 (42.11)
CEAP clinical classification				
C1	5 (21.74)	–	–	–
C2	3 (13.04)	–	–	–
C3	5 (21.74)	–	–	–
C4	6 (26.09)	–	–	–
C5	4 (17.39)	–	–	–
VCSS	5.65 (3.62)	–	–	–
Anatomical reflux				
Superficial veins	3 (13.04)	–	–	–
Deep veins	1 (4.34)	–	–	–
Superficial + deep veins	7 (30.43)	–	–	–
Superficial + perforator veins	6 (26.09)	–	–	–
Superficial + deep + perforator veins	4 (17.39)	–	–	–
Comorbidities				
Surgical removal of great saphenous vein	3 (13.04)	–	–	–
Lower limbs symptoms				
Fatigue	18 (78.26)	–	–	–
Cramps	9 (39.13)	–	–	–
Heavy legs	14 (60.87)	–	–	–
Pain	14 (60.87)	–	–	–
Skin irritation	8 (34.78)	–	–	–
Itching	11 (47.82)	–	–	–
Without symptoms	2 (8.70)	–	–	–

Quantitative variable: mean (SD); categorical variable: frequency (%). Differences between groups (CVD and control) were significant with $P < 0.05$ for two-tailed Student's t-test.
VCSS: Venous Clinical Severity Score, total scale range 0–30 (best to worst); CEAP: Clinical Etiological Anatomical Pathological Classification.

found between the CVD and the control group during the application of each maneuver.

Blood flow (peak and mean flow velocity and the flow volume) augmentations in FV during call-up maneuvers decreased with the severity of CVD, as measured by VCSS ($r=-0.51$, $P=0.026$ for peak flow velocity; $r=-0.51$, $P=0.023$ for mean flow velocity; and $r=-0.49$, $P=0.034$ for flow volume). The flow volume augmentation for the same vein also decreased with the severity of CVD, measured by CEAP clinical classification ($r=-0.64$; $P=0.03$). The GSV flow volume augmentation was not affected by the severity of the CVD disease.

Discussion

This study reveals that MLD maneuvers (call-up and reabsorption) are able to increase venous blood flow in the deep (FV) and the superficial venous systems (GSV), with no differences between maneuvers in this outcome, as well as between the CVD and the control groups. According to Leduc's technique, MLD maneuvers, especially the call-up maneuver, are believed to affect blood flow only on superficial veins.[10] To the best of our knowledge, the effect of MLD on venous hemodynamics was assessed in just one study in which MLD application produced no effect on venous return

Table 2. Venous blood flow in femoral vein.

Femoral vein		CVD group ($n = 20$) Mean (SD)	Control group ($n = 18$) Mean (SD)	P	All subjects ($n = 38$) Mean (SD)
Cross-sectional area (cm^2)	Baseline	0.47 (0.16)	0.46 (0.21)	0.992	0.45 (0.18)
	Call-up	0.52 (0.20)[a]	0.49 (0.19)	0.661	0.50 (0.19)[a]
	Reabsorption	0.60 (0.19)[a]	0.53 (0.19)[a,b,c]	0.229	0.57 (0.19)[a,b,c]
Peak flow velocity (cm/s)	Baseline	19.38 (8.77)	21.37 (9.36)	0.504	20.32 (8.99)
	Call-up	24.85 (10.92)[a]	25.67 (8.82)[a]	0.803	25.24 (9.85)[a]
	Reabsorption	25.96 (10.32)[a]	25.04 (6.83)	0.754	25.51 (8.69)[a]
Mean flow velocity (cm/s)	Baseline	12.14 (5.67)	14.20 (7.47)	0.342	13.11 (6.57)
	Call-up	16.62 (8.27)[a]	17.12 (7.15)	0.843	16.86 (7.66)[a]
	Reabsorption	15.88 (7.30)[a]	15.37 (4.76)	0.807	15.63 (6.12)[a]
Flow volume (ml/s)	Baseline	5.47 (3.40)	6.05 (4.22)	0.644	20.32 (8.99)
	Call-up	8.20 (4.72)[a]	7.99 (3.87)[a]	0.883	25.24 (9.85)[a]
	Reabsorption	9.42 (4.73)[a]	7.98 (3.89)[a]	0.320	25.51 (8.69)[a]

[a]Significantly different from baseline ($P < 0.05$).
[b]Significantly different from call-up maneuver ($P < 0.05$).
[c]Significantly different from call-up maneuver for percentage augmentation from baseline [((maneuver−baseline)/baseline) × 100] ($P < 0.05$).

Table 3. Venous blood flow in great saphenous vein.

Great saphenous vein		CVD group ($n = 20$) Mean (SD)	Control group ($n = 18$) Mean (SD)	P	All subjects ($n = 38$) Mean (SD)
Cross-sectional area (cm^2)	Baseline	0.14 (0.06)	0.09 (0.04)	0.007[b]	0.12 (0.05)
	Call-up	0.16 (0.07)	0.09 (0.04)	0.001[b]	0.12 (0.06)
	Reabsorption	0.16 (0.06)	0.10 (0.04)	0.001[b]	0.13 (0.06)
Peak flow velocity (cm/s)	Baseline	15.82 (8.06)	21.09 (17.52)	0.240	18.38 (13.66)
	Call-up	26.22 (13.65)[a]	33.31 (28.96)[a]	0.313	29.45 (22.61)[a]
	Reabsorption	28.58 (15.66)[a]	35.19 (29.05)[a]	0.393	31.84 (23.10)[a]
Mean flow velocity (cm/s)	Baseline	10.05 (4.63)	14.56 (14.29)	0.194	12.22 (10.72)
	Call-up	16.64 (8.13)[a]	24.18 (25.29)[a]	0.215	20.23 (18.92)[a]
	Reabsorption	18.09 (9.03)[a]	24.77 (23.16)[a]	0.224	21.31 (17.62)[a]
Flow volume (ml/s)	Baseline	1.35 (0.82)	1.49 (1.91)	0.784	1.42 (1.45)
	Call-up	2.69 (2.49)[a]	2.32 (2.92)[a]	0.686	2.50 (2.68)[a]
	Reabsorption	3.08 (2.80)[a]	2.35 (2.24)[a]	0.390	2.71 (2.53)[a]

[a]Significantly different from baseline ($P < 0.05$).
[b]Differences between CVD group and control (healthy) group were significant ($P < 0.05$).

in patients with chronic heart failure and lower extremity edema.[18] However, this study did not evaluate the blood flow in the lower extremity veins directly. MLD is commonly indicated for patients with CVD as a strategy to treat edema, especially when the lymphatic system is also compromised.[25] Employing MLD in patients with CVD awaiting for surgery, appears to decrease the severity of the disease and lead to an overall improvement in quality of life in these patients.[17] Also, MLD might have a real effect on improving venous hemodynamics (reflux volume index) in CVD patients.[17]

The present study reveals that MLD techniques based on manual skin-stretching of the thigh increases

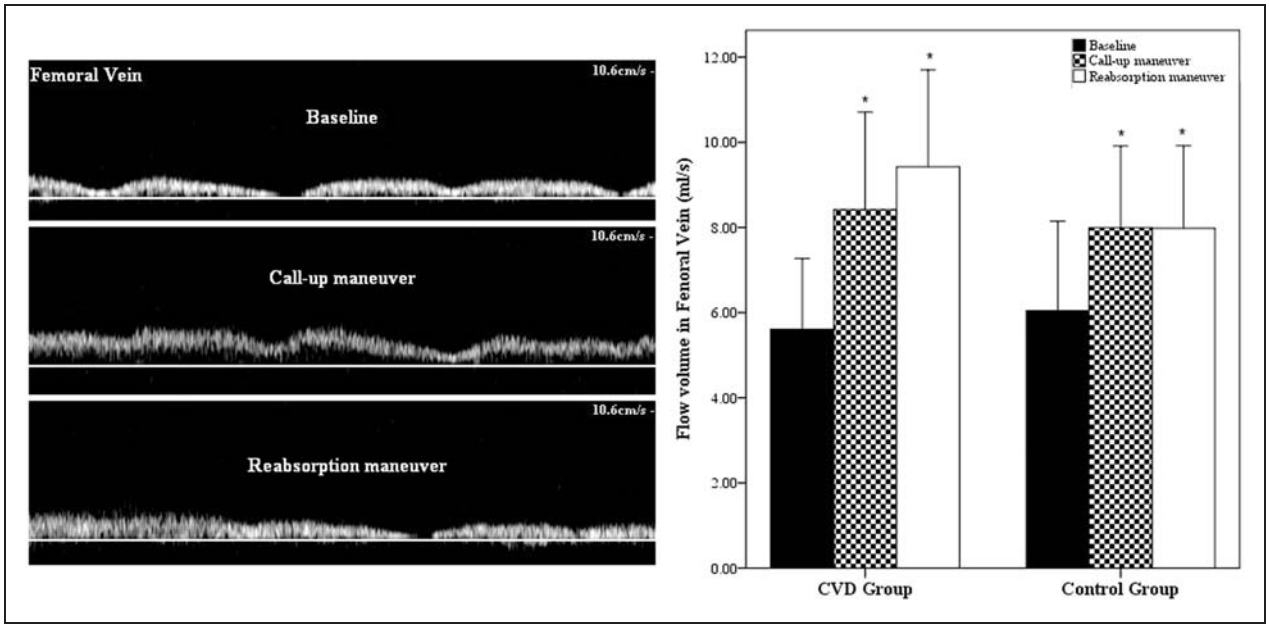

Figure 3. Left: image of blood flow velocities of FV during baseline, call-up maneuvers and reabsorption maneuvers of manual lymphatic drainage. Right: mean and error (95% of confidence interval) of flow volume in femoral vein in the CVD group (patients with chronic venous disease) and the control group (healthy subjects) during baseline and manual lymphatic drainage maneuvers (call-up and reabsorption), showing the results from Bonferroni-adjustment (*) significantly different from the baseline.

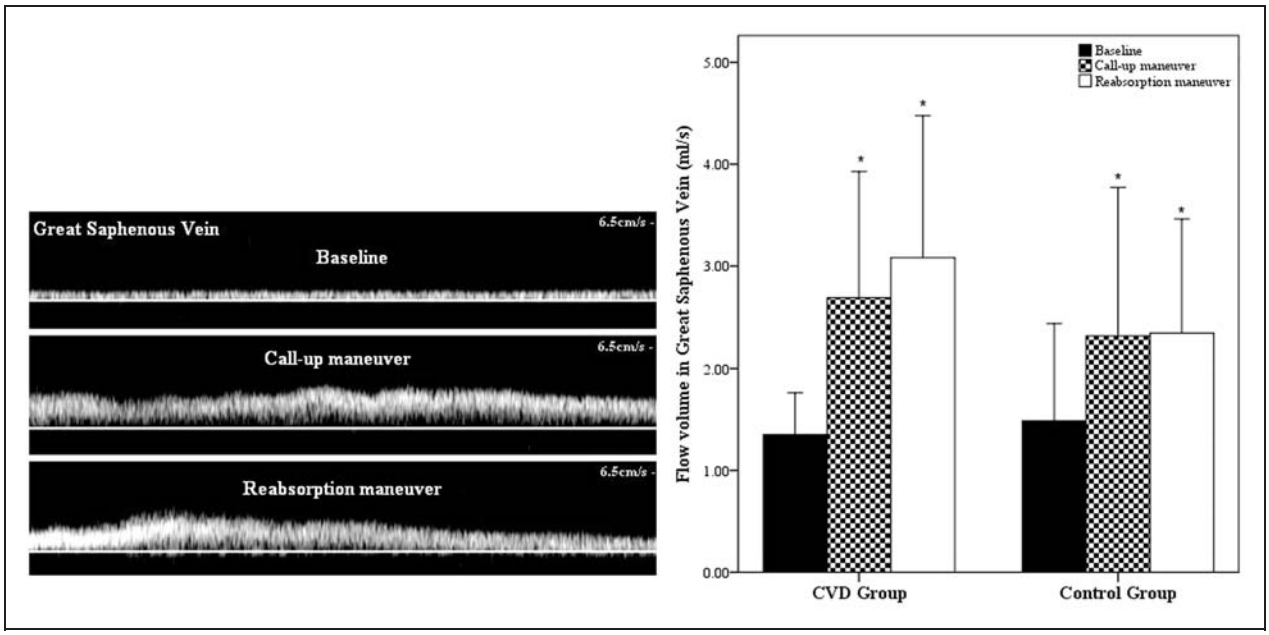

Figure 4. Left: image of blood flow velocities of great saphenous vein (GSV) during baseline, call-up maneuvers and reabsorption maneuvers of manual lymphatic drainage. Right: mean and error (95% of confidence interval) of flow volume in the GSV in the CVD group (patients with chronic venous disease) and the control group (healthy subjects) during baseline and manual lymphatic drainage maneuvers (call-up and reabsorption), showing the results from Bonferroni-adjustment (*) significantly different from the baseline.

venous blood flow in the lower extremity, in superficial veins, as has already been suggested,[10] and also in deep venous system (FV) beneath the deep fascia, which is a novel observation.

Current concepts regarding MLD indicate that each maneuver should take around 4 s from beginning to end.[19] We strictly followed this recommendation when applying the two MLD techniques in the present study,

as well as starting the technique from proximal to distal (the call-up maneuver) which enhances venous blood flow, whereas applying skin-stretching from distal to proximal (the reabsorption maneuver) only has an effect on lymph flow and fluid reabsorption.[19] Our findings do not substantiate this differential effect between the two maneuvers which, in general, enhance venous blood flow both in FV and in GSV. The only difference between the two MLD maneuvers occurred in changes of the CSA of FV and only for the control group.

Skin and deep fasciae are connected by skin ligaments on thigh, knee, popliteal fossa and leg, which give stability to the skin, like an anchor, during lower limb movements.[26] The skin-stretching applied during MLD in this study may produce enough increase in pressure on the underlying structures to enhance venous flow in superficial and deep veins, as does the physiological skin-stretching during movement.[27]

Venous blood flow was found to be similar in the CVD and the control groups, which is a typical observation when venous blood flow is assessed at rest.[28] Nevertheless, the CSA of GSV was found to be higher in the CVD group which is in accordance with previous observations,[29] revealing that the diameter of GSV can be a good predictor of the presence/absence and severity of CVD.

With the severity of CVD, the percentage augmentation of flow volume during reabsorption maneuvers appears to decrease. The severity of the disease is related to the difficulty in evacuating the venous blood from the periphery towards the heart,[30] and with a corresponding increase in the reflux volume index and venous stasis.[30,31] Moreover, the link between blood flow velocity, deep vein thrombosis and the risk of pulmonary embolism is well known.[22] The triad composed by stasis, vessel damage, and hypercoagulability is accepted as a major factor in thrombogenesis.[22] Preventing venous stasis is a main goal in CVD treatment and decisive in preventing venous complications. In this regard, MLD maneuvers maybe an alternative treatment procedure to enhance venous flow. Nevertheless, this intervention needs specialized professionals, and could be an expensive health care treatment. In addition, middle and long-term effects of MLD in venous flow are unknown. Teaching caregivers or patients simple lymphatic drainage, despite the lower efficacy showed in the treatment of lymphedema, when compared with MLD applied for professionals,[9] could be an alternative.

Intermittent pneumatic compression, with a pressure setting of 80 mmHg, increases venous flow velocity in FV and in GSV, but produces an increase in flow volume just in the FV, belonging to the deep venous system.[32] With a pressure setting of 40 mmHg, venous flow velocity at the FV rises to 35–60 cm/s and raising the pressure setting to 120 mmHg, flow velocity reaches 100 cm/s.[22] With foot compression, the increase typically goes up to 20–40 cm/s in the FV. In this study, the peak flow velocity in FV during the MLD maneuvers reached around 25–26 cm/s, slightly lower than with intermittent pneumatic compression at low pressure setting. Compression (bandages and stoking) is the conservative treatment more often advocated for CVD[30] which also increases venous flow velocity in GSV and the Popliteal vein.[33] When compression exceeds the 40 mmHg, then the CSA of GSV and FV starts to decrease,[34] showing that the pressure applied in this study was within the range indicated for this technique (lower than 40 mmHg).[7] Although compression above 40 mmHg produces large increase in blood flow velocity,[22,34] this is limited to deep veins. The manual maneuvers employed in the present study produced mild enhancement in venous blood flow velocity and flow volume, but in this case in both the deep and the superficial venous systems.

The mean and peak flow velocity in FV also increase during the active and the passive movement of the ankle joint.[35] The venous blood flow velocity registered during the active movement of the ankle increased by 20–40%,[35] which is similar to the increase in blood flow velocity found during the call-up and reabsorption maneuvers in this study. The increase in blood flow velocity in the superficial GSV during active and passive movements of ankle is also similar to that observed here using call-up and reabsorption maneuvers.[36] In the standing position and during active tip-toe movements, blood flow velocity in the popliteal vein also increases in response to calf muscle pump contractions,[28] but in this case and contrary to what was observed in the present study, healthy subjects demonstrated larger enhancement in venous blood flow as a result of the tip-toe movement.[28]

There are few studies that describe and demonstrate the efficacy of MLD maneuvers on venous blood flow. Therefore, this study is a preliminary attempt to assess the potential role of MLD in treating CVD patients. The mechanisms explaining the increases in venous flow during MLD are still unknown. A likely mechanism would be that skin traction increases the pressure over superficial vessels reducing their caliber and leads to an increase in blood flow velocity. The increased blood flow in the superficial veins would result in higher blood flow across perforating veins and into the deep veins, thereby raising blood flow in the deep venous system as well. The pressure applied to the skin would probably cause movement in the muscles underneath, and pressure would also increase in deep seated structures and deep veins further stimulating blood flow. Also, muscle tone might increase during the interval of time that MLD techniques are being applied.

Although participants were instructed to remain as relaxed as they possibly could during the MLD sessions, unnoticed muscle contraction could have occurred either induced by the manual stimulation, or in response to the movement of the lower extremity, which could have contributed to the observed increase in venous blood flow.

The major limitation in this study is the non-existence of data regarding the level of bias and reliability of the ultrasound measurements of blood velocity and veins' CSA during manual maneuvers. Future studies should evaluate the test–retest accuracy of these measurements.

In conclusion, the call-up and reabsorption MLD-maneuvers, applied to the medial aspect of the thigh, improve venous blood flow in FV and GSV in CVD patients and healthy subjects. The blood flow-enhancing effect of MLD was similar between the CVD patients and the healthy participants. However, the efficacy of the reabsorption maneuvers in increasing venous blood velocity decreased with CVD severity. Future studies should evaluate the effect of MLD maneuvers in venous blood flow when applied to regions of the lower extremity other than the medial aspect of the thigh.

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Conflict of interest statement

The authors have no conflict of interest to report.

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10.3 Annex 3 - Chronic Venous Disease Quality of Life Questionnaire (CIVIQ-20)

Auto-Questionário de Qualidade de Vida na Insuficiência Venosa

Muitos portugueses queixam-se de dores nas pernas. Procuramos saber qual a frequência dos problemas das pernas, e de que maneira estes problemas podem afectar a vida quotidiana dos que sofrem deste problema.

Vai encontrar adiante, um número de sintomas, sensações ou incómodos que pode ou não sentir e que podem tornar a vida do dia a dia mais ou menos difícil. Para cada sintoma, sensação ou incómodo mencionados, pedimos-lhe que responda a duas perguntas:

1- Na coluna I, indique se sentiu realmente o a frase descreve, em caso afirmativo, e com que intensidade. Cinco respostas são previstas, coloque um círculo em volta da que melhor corresponda à sua situação:

   1 - se não se considerar afectada(o) pelo sintoma, ou a sensação ou incomodo descrito.

   2, 3, 4 ou 5 se os tiver experimentado com maior ou menor intensidade.

2- Na coluna II, indique a importância que a atribui a este sintoma, sensação ou incómodo na sua vida quotidiana.

Algo pode ser sentido intensamente, sem no entanto ocupar um lugar importante na sua vida de todos os dias. Para avaliar a importância que dá a cada um dos sintomas ou sensações experimentados, foram previstas cinco respostas que vão de 1 (nenhuma importância) a 5 (extrema importância). Faça um círculo naquela que parece corresponder ao seu caso.

Coluna I					Coluna II				
P1 a) Nas últimas 4 semanas sentiu **dores** nos **tornozelos** ou nas **pernas?** E qual foi a intensidade dessas dores? *(Faça um círculo no número correspondente à sua resposta.)*					**P1 b)** Que importância dá a estas dores na sua vida actual? *(Faça um círculo no número correspondente à sua resposta.)*				
Nenhuma dor	Dores leves	Dores moderadas	Dores importantes	Dores intensas	Nenhuma importância	Pouca importância	Importância moderada	Muita importância	Importância extrema
1	2	3	4	5	1	2	3	4	5

P2 a) Nas últimas 4 semanas em que medida se sentiu incomodado(a) no seu **trabalho** ou nas suas outras **actividades quotidianas devido aos seus problemas de pernas**? *(Faça um círculo no número correspondente à sua resposta.)*					**P2 b)** Que importância dá a este incómodo na sua vida actual? *(Faça um círculo no número correspondente à sua resposta.)*				
Nenhum incómodo	Um pouco incomodado(a)	Moderadamente incomodado	Muito incomodado(a)	Extremamente incomodado(a)	Nenhuma importância	Pouca importância	Importância moderada	Muita importância	Importância extrema
1	2	3	4	5	1	2	3	4	5

P3 a) Nas últimas 4 semanas, aconteceu-lhe **dormir mal** devido aos seus problemas de pernas, e com que frequência? *(Faça um círculo no número correspondente à sua resposta.)*					**P3 b)** Que importância dá a estas perturbações do sono na sua vida actual? *(Faça um círculo no número correspondente à sua resposta.)*				
Nunca	Raramente	Frequentemente	Muito frequentemente	Todas as noites	Nenhuma importância	Pouca importância	Importância moderada	Muita importância	Importância extrema
1	2	3	4	5	1	2	3	4	5

	P4 a) Nas últimas 4 semanas em que medida os seus **problemas de pernas o (a) incomodaram durante os movimentos ou actividades** indicadas a seguir? *(Para cada enunciado inscrito na coluna esquerda, indique em que medida é incomodado(a, fazendo um círculos no número escolhido.)*					**P4 b)** Que importância dá a este incómodo na sua vida actual? *(Indique a importância atribuída a cada incomodo fazendo um circulo no número escolhido)*				
	Nenhum incómodo	Um pouco incomodado(a)	Moderadamente incomodado(a)	Muito incomodado(a)	Impossível de fazer	Nenhuma importância	Pouca importância	Importância moderada	Muita importância	Importância extrema
Permanecer muito tempo em pé	1	2	3	4	5	1	2	3	4	5
Subir encostas ou escadarias	1	2	3	4	5	1	2	3	4	5
Agachar-se /ajoelhar-se	1	2	3	4	5	1	2	3	4	5
Caminhar com passo decidido	1	2	3	4	5	1	2	3	4	5
Viajar de carro, de comboio	1	2	3	4	5	1	2	3	4	5
Realizar tarefas domésticas (cozinhar, levar uma criança nos braços, passar a ferro, limpar o chão ou os móveis, fazer bricolage, ir as compras…	1	2	3	4	5	1	2	3	4	5
Ir ao café, a festividades (casamentos), convívios…	1	2	3	4	5	1	2	3	4	5
Fazer desporto, esforços físicos importantes (carregar pesos, deslocar moveis…)	1	2	3	4	5	1	2	3	4	5

	P5 a) Os problemas de pernas podem igualmente afectar o seu ânimo. Em que medida as frases abaixo indicadas correspondem ao que experimentou nas 4 últimas semanas? *(Para cada enunciado inscrito na coluna esquerda, faça um círculo no número escolhido.)*					**P5 b)** *(Para cada sensação experimentada)* Que importância representa isto para si na sua vida actual? *(Indique a importância atribuída a cada sensação experimentada fazendo um circulo no número escolhido)*				
	Nada	Um pouco	Moderadamente	Muito	Completamente	Nenhuma importância	Pouca importância	Importância moderada	Muita importância	Importância extrema
Sinto-me nervoso(a), tenso(a)	1	2	3	4	5	1	2	3	4	5
Canso-me depressa	1	2	3	4	5	1	2	3	4	5
Sou um fardo para os outros	1	2	3	4	5	1	2	3	4	5
Devo tomar sempre precauções (por exemplo esticar as pernas, evitar permanecer muito tempo em pé…)	1	2	3	4	5	1	2	3	4	5
Sinto embaraço em mostrar as pernas	1	2	3	4	5	1	2	3	4	5
Irrito-me facilmente, estou de mau humor	1	2	3	4	5	1	2	3	4	5
Tenho a impressão de ter uma deficiência	1	2	3	4	5	1	2	3	4	5
Sinto dificuldade para começar o dia	1	2	3	4	5	1	2	3	4	5
Não me apetece sair de casa	1	2	3	4	5	1	2	3	4	5