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DOTTORATO DI RICERCA IN
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CICLO XXIX

COORDINATORE Prof. Francesco Bernardi

LOWER LIMB VENOUS KINETICS

and

THEIR IMPACT ON DRAINAGE DIRECTION

Settore Scientifico Disciplinare MED/22.

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In dedication to my parents

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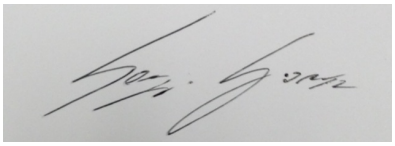
Declaration and claims to originality

The present thesis is original, conceived and developed by the author, Sergio Gianesini.

All else is acknowledged and referenced properly.

No parts of this thesis have been submitted for other degree application at this or any other university.



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List of abbreviations

ċ	*Fluid density*
ċ	*Viscosity*
ċ P	*Pressure gradient*
A	*Cross sectional area*
AASV	*Anterior Accessory Saphenous vein*
AASV	Anterior Accessory Saphenous Vein
AC	*Anatomical compartment*
AC1	Anatomical Compartment 1 (deep venous system)
AC2	Anatomical Compartment 2 (saphenous system)
AC3	Anatomical Compartment 3 (tributary system)
ADF	Active foot dorsi-flexion
AP	*Athmosperic Pressure*
C	*Compliance*
CR	Compression relaxation
CVD	Chronic venous disease
CVI	*Chronic Venous Insufficiency*
DTAV	Diastolic time average velocity
DUS	Doppler Ultrasonography
ECD	*Echo-color-Doppler*
EDV	*End diastolic velocity*
EVP	*External Venous Pressure*
F	*Force*

FV	Femoral vein
GSV	*Great sapheonus vein*
GSV	Great Saphenous vein
HP	*Hydrostatic Pressure*
ILV	*Iliac vein*
IVP	*Internal Venous Pressure*
L	*Length*
LL.GSV	Great saphenous vein at the mid-leg level
LP	*Lateral Pressure*
lp	*lateral pressure*
MMP	*Metallopoteninases*
N	Network
OP	*Oncotic Pressure*
PDV	*Peak diastolic velocity*
PSV	*Peak systolic velocity*
PTV	Posterior tibial vein
PV	Popliteal vein
R	*Resistance*
r	*Radius*
RI	*Resistance index*
RT	Reflux time
SFJ	*Sapheno-femoral junction*
SFJ.FV	*C*ommon femoral vein at the sapheno-femoral junction
SFJ.GSV	Great saphenous vein at the sapheno-femoral junction
SSV	Small saphenous vein

T	Tributary
TAV	*Time average velocity*
TL.GSV	Great saphenous vein at the thigh level
TMP	*Transmural pressure*
TP	*Tissue Pressure*
v	*Velocity*
V	*Volume*

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CHAPTER 1

PHYSIOLOGY OF THE VENOUS SYSTEM

1.1 Venous Anatomy

In the description of the lower limb venous anatomy three different compartments can be identified, based on their relationship to the muscular fascia.[1]

The *deep anatomical compartment (*AC1*)* is located below the muscular fascia and includes the deep venous systems vessels (femoral, popliteal, soleal, gastrocnemial and tibial veins).

The saphenous system represents a separate anatomical compartment (AC2), characterized by its lying in between the muscular fascia layers (*Fig. 1.1.1, 1.1.2*).

AC2 consists of the Great Saphenous Vein (GSV), Small Saphenous Vein (SSV), Anterior Accessory Saphenous Vein (AASV) and Giacomini Vein.

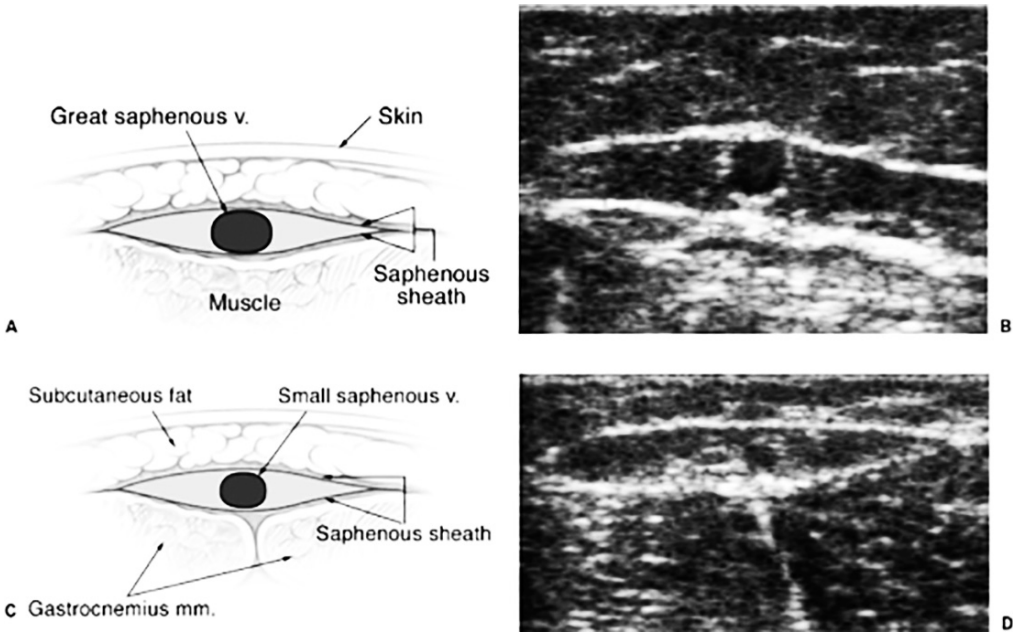

Fig. 1.1.1 Doppler Ultrasonography (DUS) appearance of the Great (GSV) and Small (SSV) Saphenous vein as seen on axial DUS*. (A), (C) Note the intr-afascial location of the GSV and SSV outside the muscular compartment and inside the fascia layers.*[2]

(B), (D) DUS axial imaging of the GSV and SSV inside the fascia layers, respectively.[2]

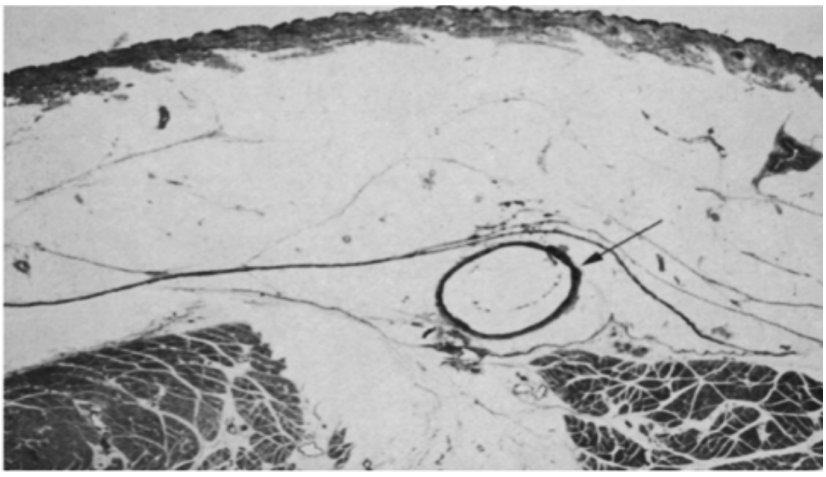

Fig. 1.1.2 Histological transverse sections of the GSV*: a fibrous structure envelops the GSV and holds it against the deep fascia. Tributary veins are located above the fascia, in the subcutaneous tissue.*[3]

The third anatomical compartment (AC3) is represented by the tributary system, above the fascia, being constituted by the veins surrounded just by subcutaneous fat.

More than 60 perforating veins connects the three anatomical compartments, by perforating the muscular fascia, so presenting interconnections for pressure gradients development and consequent drainage propulsion toward the heart.[3,4]

Normal blood flow is from the most distal lower extremity toward the heart and from the most superficial (AC3) toward the deepest compartments (AC2 and then AC1).

All the three networks are connected by perforating veins, physiologically draining from AC3 to AC2 and to AC1 (Fig. 1.3). Whatever subversion of this hierarchical order of the venous networks

emptying can be considered as pathological.

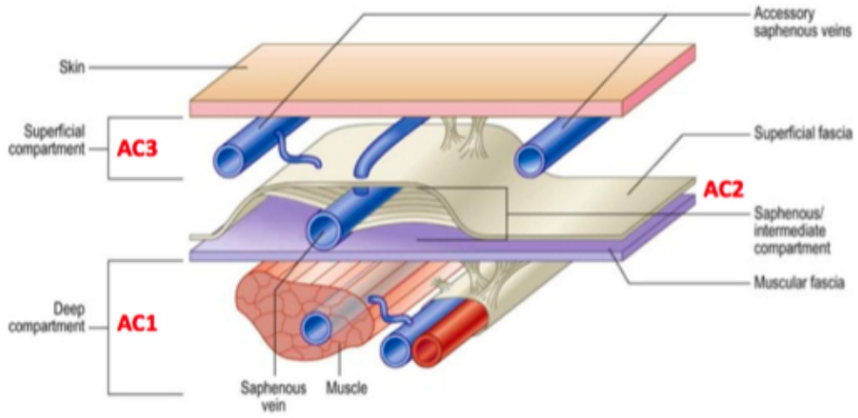

Fig. 1.1.3 Anatomical compartments of the lower limb venous system*: AC1 includes the deep venous system below the muscular fascia. AC2 is represented by the saphenous system, in between the fascia layers. AC3 includes the superficial tributaries, above the muscular fascia.*[5]

The anatomical features of the different compartments influence their hemodynamics.

AC1 is surrounded by the muscle, thus protected by excessive dilation and directly influenced by the pressure gradient that follows the muscle contraction/relaxation.

AC2 is directly linked to the muscle mass by its laying inside the muscular fascia doubling.

This feature indirectly transmits the muscle systole/diastole to the saphenous network, even if in a lower grade compared to the deep venous system.

At the same time, the two fascia layers wrap the saphenous system like a natural elastic stocking, limiting its excessive dilation under venous hypertension conditions.

Tributary veins of the AC3 run above the muscular fascia, surrounded just by subcutaneous fat, thus unprotected by neither muscular or fascial structure.

In this anatomical district, muscular systo-diastole has its minimum effect, while pressure gradients can exert their full action.

1.2 Laws of physics inside the venous system

According to ***Poiseuille's Law***, in order to create a flow, a pressure gradient is needed.

The pressure gradient (ċ P) is directly related to the applied force (F) and the resistance (R).

Poiseuille's law

$$\dot{c}\ P = F \times R$$

The resistance to flow is directly related to the vessel length (L) and viscosity (ċ), while it's inversely related to its radius (r), according to the following formula:

$$R = 8\dot{c}\ L/\dot{c}\ r^4$$

Considering that pressure (P) can be defined as a force on a surface, many clinical implications follow these first formula already.

In fact, the pressure gradient is directly influenced by the force exerted by the heart systole, by the thoracic aspiration following the breathing act and by the peripheral muscles contraction.

At the same time, the pressure is inversely related to the surface, which leads to the implication of the venous tone and of the wall compliance: two variable factors able to influence the pressure gradient, thus the flow (*Fig. 1.2.1*).

The same vessel calibre influences the resistance formula, so adding another variable in the flow determination.

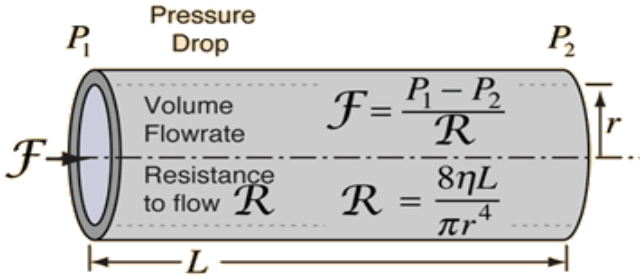

***Fig**. 1.2.1 Poiseuille's law and consequent implication in venous drainage.*

Together with an energy gradient, another necessary element in producing a flow is the vessel capacity to receive a certain amount of fluid.

Considering the intrinsic collapsible vein nature, in a beginning filling phase a great capacity variability is possible with little changes in venous pressure, turning the vein from an elliptical to a circular shaped vessel: a property conferring the blood reservoir function to the venous system.

Much more pressure is required to stretch the vessel once it has become circular.

The physical property of a vessel to increase its volume with increasing trans-mural pressure is known as compliance (*C*) and is expressed by the change in volume (≥ *V)* divided by the change in pressure (≥ *P*).

$$\textbf{\textit{Compliance}} = \geq V / \geq P$$

Compliance is strictly linked not only to the filling degree, but also to the geometric vessel properties (length and radius), together with its wall elasticity.

A pressure-diameter curve (*Fig. 1.2.2*) highlights the not linear relationship in the initial filling phase, which is due to the great increase in vessel calibre following tiny pressure augmentations.

On the contrary, in a distension phase, starting from pressure values around 20 mmHg, a volume/pressure linearity has been demonstrated. [6]

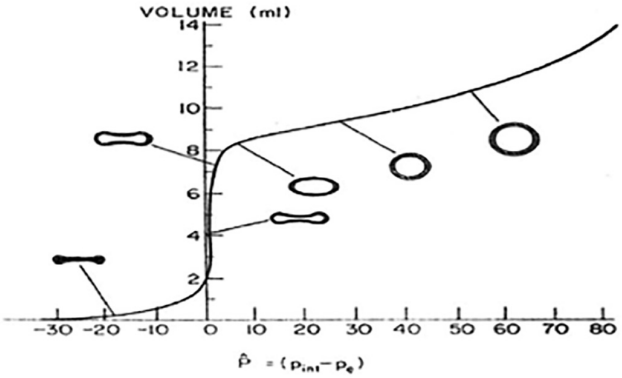

Fig. 1.2.2 Compliance curve. *The pressure-diameter curve highlights an exponential pressure increase over a little volume amount in an initial filling phase. After the achievement of a certain distension phase the volume-pressure ratio (compliance) shows a linear relationship.*[6]

The pressure gradient is exerted both longitudinally along the vessel and transversally on the venous wall.

This last energy development is associated to the so called Trans-mural pressure (TMP) and it represents a fundamental parameter both in hemodynamics and in fluid exchanges.

TMP is the difference between the internal venous pressure (*IVP*), acting on the internal vessel side to expand it, and the external venous pressure (*EVP*), pressing on the external parietal wall to collapse it (*Fig. 1.2.3*). TMP and vessel permeability represent the intra-extra-vascular exchanges determinants (***Starling's law***).

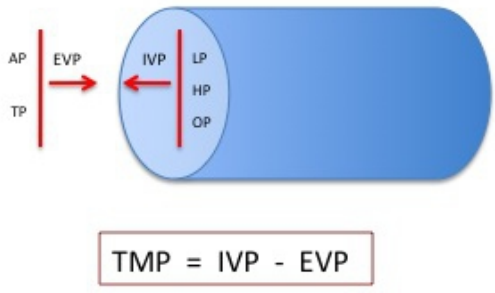

Fig. 1.2.3 Trans-mural pressure. *AP: athmospheric pressure. TP: tissue pressure. EVP: external venous pressure. IVP: internal venous pressure. LP: lateral pressure. HP: hydrostatic pressure. OP: oncotic pressure. TMP is the crucial parameter in tissue drainage and venous calibre regulation.*

Fundamentals components of the TMP follows the ***Bernoulli's principle***, both in static and dynamic conditions.

According to this law of physics, an increase in the speed of a fluid occurs simultaneously with a decrease in the fluid's potential energy.

It's a derivation from the principle of the conservation of energy, which states that, in a steady flow, the sum of all forms of energy in a fluid is constant.

This requires that the sum of kinetic and potential energy remains constant.

In ideal conditions, it states that the energy factors governing the venous hemodynamic are the kinetic energy ($\geq v^2/2$; $\geq$ represents the fluid density, v the fluid velocity) together with the potential energy.

This last one is constituted by the lateral pressure (lp), linked to the vessel wall elastic properties, and gravitational pressure, produced by the blood column weight.

The sum of them ($\geq v^2/2 + lp + \geq gh$) is constant at any point.

Bernoulli's principle: $\geq v^2/2 + lp + \geq gh = \text{constant}$

This means that in venous stasis condition the potential energy will be at its maximum, while it will decrease proportionally to the flow velocity increase.

The obvious but determinant consequence is that the lateral pressure, exerted on the venous wall, will decrease proportionally to the velocity reached by the fluid (Fig 1.2.4).

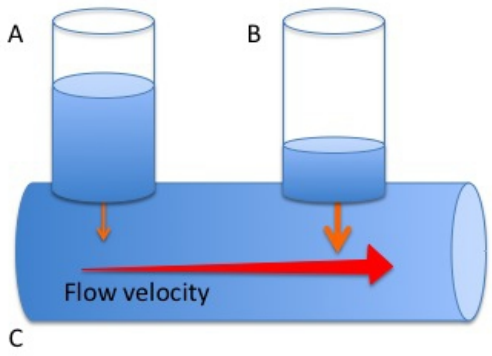

Fig. 1.2.4 Bernoulli's principle related Lateral Pressure drop. *Decreasing lateral pressure values, according to flow velocity increase.*

According to Bernoulli's principle, in two communicating vessels, the one presenting a higher flow velocity will display a lower lateral pressure: a gradient will be created and the blood will flow from the slower to the faster vessel.

The aspiration effect performed by the higher velocity vessel is universally known as the *Venturi's principle*.

This last one is strictly linked to the *Castelli's law* (*Fig. 1.2.5*) which states that flow velocity (v) is inversely proportional to the vessel cross sectional area (A).

Castelli's law: $A_1v_1 = A_2v_2 =$ Flow

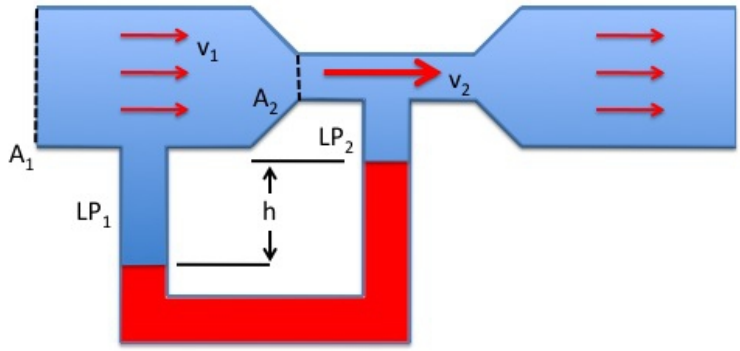

Fig. 1.2.5 Castelli's law and Venturi's effect. *Flow velocity (v) inverse proportionality to vessel cross sectional area (A) (Castelli's law) and consequent lateral pressure (LP) variation ($LP_1 > LP_2$) leading to the fluid aspiration determined by the Venturi's effect.*

The implication is that, whenever the vessel divides into several branches, if the sum of these last ones sections will be smaller an increased flow velocity will be expected; of course the opposite will be realized in case of a sections total area increase.

In the Bernoulli's principle, the counterpart of the kinetic energy is represented by the potential energy, thus by the hydrostatic component.

Hydrostatic energy is created inside the veins by the different hydrostatic columns in between the valvular planes.

The columns present different energy states, which vary just according to the same column height.

In fact, in this static situation, the only energetic level determinant is the potential gravitational energy value, which is expressed by the following formula:

Potential gravitational energy $= \geq gh$

($\geq$ represents the fluid density, g the gravity constant, h the height above the surface)

Whenever the different venous systems are in communication (Fig. 1.2.6) fluid flows from the higher to the lower column, than settling into a balanced common energetic state, in which all the columns heights are equal.

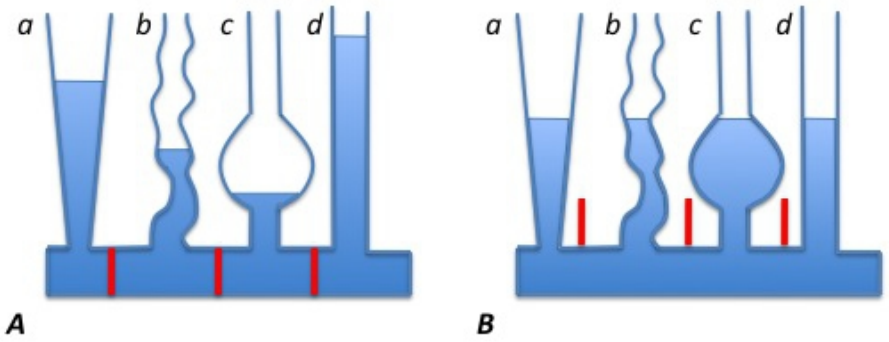

Fig. 1.2.6 The communicating vessel principle*. A) Not communicating hydrostatic columns presenting different heights, which leads to different energetic states (column d presents the highest energetic value).* ***B****) Communicating columns in which flow moves from the higher to the lower energetic state systems, until a common energetic balance is reached.*

In the lower limb system, this phenomenon is translated into a blood flow from the system presenting a higher blood column to the one with a smaller column.

Different valve density in the deep versus the superficial system gives a rationale to such blood movement during the diastolic phase (*Fig. 1.2.7*)

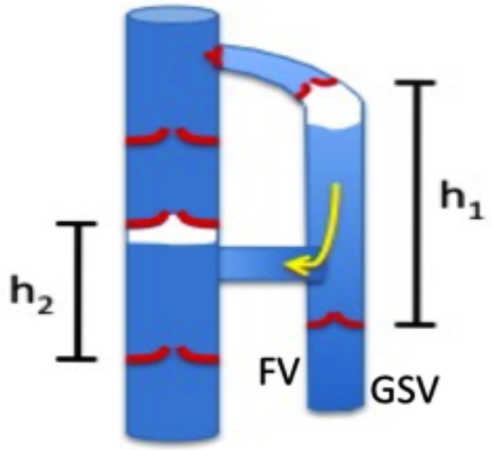

Fig. 1.2.7 Valve density and hydrostatic pressure: considering that the hydrostatic pressure is directly related to the only variable of the column height (h), during the diastolic phase the venous blood flows from the higher to the lower column, moving from a system with higher energy values to ones at a lower energy state.

Certainly, up to this point, all of our physic laws applications have been made considering the vessel and the blood as an ideal conduit and liquid, respectively.

To the contrary, in the human body environment the vessel produces friction through blood contact, while this last one possesses complex visco-elastic properties.

An extension of the thermodynamics second principle, the entropy law, states that in case of not ideal conduits or liquids part of the energy is dissipated as heat generation, so increasing the amount of no more available energy (entropy).

The human body solution to counteract this energetic dissipation has been the creation of the several pump mechanisms placed in series all along the cardiovascular system.

1.3 The "valvulo-muscular" pumps

Tissue drainage and venous flow maintenance are based on valvular and muscle function.

While the first avoids reflux, the second one promotes the systolic push.

Both systems are so tightly integrated to be considered as a singular "valvulo-muscular" unit.

A system of serial pumps propels the venous blood from the most distal area of the body toward the heart.

The same heart represents the first propulsive unit, pushing the venous from behind by the so called *vis a tergo* (i.e. force from behind).

The heart is the fundamental blood propeller providing volume, pressure and flow to the system in the lying down position, when the hydrostatic pressure is null.

Moreover, also the right heart side greatly influence the venous hemodynamic, increasing the central venous pressure during its systole and the venous flow during its diastole.

The close link between heart pump and venous circulation is testified by the evident cardiac pulsations in lower limbs venous tracings and by the venous oedema highlighted in congestive heart failure patients.

A second pump is the thoraco-abdominal pump. It represents a force from the front of the venous circulation, influencing the venous return by means of the diaphragm movements.

During inspiration, intra-abdominal pressure increases, so compressing the inferior vena cava and reducing the venous blood flow: usually the venous outflow from the lower limbs can temporaly cease.

During expiration, the intra-abdominal pressure falls again, the inferior vena cava expands and the venous blood from the lower limbs can flow to the heart.

The third pump is constituted by the peripheral muscles, preforming a second "systole" inside the venous system ,thus being called "the peripheral heart".

In fact, the muscle pump, which is mainly developed in the calf, assumes a main antagonist role against the force of gravity. The soleal and gastrocnemial contractions exerts an external venous pressure between 40 to 200 mmHg, so reducing the transmural pressure and displacing the blood toward the heart.

The so generated pressure wave will be transmitted to the surrounding veins proportionally to their own proximity to the muscular fascia investments (AC1 > AC2 > AC3, see anatomy chapter).

The foot represents a peculiar anatomical venous site, where physiologically the blood can empty from the inner side toward the outer surface.

Axial veins are prevalent in the plantar region, constituting a sort of "sponge" that can be squeezed at every footstep, so providing blood propulsion (Fig. 1.3.1).

This venous network is called Lèjar sole and, even if not the main pump of the system, represents a fundamental factor influencing venous return.

The venous anatomy and functionality of the foot also explains the possible correlation among postural disorders and venous insufficiency.[7]

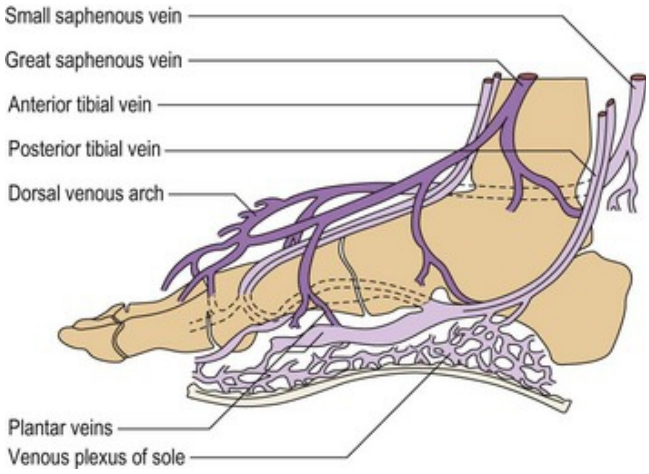

Fig.1.3.1 Plantar venous pump: *At the foot, the plantar venous plexus (Lèjar sole) represents a fundamental pump in venous drainage propulsion.*

In conclusion, the veno-muscular pumps play the role of a peripheral heart, which, combined with the venous valves, avoids gravitational reflux and promotes venous drainage.

The tight interconnection among muscular and venous function is demonstrated also by the potential consequences following joints and muscular impairment.

For example, ankle range of motion has demonstrated to be directly correlated with lower limb drainage.[7,8]

1.3 Needs in venous physiology literature

In an apparent paradox, the scientific literature presents more data regarding pathological rather than physiological scenario in lower limbs venous hemodynamics.

One of the main facts leading to this paradox regards the extreme variability of the venous system: a feature that impedes an easy standardization of the network and of its consequent physical characteristics.

Considering the several mechanisms involved in the venous flow creation, another difficult point comes from the need of homogeneity in the same flow elicitation.

Different manoeuvres to elicit the flow can create different effects. At the same time, up to now, no manoeuvres are able to perfectly reproduce the activation of the muscle pumps as in a real life scenario.

Another aspect to be remembered is the application of the Newtonian laws of physics inside the human body.

Blood is not a Newtonian fluid and the vessels are not ideal conduits.

Lump models have provided fundamental advancement in hemodynamics interpretation, but the real application of the laws of physics in the venous system still needs further investigations.[9]

In particular, in this analysis, it must be taken into consideration that blood flow is not steady, rather pulsatile and that the vessels are elastic, multi-branched and constantly changing not only diameter but also shape.

An extreme need of objective data is present in modern hemodynamics interpretation.

The present work aims to provide further clues, paving the way for a deeper understanding of both the physiological and pathological venous functioning.

CHAPTER 2

CHRONIC VENOUS INSUFFICIENCY PATHO-PHYSIOLOGY

2.1 Chronic venous insufficiency: an inflammatory disease

Lower limb chronic venous insufficiency (CVI) can be defined as whatever subversion of the hierarchical order of venous emptying, leading to venous hypertension.

Venous hypertension is responsible for a vicious circle promoting inflammation.

The vessel dilation, consequent to hypertension, leads to valve leakage.

A pathological share stress occurs on the vessel wall, triggering inflammation.

The same inflammation progressively damage both the valve and the wall structure, worsening the reflux.

At this point a capillary hypertension is also generated, leading to capillary leakage, thus edema.

The same edema worsens the inflammation, so progressively increasing the grade of venous hypertension (Fig. 2.1.1).[10]

Regarding the primitive ethiology of this phenomenon controversial theories exist about the origin from a valve malfunctioning rather than a primitive venous wall degeneration.

As a matter of fact there is a vicious circle between structural changes in valves and venous wall and hemodynamic forces leading to reflux and venous hypertension.[11]

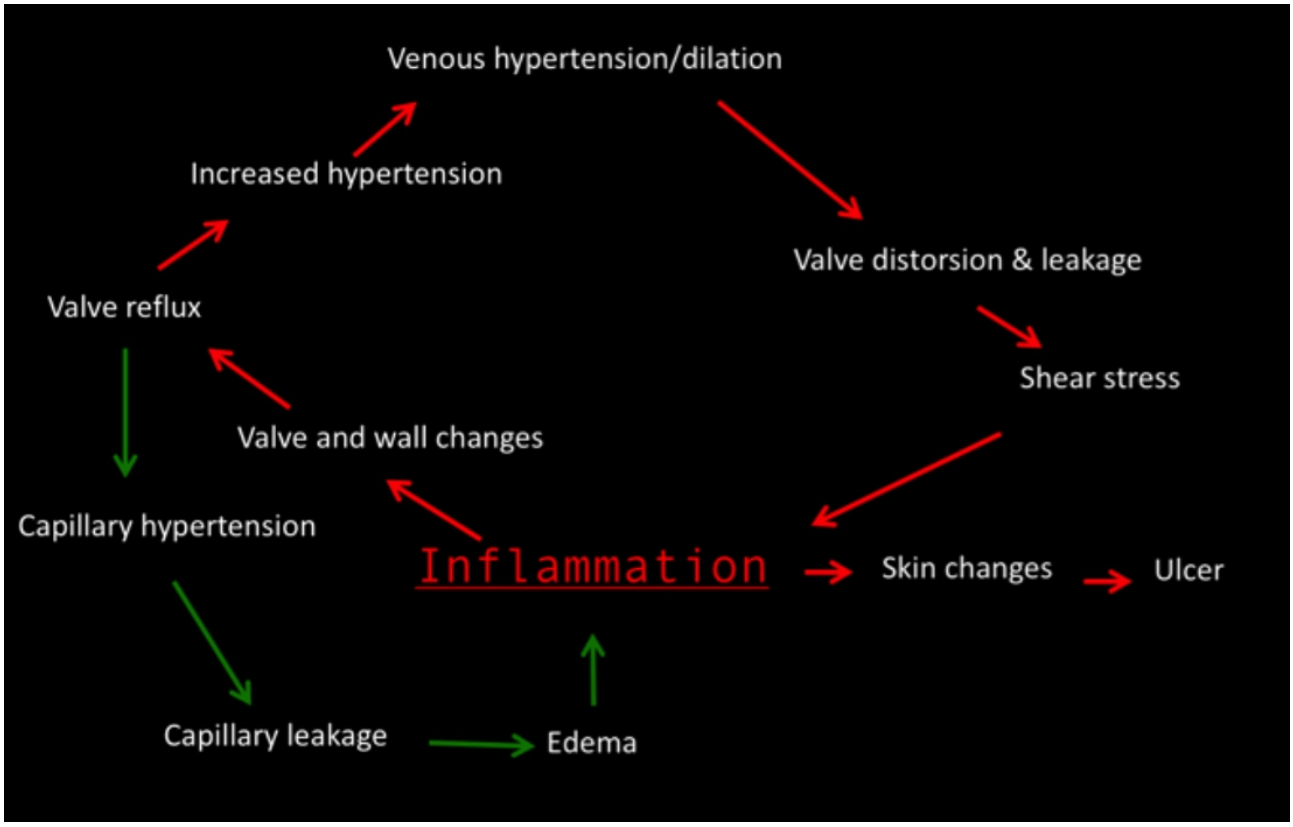

Fig. 2.1.1 The vicious circle of venous hypertension/venous inflammation: inter-relationship among venous hypertension and venous inflammation, in a vicious circle leading to progressive drainage impairment and edema formation.[10]

henever CVI hypertension and inflammation is translated in a clinical condition, chronic venous disease (CVD) occurs.

CVD presents a high prevalence, with up to 56% of males and 60% of females involvement.[12]

A consequent significant socioeconomic impact is associated with this disease, that, in its latest stage leads to venous ulceration: a condition that require the involvement of 1% of the health care budgets, in many industrialized countries.[13]

CVD clinical signs and symptoms develop from venous hypertension and inflammation.[14]

The major contributing factors of CVD are reported in Fig. 2.1.2.

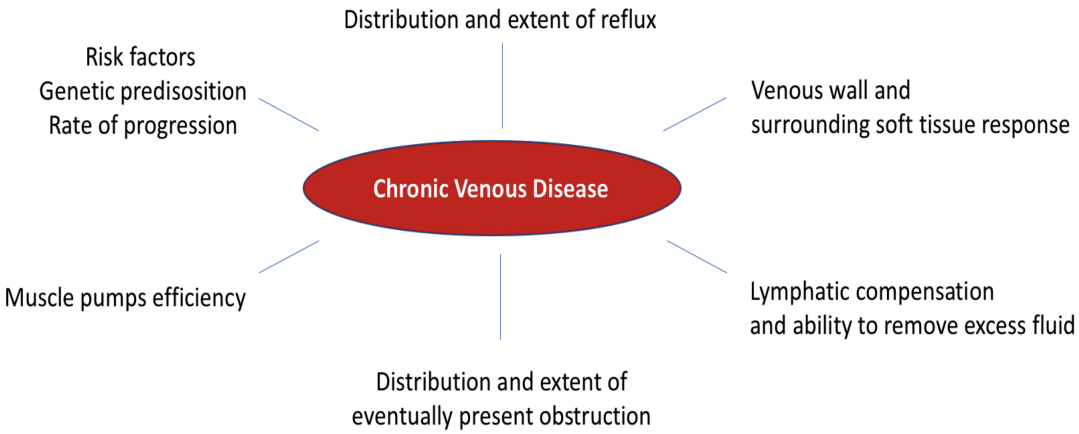

Fig. 2.1.2 *Factors influencing CVD development.*

The distribution and extent of the venous reflux represent a main factor in symptomatology development, together with the eventually present venous obstruction, leading to hypertension. Yet, a direct correlation among symptomatology and venous hypertension has not been found. This aspect suggests the extreme complexity behind lower limb venous patho-physiology, warranting further investigations on the topic.

Other factors to be taken into account are the local response of the venous wall and of the surrounding tissue, together with the ability of the interstitium to drain the excessive fluid accumulated as a consequence of the hypertension.

Muscle pumps efficiency is crucial in the same fluid propulsion too, while individual risk factors, genetic predisposition and rate of disease progression create heterogeneity in CVD clinical manifestation.

Multiple studies have shown that CVD is an inflammatory disease, as it is evident in the vein wall and in the perivenous space.[10,15]

Monocytes/macrophages are easily found in the affected proximal vein wall and in the valve sinus. Mast cells are widely distributed throughout the vein wall.[16,17]

The endothelial lining represents a fundamental translation point from physical forces to biochemical messages.

The so-called “bio-signalling” leads to several cytokines and inflammatory mediators activation, following the pathological hemodynamics changes.[18,19]

A direct interconnections exists among hemodynamics forces and biological signalling.

Many arterial investigations have already demonstrated the direct relationship among haemodynamic forces and endothelial expression:[20] while a laminar flow is associated with an anti-inflammatory vessel wall,[21] an oscillatory flow is linked to a pro-inflammatory endothelial lining.[22]

On the other side, these kinds of investigations have been definitely rare on the venous field,

remaining mainly limited to animal models.[23-25]

Nevertheless, preliminary evidences have been produced ex-vivo, demonstrating how CVD patients endothelial cells exhibit a pro-inflammatory phenotype.[26]

Moreover, always in an ex-vivo setting, a correlation among reflux as an oscillatory flow and cytokine release was observed, showing how the reflux time is directly related to the endothelial PDGF release from the varicose vein. [26]

As demonstrated also by a lymphnodes activation during CVD, inflammation parallels CVD severity.[27]

Venous symptomatology is linked to the activation of nerve endings along the venous wall.

An interaction among venous nociceptors and inflammatory reaction is postulated and considered associated with the perceived symptoms.[28]

Venous stasis and hypertension lead to a wall response leading to the release on inflammatory mediators linked to the release of algogenic factors, stimulating the nerve endings, thus the symptoms perception.

The degree of such stimulation may depend on the location and sensitivity of these nerve endings, together with the simultaneous presence of co-factors such as hormonal stimuli (menstrual cycle) and environmental habits (for example, prolonged standing).

All these variables can explain why there can be such a discrepancy among symptoms intensity and clinical severity.

In most severe CVD stages, the perivenous tissue becomes infiltrated by a fibrous reaction, potentially reducing the functionality of the nerve transmission. In this case, less symptomatology will be reported by the patient, despite the severity of the condition.

In other types of nerve damaged associated with advanced CVD, a pain threshold can be reduced by the nerve damage, so leading to a particularly intense symptomatology.[14]

2.2 Laws of physics implication in pathological scenarios

In case of a pressure gradient subversion, according to the *Poiseuille's Law*, a pathological flow will be generated in the opposite direction compared to the physiological drainage direction.
In according with the *communicating vessel principle*, the pressure gradient subversion can be generated by an incompetent valve leading to an increase in the hydrostatic column, thus to an outflow toward the most superficial rather than the deepest venous compartment.
The progressive venous calibre dilation means an increase of the vessel radius, which is inversely and exponentially correlated to the resistance.
The consequent drop of resistance favours a drainage toward the incompetent and dilated varices, according to the resistance formula (see chapter 1.2).

Once the vessel has reached its maximum dilation, the compliance curve indicates an almost linear increase with the pressure values. On this basis, venous hypertension can be triggered by volume overload inside a varicose and dilated network.
Small veins can dilate inside their compartment, following the venous pressure overload.
The sum up of their sections increases. Based on the *Bernoulli's principle* and the *Castelli's law* the velocity inside this compartment slows down, potentially leading to venous stasis.
In these conditions, the flow changes from laminar to turbulent, so leading to a pathological shear stress on the wall and to the consequent inflammatory mediators release.

2.3 Venous Ultrasound Scanning

Together with an accurate visit, echo-color-Doppler (ECD) scanning represents the most clinically useful test for detecting, localizing and evaluating venous flow.[29]

Spectral analysis of the ECD signal characterize the flow in its direction and patterns.

A color-coded flow map can simplify the examination by immediately distinguishing the flow direction, even in multiple vessels at the same time.[30]

An inverted spectrum or color flow map indicates a retrograde flow.

If a retrograde flow lasts more than a specific time, it is considered reflux, with different values according to the anatomical localization.

In particular, the reflux time cut-off for the superficial venous system is longer 0.5 seconds.[31]

The goal of the ECD examination is to identify the source of the the pressure gradient subversion, i.e. the site where a valve/wall derangement triggers a reflux.

The patient must be assessed in a standing position whenever looking for CVI signs.

After the vein segment has been identified, flow can be elicited by active or passive manoeuvres, based on the activation of the muscular pumps actively by the patient or passively by the operator.

The deep venous system is evaluated for obstruction and reflux.

Next, the AC2 and AC3 compartments are scanned.

The veins are assessed by tracing its course.

The caliber is then assessed, as changes in caliber can provide important clues to physiologic disturbance.

Peripheral to the takeoff of incompetent tributary veins, the caliber of the vein often decreases. Conversely, the caliber of the saphenous vein generally increases at the level of a significant incompetent perforator vein.

A careful search should be made at points of dilatation for this important source of reflux.[2]

The spectral analysis includes objective parameters assessment, addressed to the flow characterization: reflux time (RT), peak systolic velocity (PSV), end diastolic velocity (EDV), resistance index (PSV/PSV-EDV)(Fig.2.2)

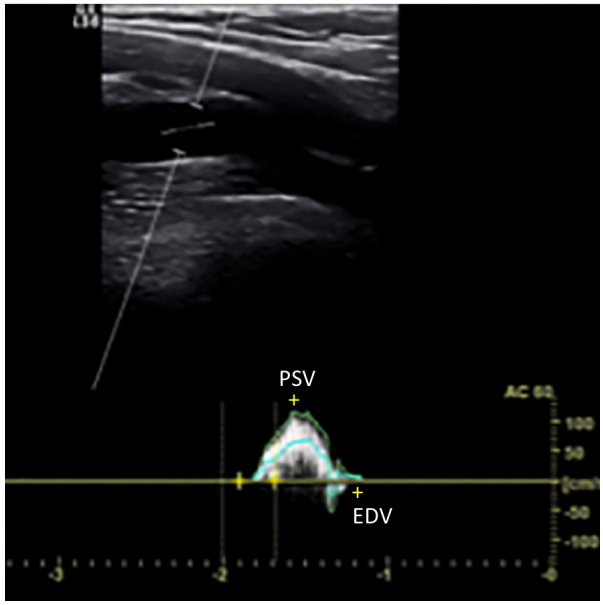

Fig. 2.3.1 Objective sonographic parameters assessment*: ultrasound scanning allows measurement of objective parameters such as peak systolic velocity (PSV)(highest velocity during the systolic phase) and end diastolic velocity (EDV)(velocity at the end of the diastole). From PSV and EDV, the resistance index can be calculated (PSV/PSV-EDV).*

2.4 Still missing literature in venous patho-physiology

Primary venous reflux pathogenesis remains largely controversial even nowadays: the descending vs ascending theory remains a discussion topic of high actual interest.

The descending or valvular hypothesis was first described by Trendelenburg in the19th century: the reflux begins because of an incompetent terminal sapheno-femoral valve, which is overwhelmed by the hydrostatic column pressing on it. Reflux than progresses in a retrograde direction, progressively altering more distal valves function.

The ascending theory was proposed in the eighties and found its basis on histological, biochemical and functional investigations demonstrating how venous wall can undergo pathological alterations in segmental localizations, irrespectively of the site and functional state of the valves. In this pathophysiological explanation, the reflux beginning results to be a local alteration, possibly developing in any part of the lower limb.

Even if the varicose veins genesis remains not clearly defined, recent researches proposed a unifying pathogenetic theory: primary structural changes of the valvular structure lead to an intial reflux which becomes responsible of secondary focal wall abnormalities, which in turn lead to a further increased reflux.

An increased metalloproteinases (MMPs) activity, following high wall tension values, has been recently demonstrated: the consequent derangement of the endothelium and smooth muscle cells becomes responsible of the altered venous constriction/relaxation properties, together with the leukocyte chemotaxis.

A vicious circle involving valvular incompetence, venous wall alteration, vessel dilation and increasing reflux is created.

Thus, parietal damage leading to vessel dilation seems to be antecedent to the valvular incompetence instauration; an events cascade is than executed by the MMPs activation, with consequent progressive venous drainage impairment.[32]

At the same time, an extreme need of objective data is present in modern hemodynamic interpretation.

Up to now, this last one has been based mainly on the reflux time values.

Nevertheless, preliminary evidences demonstrate the role also of the velocity of flow.

As demonstrated by Marston, deep venous system velocity represents an outcome determinants in patients undergoing superficial venous ablation.[33]

At the same time, according to the work by Tisato, a correlation was found among specific inflammatory cytokines modulation and EDV.[34]

Aim of this thesis work is to provide further objective data in physiological and pathological scenarios, paving the way for an appropriate application of the laws of physics inside the venous system.

CHAPTER 3

THE INVESTIGATION

3.1 Background

Consistent lower limbs venous system knowledge is nowadays accessible thanks to the hi-tech diagnostic instrumentation.[29,35,36]

Nevertheless, even if the venous flow haemodynamics have been deeply investigated in pathological conditions like the reflux one,[31]

there is still a significant literature lack concerning the physical laws governing the flow and its physiological characteristics.

In particular, apart preliminary assessments of venous pressure inside the deep and saphenous systems.[37,38] and very rare data on the femoral vein velocity,[39-42] investigations regarding the whole

lower limbs venous system flow kinetics and the consequent physical implications on the drainage are missing.

The actual anatomical and physiological interpretation of the lower limb venous network indicates a draining direction from the most superficial to the deepest lower limbs compartments: from the tributaries network (AC3), toward the saphenous system (AC2) and the deep venous system (AC1).[43]

Nevertheless, evidences concerning these networks kinetics are lacking.

Velocity and calibres assessments along the different segments of these compartments could provide useful data for a detailed lower limb venous hemodynamics model description and for a deeper insight into venous pathophysiology.

Ultrasound scanning, under determined circumstances, have already demonstrated to be a reproducible and effective tool in venous hemodynamics assessment.[44]

Aim of the present thesis is to determine the flow velocities along the venous segments of the lower limb network, both in physiological and pathological scenarios. Secondary endpoint is the detection of eventual hemodynamic differences inside the anatomical compartments, so providing clues for the identification of the physical model governing the flow direction.

The first part of the work report the velocity values of the different venous segments.

A statistical analysis highlights eventual correspondence among velocity values and anatomical compartment localization.

In the second part of the investigation, velocity values are reported from incompetent saphenous tributaries and compared with cranial and caudal saphenous segments, in order to evaluate the potential role of the Venturi's effect inside pathological network.

3.2 Methods: physiological cases

Study population

Twenty-six healthy subjects (8 males, 18 females, mean age 25 ± 5 years, BMI: 21±2) underwent an echo-colour-Doppler scanning (ECD) of the lower limbs venous system.

All the subjects were evaluated among 11 am and 3 pm, always in the same controlled room temperature.

All subjects who took part in this study were healthy medical students.

ECD protocol

The entire cohort was investigated while standing up, after 1 minute of immobility (MyLab 70 Esaote, Italy, multi-frequency linear array transducer, 7–12 MHz).

The assessing protocol included the recording of peak systolic velocity (PSV), time average velocity (TAV) and diameter of all the following venous segments in the deep venous compartment (AC1): external iliac vein immediately above the inguinal ligament (ILV), common femoral vein (SFJ.FV) at the sapheno-femoral junction (SFJ), femoral vein at the mid-thigh (FV), popliteal vein (PV), posterior tibial vein at the ankle (PTV). Same parameters were assessed in the following tracts of the saphenous system (AC2): great saphenous vein (GSV) at the saphenous side of the terminal valve (SFJ.GSV), GSV at the thigh-level (TL.GSV), GSV at the mid leg-level (LL.GSV), mid-leg small saphenous vein (SSV), whatever ECD detectable GSV and/or SSV. PSV, TAV and diameter were assessed also in the tributary system (T) (AC3)(Fig. 3.2.1).

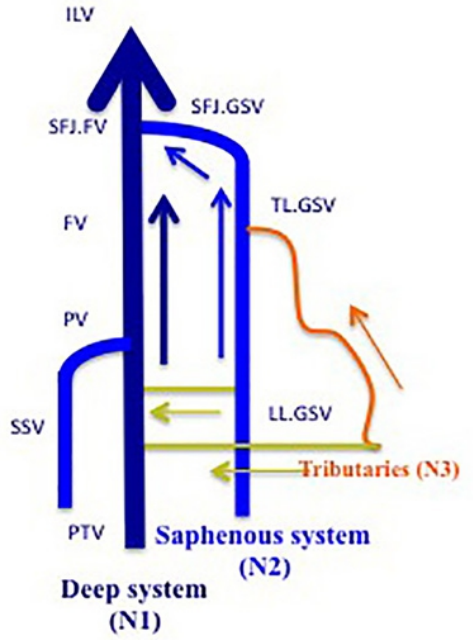

Fig. 3.2.1 Lower limb venous networks. *Venous network subdivision in 3 anatomical compartments and the related segments in which velocity was assessed. N1: deep venous system. N2: Saphenous System. N3. Tributary system. ILV: external iliac vein. SFJ.FV: Femoral vein at the sapheno-femoral junction. FV: Femoral vein at mid-thigh. PV: popliteal vein. PTV: posterior tibial vein at the ankle. SFJ.GSV: great saphenous vein at the sapheno-femoral junction. TL.GSV: great saphenous vein at the thigh level. LL.GSV: great saphenous vein at the leg level. SSV: small saphenous vein.*

The flow was always elicited both by active foot dorsi-flexion (ADF) and passive manual compression/relaxation (CR) manoeuvres (Figure 3.2.2).

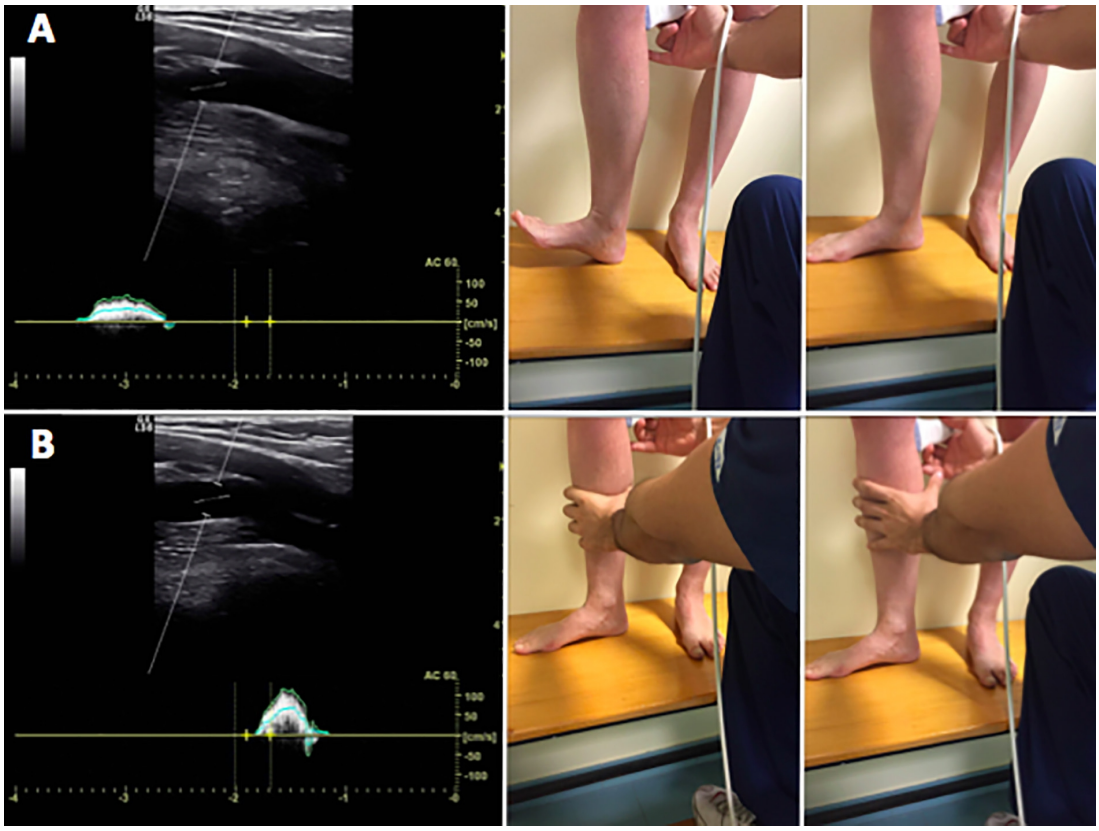

Fig. 3.2.2 Assessment of the same vein segment using different manoeuvres to elicit the flow*: A) ADF manoeuvre to elicit the flow in the popliteal vein. B) Same segment assessment evoking the flow by CR manoeuvre*

All the veins were assessed by means of the following ECD setting: longitudinal scanning, PRF value in between 0.7 and 1 KHz, sample volume opened up to the vessel wall limits, insonation angle parallel to the vessel direction.[45]

All those subjects presenting anatomical conditions forcing the sonographer to set an insonation angle lower than 45 degree or higher than 60 degree were considered as drop out for lack of measurement precision.[46]

Three equally trained and experienced assessors performed the scanning.

In order to establish the measurement reproducibility the 3 sonographers performed 5 assessments of PSV and TAV at FV, TL.GSV and T, evoking the flow both by CR and ADF.

Statistical analysis

Preliminary data analysis has been performed in order to choose the proper statistical methodology. Shapiro-Wilk test was used for the normality detection. Since the results were inhomogeneous over the analysed data, we choose to use both parametric and non-parametric tests when necessary.

Correlation between velocity (both TAV and PSV) and vein diameter has been evaluated using the Pearson's correlation test for both CR and ADF manoeuvres.

ANOVA (parametric) or Kruskal Wallis (non parametric) tests were used to compare the velocity values of the assessed venous segments (ILV, SFJ.FV, FV, PV, PTV, SFJ.GSV, TL.GSV, LL.GSV, SSV, TRIBUTARY).

The dependency of the velocity (both TAV and PSV) with respect to the veins list has been plotted using box plot. The bottom and top of the box are the first and third quartiles; the band inside the box is the median. The lines extending vertically from the boxes (whiskers) represent the lowest datum within 1.5 interquartiles of the lower quartile, and the highest datum still within 1.5 interquartiles of the upper quartile.

Finally, for each vein velocity averaged values (both TAV and PSV) and standard deviation have been calculated for both CR and ADF manoeuvres. Also veins diameter averaged values together with standard deviation have been calculated.

Wilcoxon and T-test were used to compare PSV and TAV obtained by ADF and CR ($p<0.05$).

Assessment reproducibility was tested by paired one-way ANOVA.

A value of $P<0.05$ was considered significant.

3.3 Results: physiological cases

Three of the 52 assessed lower limbs presented venous reflux, so being excluded from the study.

Comparison among TAV evoked by CR and ADF showed no statistical differences with the exception of the following segments: ILV, FV, PTV and T ($p<0.05$) (Table 3.3.1).

Comparison among PSV evoked by CR and ADF showed no statistical differences with the exception of the following segments: FV, SSV and T ($p<0.05$) (Table 3.3.1).

Table 3.3.1. Velocity Mean and Standard Values of CR and ADF manoeuvre.

Manoeuver	Parameter	ILV (cm/sec)	SFJ.FV (cm/sec)	FV (cm/sec)	PV (cm/sec)	PTV (cm/sec)	SFJ.GSV (cm/sec)	TL.GSV (cm/sec)	LL.GSV (cm/sec)	SSV (cm/sec)	TRIBUTARY (cm/sec)
CR	TAV	35±8	36±10	41±12	35±13	25±11	18±8	17±8	15±5	12±4	5±2
CR	PSV	100±23	101±25	109±39	89±27	45±18	37±21	40±16	35±14	24±9	12±4
ADF	TAV	42±13	37±10	34±10	32±12	19±10	15±7	18±9	16±7	12±5	9±4
ADF	PSV	103±24	100±32	90±31	82±28	41±20	37±17	42±22	30±11	21±8	15±4

The venous diameters increased moving from the most superficial (AC3) to the deepest compartments (AC1) ($P<.0001$) and demonstrated to be directly related to the corresponding TAV and PSV velocity values, both at the CR ($r2= 0.79$, $r2= 0.87$) and ADF ($r2= 0.95$, $r2= 0.96$).

Detailed mean and standard deviation (SD) values of PSV and TAV, evoked both by CR and ADF, are reported in Table 3.3.1 and plotted in Figure 3.3.1.

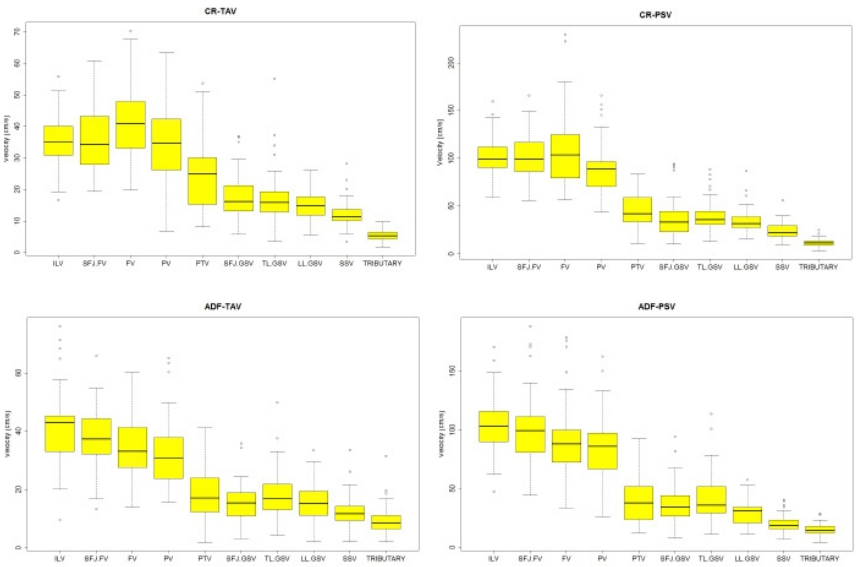

Fig. 3.3.1 Boxplot of TAV and PSV mean and standard deviation values at CR and ADF manoeuvre.

The comparison among PSV and TAV values, evoked both by CR and ADF, along the different vein segments are reported in Table 3.3.2, Table 3.3.3, Table 3.3.4 and Table 3.3.5.

Table 3.3.2 Kruskal-Wallis test: CR-TAV.

	ILV	SFJ.FV	FV	PV	PTV	SFJ.GSV	TL.GSV	LL.GSV	SSV	TRIBUTARY
ILV		0.96	**0.022**	**0.034**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
SFJ.FV			**0.025**	**0.026**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
FV				**0.017**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
PV					**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
PTV						**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
SFJ.GSV							0.696	0.141	**<0.001**	**<0.001**
TL.GSV								0.106	**<0.001**	**<0.001**

LL.GSV	**0.002**	**<0.001**
SSV		**<0.001**

Table 3.3.3 Kruskal-Wallis test: CR-PSV.

	ILV	SFJ.FV	FV	PV	PTV	SFJ.GSV	TL.GSV	LL.GSV	SSV	TRIBUTARY
ILV		0.918	0.765	**0.008**	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
SFJ.FV			0.624	**0.008**	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
FV				**0.006**	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
PV					<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
PTV						**0.016**	**0.040**	**0.007**	<0.001	<0.001
SFJ.GSV							0.167	0.133	<0.001	<0.001
TL.GSV								**0.043**	<0.001	<0.001
LL.GSV									<0.001	<0.001
SSV										<0.001

Table 3.3.4 *Kruskal-Wallis test : ADF-TAV.*

	ILV	SFJ.FV	FV	PV	PTV	SFJ.GSV	TL.GSV	LL.GSV	SSV	TRIBUTARY
ILV		0.151	**0.007**	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
SFJ.FV			0.087	0.008	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
FV				0.134	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
PV					<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
PTV						0.054	0.085	0.129	<0.001	<0.001
SFJ.GSV							0.058	0.164	<0.001	<0.001
TL.GSV								0.238	<0.001	<0.001
LL.GSV									**0.004**	<0.001
SSV										<0.001

Table 3.3.5 Kruskal-Wallis test: ADF-PSV

	IL V	SFJ.FV	FV	PV	PTV	SFJ.GSV	TL.GS V	LL.GSV	SSV	TRIBUTARY
ILV		0.306	**0.006**	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
SFJ.FV			**0.031**	**0.006**	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
FV				0.337	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
PV					<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
PTV						0.248	0.442	**0.007**	<0.001	<0.001
SFJ.GSV							0.298	**0.011**	<0.001	<0.001
TL.GSV								**0.004**	<0.001	<0.001
LL.GSV									<0.001	<0.001
SSV										<0.001

Taking into consideration the anatomical subdivision of the three networks (AC1, AC2, AC3), the following venous segments presented velocities values that were significantly different from the remaining venous tracts of their own anatomical compartments: FV, PV, PTV, SSV (TAV at CR); PV, PTV, SSV (PSV at CR); FV, PTV (TAV at ADF), PV, PTV (PSV at ADF).

No significant differences were reported comparing the velocities with the gender and with the right vs left limb.

No significant differences were reported among the data reported from the three assessors.

3.4 Methods: pathological cases

In the pathological scenario, venous Doppler scanning was performed on 40 lower limbs of 28 patients affected by superficial chronic venous disease (CVD) (mean age 56+6, M/F: 1/1, BMI 23+2, C2-6Ep,As,Pr).

ECD protocol

The entire cohort was investigated while standing up, after 1 minute of immobility (MyLab 70 Esaote, Italy, multi-frequency linear array transducer, 7–12 MHz).

All the patients were evaluated among 11 am and 3 pm, always in the same controlled room temperature.

The investigation was focused on the incompetent superficial venous tributaries and their confluence with the GSV.

Diameters, peak systolic velocity (PSV), peak diastolic velocity (PDV), end diastolic velocity (EDV), reflux time (RT), resistance index (RI) and diastolic time average velocity (DTAV) were measured in three different groups of venous segments: great saphenous vein (GSV) at 2 cm above the origin of the incompetent tributary (T)(Group-A), GSV at 2 cm below the origin of the incompetent T (Group-B), incompetent tributary at 2 cm from its origin from the GSV (Group-C) (Fig. 3.4.1).

Manual compression/relaxation (CR) manoeuvres was used to elicit flow.

The scanning and data collection were performed always by the same highly experienced assessor.

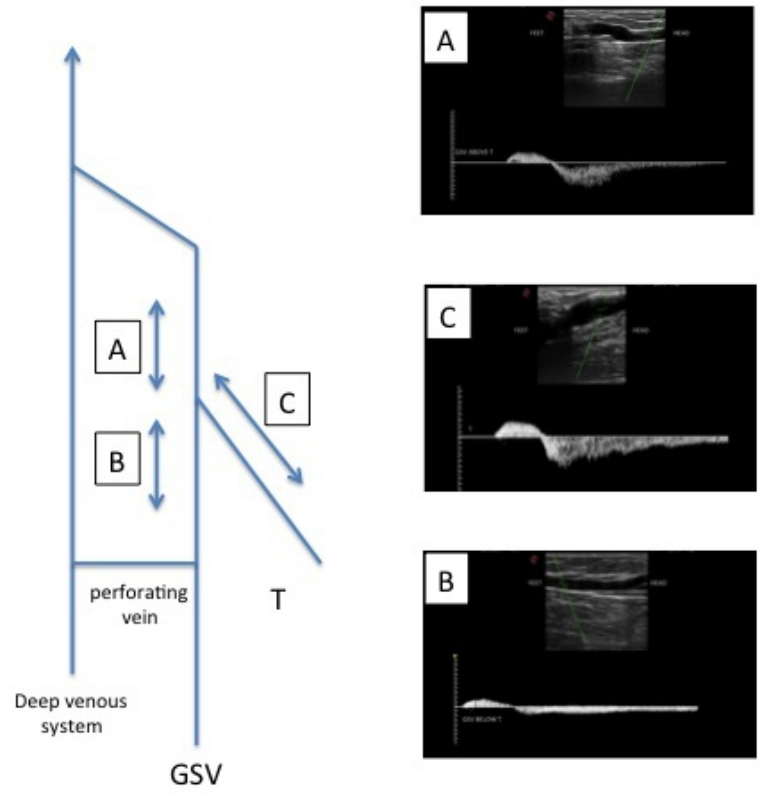

Fig. 3.4.1*: Refluxing venous network. Double arrows indicate the points of assessments at 2 cm from the emergence of the incompetent great saphenous vein (GSV) tributary (T). Group-A: GSV at 2 cm above the origin of T. Group-B: GSV at 2 cm below the origin of T. Group-C: T at 2 cm from its origin from the GSV.*

Statistical Analysis

InStat GraphPad (GraphPad Software, Inc.La Jolla, CA 92037 USA) was used for statistical analysis. The data were expressed as mean ± standard deviation. In order to verify the data distributions Kolmogorov-Smirnov test was used. The differences in PSV, PDV, EDV, DTAV, RI and Diameter were evaluated by Student's t-test and Wilcoxon test as appropriate. Statistical significance was defined as $p < 0.05$.

3.5 Results: pathological cases

No significant differences were demonstrated among PSV of group-A and C (p:ns).

PSV in group-B (16.7±6.6 cm/s) was significantly lower than in group-A (30.5±12.1 cm/s; p=0001) and C (28.1±15 cm/s; p=0.0001) (Fig. 3.5.1).

PSV in group-A was not significantly different from group-C.

PDV was significantly higher in group-C (-60.2±25 cm/s) compared to group-A (-36.8±12.9 cm/s; p=0.0001) and group-B (-15.1±4.4 cm/s; p=.0001).

There was no significant difference in EDV among the three groups.

DTAV was significantly higher in group-C (-21.3±8.5cm/s) compared to group-A (-15.7±5.2 cm/s; p=.0001) and group-B (-11.1±2.9 cm/s; p=.0001). In group-B, DTAV was significantly lower than in group-C (p=.0001).

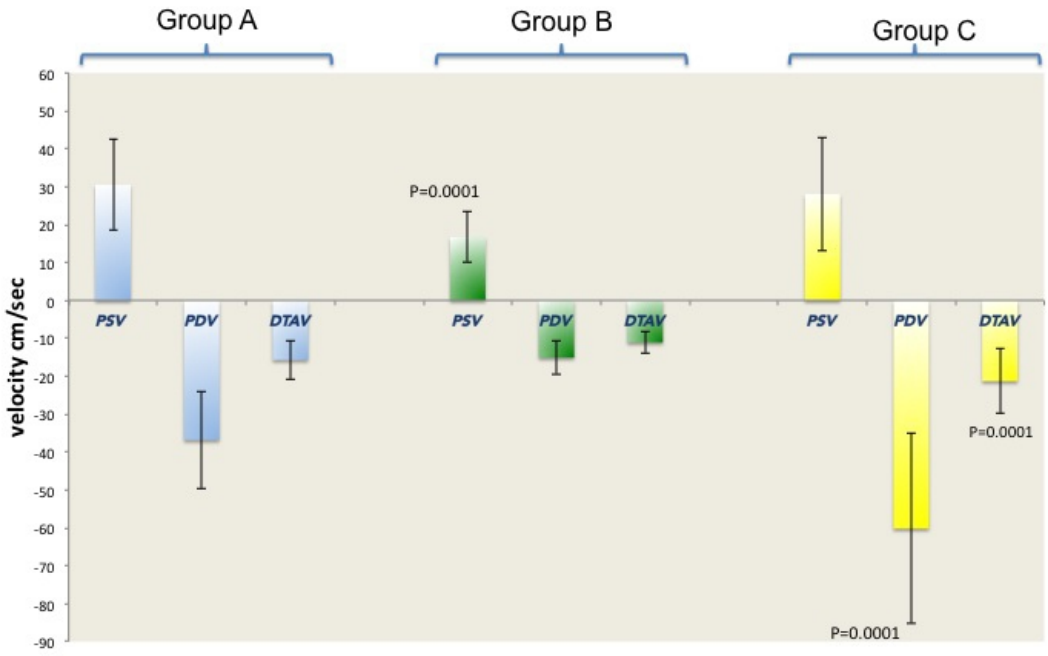

Fig. 3.5.1 PSV, PDV DTAV comparison among group A, B,C.

RT was not significantly different among the 3 groups.

RI was significantly higher in group-B (1.7±0.8) compared to group-A (1.4±0.2) (p=.04).

RI in group-A was not significantly different from group-C (1.4±0.1).

Group-B and C RI was significantly different (p=.03) (Fig. 3.5.2).

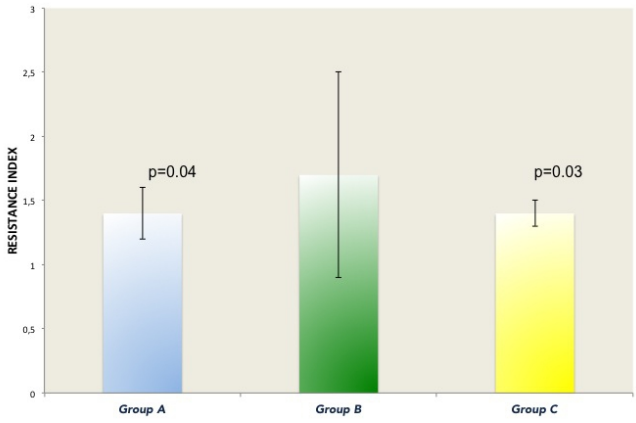

Fig. 3.5.2 RI comparison among group A, B, C

Venous diameter was significantly larger in A (5.9±0.9 mm) compared to C (3.8+0.8 mm) (p=.0001). Group-A and B (3.8+0.5 mm) diameters were significantly different (p=.0001). Group-B diameter was not significantly different from group-C (Fig. 3.5.3).

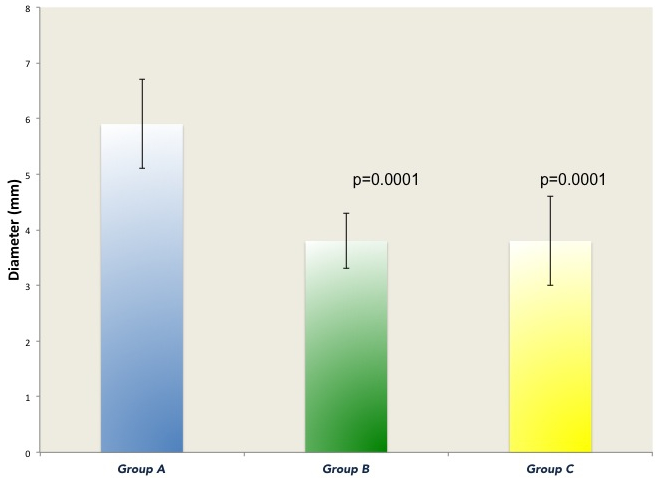

Fig. 3.5.3 Diameters comparison among group A, B, C.

CHAPTER 4

DISCUSSION

Venous system remains one of the most intriguing and charming field of medical research.

As reported by the cadaveric study of Thomson,[3] even the anatomy is so diverse from case to case that no dissected limbs present the same superficial venous arrangement.

Moreover, the blood is not a Newtonian fluid and that the venous vessel is not an ideal conduit, so making the application of the laws of physics in the drainage interpretation even more difficult.

The Bernoulli theorem, Communicating vessel penomenon and Castelli's law are fundamentals principles to be taken into consideration.[47,48]

Some pioneering papers have proposed lower limbs venous haemodynamics lump models[9]

but, to the best of our knowledge, a detailed description of the venous velocities in the different anatomical compartments is still lacking, together with the potential consequences on the drainage direction.

In particular, only few reports were conducted on the physiologic lower limbs venous flow characteristics, providing only partial data regarding the normal velocity range into the different anatomical compartments.[39,40,41,42]

Moreover, no investigations of this type were performed with healthy subjects standing still, evoking the flow by two different manoeuvres.

The first part of this thesis reports the different venous segments velocity physiological values, together with the diameter assessment of the different vein segments.

The vessel calibres demonstrated overlapping values with the literature concerning the common femoral and great saphenous vein,[40,49-51] while implementing the available data regarding the remaining lower limbs venous tracts.

According to the literature, focal venous dilation and segmental hypoplasia can be found in 12.4% of healthy subjects and in 24.6% of varicose veins patients.[52]

In the herein assessed physiological cases, no significant segmental calibres variations were reported. Further studies should be addressed to evaluate the impact of these anatomical features on lower limbs venous kinetics, so providing further clues regarding the application of the laws of physics into the venous network.

In the study, the anatomical venous network compartmentalization, according to the vessel localization under (AC1), in-between (AC2) and above the muscular fascia (AC3), shows an overlapping with the velocity values groups division.[1,53,54]

FV, PV, PTV and SSV constitute a main exception to this observation since presenting significantly different velocity values compared to the other AC1 network vessels ($P<.05$). This feature requires further investigation. In fact, PTV is the only segment that was evaluated in this analysis to be located above the plantar pump and below the calf pump. Moreover, PTV is a usually duplicated vessel.[55-57]

At the same time, SSV present a wide variability of anatomical junctions with the deep system: a fact that could influence its hemodynamics.[58]

FV and PV belong to an intriguing anatomical site, whose valves presence and location variability could represent a clue for this result explanation.[59,60]

In fact, together with their anatomical variable distribution, valvular regions present potential confounding factors in the hemodynamics analysis, also due to the flow acceleration through the leaflets tract narrowing and to the valvular pockets turbulence.[61]

Independently by these considerations, the different anatomical venous networks resulted to be associated with different velocity values, which follow the drainage hierarchical order: a tendency that is evident looking at the decreasing values reported in Figure 3.3.1.

A fundamental outcome was the detection of a constantly slower velocity in the tributaries system compared to the saphenous and deepest one, both for TAV and PSV.

In this scenario the Venturi's effect must be taken into consideration, with a suction action exerted on the slower flow by the fastest one.

Of course, further investigations are mandatory to increase the accuracy of this observation. In particular, the Castelli's law states that the sum of the different vessels sections multiplied for the sum of the corresponding velocities is constant. For this reason it will be mandatory to find out a way to assess not just a single vein segment of each network, rather the sum of all the different vessels that are localized inside the same compartment (Fig. 4.1).

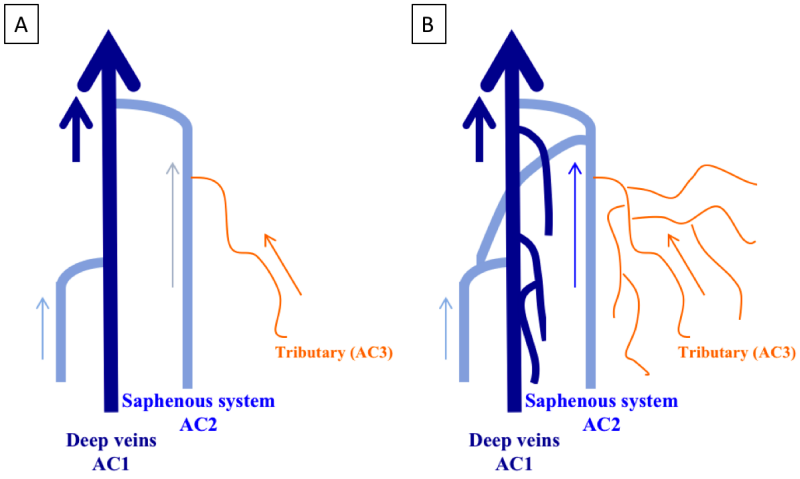

Fig. 4.1 Castelli's law application in the lower limb venous system*: the product among the vessel section and the velocity inside it is a constant. In lump models of the venous system it is fundamental to consider the sum up of all the sections belonging to the same anatomical compartments.* ***A)*** *Elementary models are available, taking into consideration single vessels inside the compartment.*

B) *More complex models are needed, evaluating the sum-up of all the sections inside a specific anatomical compartment.*

Secondary endpoint of this investigation is the demonstration of not significant differences in the TAV and PSV values whenever obtained by CR rather than ADF. An exception is represented by the ILV, FV, PTV and T (Table 3.3.1).

Considering the pathological data set of this investigation, the hemodynamic role of the incompetent tributary in the pressure gradient subversion becomes evident.

PSV is not significantly different comparing the GSV above the T and the T itself.

To the contrary, PSV becomes significantly lower inside the GSV below the confluence with the T.

The phenomenon indicates a preferential pathological route of drainage toward the most superficial compartment (from AC2 to AC3), caused by the pathological pressure gradient triggered by the T incompetnce.

The interpretation is supported by a higher RI in the GSV tract below the T, while no significant difference is reported whenever comparing GSV above the T with T itself.

The increased kinetic component in the pathological superficial compartment is demonstrated also by the significantly higher values of PDV and DTAV in the T network compared to the saphenous one.

According to the Bernoulli's principle, the kinetics component in the refluxing AC3 is higher than in AC2, thus leading to a lateral pressure drop and a potential Venturi's effect of aspiration from the saphenous system toward the incompetent varicose vein.

The diameter resulted not significantly different whenever comparing the T with the GSV below its confluence. To the contrary, GSV diameter above the confluence resulted significantly bigger than the T. This last finding can represent another factor responsible for the acceleration inside AC3, according to the increased velocity through a smaller section.

Whenever considering velocity as a guiding parameter for the venous network interpretation, it must be stated that a fundamental bias can rise by the same data acquisition tool. Even modern linear probes use large Doppler apertures in order to perform a proper beam steering and depth

penetration. Consequently, these large apertures create a spectral spread that is unrelated to the blood cell velocity. The phenomenon is called intrinsic spectral broadening and it occurs more or less in all the transducers, causing values overestimation.[62,63]

Recently, interesting technical instrumental innovations are offering the solution for a more precise assessment, paving the way for a more significant collection of data, also in settings that are similar to the ones herein presented.[64]

Other potential biases are represented by the use of multiple assessors and by the prolonged standing up time that the exam requested. The recording of all the physiological segments required more than 30 minutes of evaluation time. During this prolonged stasis the haemodynamics could present an intrinsic variability.

The herein presented physiological data set of velocity and diameter can be taken into consideration for further investigations of all the three lower limbs venous compartments. These data provided also a physiological starting point for further assessments in the pathological scenario of kinetics subversion inside refluxing patterns.

Future investigations regarding obstructive disease kinetics subversion can consider these data as a starting point

Moreover, the physiological and pathological reported values could become a reference point in the comparison with pre and post-operative hemodynamics.

This acquisition, even together with the same study biases, open new questions, while paving the way for a deeper understanding of the intriguing laws governing the flow haemodynamics.

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Appendix I

Publications in journals as PhD candidate

December 2013 – 2016

1. ***CHIVA strategy in chronic venous disease treatment: instructions for users.*** Gianesini S, Occhionorelli S, Menegatti E, Zuolo M, Tessari M, Spath P, Ascanelli S, Zamboni P. Phlebology 2014 apr 22 [Epub ahead of print]

2. ***Ultrastructure of internal jugular vein defective valves.*** Zamboni P., Mascoli F, Salvi F, Menegatti E, Gianesini S, Tisato V, Secchiero P. *Phlebology*, 2014 Jun 27. pii:0268355514541980
3. ***Modulation of circulating cytokine-chemokine profile in patients affected by chronic venous insufficiency undergoing surgical haemodynamic correction.*** Tisato V, Zauli G, Gianesini S, Menegatti E, Brunelli L, Manfredini R, Zamboni P, Secchiero P. *J Immunol Res 2014;2014:473765.*
4. ***A phlebo-lymphology humanitarian trip to Matagalpa, Nicaragua***. Gianesini S, Cavezzi A, Mosti G, Tessari L, Zini F, Urso S, Campana F, Tessari M, Della Caneva P, Espinoza F, Rocha R, Neuhardt D, Mowatt-Larssen E, Zygmunt J, Cortesi S, Morrison T, Morrison N. *Veins&Lymphatics. DOI: http://dx.doi.org/10.4081/vl.2015.4851*
5. ***Venous anomalies as potentially lethal risk factors during ordinary catheterization*** Savino Occhionorelli, Sergio Gianesini, Lorenzo Marinelli, Marianna Daniele, Sara Chierici, Paolo Zamboni. *Veins&Lymphatics DOI: 10.4081/vl.2015.5098*
6. ***Laser-assisted strategy for reflux abolition in a modified CHIVA approach*** Sergio Gianesini, Erica Menegatti, Michele Zuolo, Mirko Tessari, Paolo Spath, Simona Ascanelli, Savino Occhionorelli, Paolo Zamboni. *Veins&Lymphatics DOI: 10.4081/vl.2015.5246*
7. ***Elastic stockings effect on leg volume variability in healthy workers under prolonged gravitational gradient exposure***. Mirko Tessari, Sergio Gianesini, Erica Menegatti, Michele Zuolo, Anna Maria Malagoni, Maria Elena Vannini, Paolo Zamboni. Veins&Lymphatics doi:10.4081/vl.2015.5182
8. ***Histologic and sonographic features of holmium laser in chronic venous disease treatment.*** Gianesini S, Gafà R, Occhionorelli S, Menegatti E, Malagoni AM, Spath P, Zamboni P. Int. Ang. in press

9. ***An apparently untreatable ulcer of the face.*** Borghi A, Gianesini S, Pedriali M, Stefanelli A, Mangiola G, Dalla Caneva P, Lanza G, Virgili AR, Zamboni P. International Wound Journal. 2016 Jan 10. doi: 10.1111/iwj.12567
10. ***Pelvic Congestion Syndrome: does one name fit all?*** Gianesini S, Antignani PL, Tessari L. Phlebolymphology, in press
11. ***Comparison among 18 mmHg and 23 mmHg elastic stockings effect on leg volume and tiredness after golf.*** Gianesini S, Tessari M, Menegatti E, Spath P, Vannini ME, Occhionorelli S, Zamboni P. Int Angiol 2016, in press
12. **Venous hemodynamic changes in lower limb venous disease: the UIP consensus according to scientific evidence**
    Franceschi C, Cappeli M , Gianesini S, Mendoza E, Ermini S, Passariello F, Zamboni P. Int. Ang. 2016 June;35 (3):236-352
13. **Surgical techniue for deep venous reflux supression in femoral vein deuplication.**
    Zamboni P, Gianesini S. Eur J vasc Endovasc Surg 216, in press
14. **Autologous adipose-derived stem cells: basic science, technique, and rationale for application in ulcer and wound healing.** Zollino I., Zuolo M, Gianesini S, Pedriali M, Sibilla AG, Tessari M, Carinci F, Occhionorelli, Zamboni P
    Phlebology 2016 in press
15. **Mini-invasive high-tie by clip apposition versus crossectomy by ligature: Long-term outcomes and review of the available therapeutic options.** Gianesini S, Menegatti E, Malagoni AM, Occhionorelli S, Zamboni P. Phlebology 2016 in press
16. **Foam sclerotherapy safety**: news from the lab. Tessari L, Gianesini S. Flebologia 2016;42(1):41-43.
17. **Clinical applicability of assessmnet of jugular flow ove the individual cardiac cycle**

compared with current ultrasound methodology Sisini F, Tessari M, Menegatti E, Vannini ME, Gianesini S, Tavoni V, Gadda G, Gambaccini M, Taibi A, Zamboni P. Ultrasound Med Biol 2016 in press

18. **Fixing the jugular flow reduces ventricles volume and improves brain perfusion**. Zamboni P, Menegatti E, Cittanti C, Sisini F, Gianesini S, Mascoli F, Salvi F. J Vasc Surg: Ven & Lymph 2016 in press
19. **Oscillatory flow suppression improves inflammation in chronic venous disease-** Zamboni P, Spath P, Tisato V, Tessari M, Dalla Caneva P, Menegatti E, Occhionorelli S, Gianesini S, Secchiero P.
20. **A specifically designed aquatic exercise protocol to reduce chronic lower limb edema**. Gianesini S, Tessari M, Bacciglieri P, Malagoni AM, Menegatti E, Occhionorelli S, Basaglia N, Zamboni P. Phleb. 2016 in press
21. **A novel concept of pneumatic pump in the outpatient management of mixed leg ulceration: a pilot study.** Tessari M, Gianesini S, Spath P, Menegatti E, Sibilla MG, Zamboni M, Malagoni AM, Zamboni P. Veins & lymph 2016 doi:10.4081/vl.2016.5266 in press
22. **Femoral vein valve incompetence as a risk factor for junctional recurrence**. Gianesini S, et al. Phlebology. 2016 in press
23. **Lower libs kinetics and consequent impact on drainage direction.** Gianesini S, et al. Phlebology 2016

Appendix II

Chapters in books as PhD candidate

January 2014 –December 2016

1. **International Union of Phlebology 2013**

Byung-Boong LEE, Andrew N. NICOLAIDES, Kenneth MYERS, Mark MEISSNER, Evi KALODIKI, Claudio ALLEGRA, Pier Luigi ANTIGNANI, Niels BÆKGAARD, Kirk BEACH, Giovanni BELCARO,

Stephen BLACK, Lena BLOMGREN, Eliete BOUSKELA, Massimo CAPPELLI, Joseph CAPRINI, Patrick CARPENTIER, Attilio CAVEZZI, Sylvain CHASTA NET, Jan T. CHRISTENSON, Demetris CHRISTO POULOS, Heather CLARKE, Alun DAVIES, Marianne DE MAESENEER, Bo EKLOF, Stefano ERMINI, Fidel FERNÁNDEZ, Claude FRANCESCHI, Antonios GASPARIS, George GEROULAKOS, **Sergio GIANESINI**, Athanasios GIANNOUKAS, Peter GLOVICZKI, Ying HUANG, Veronica IBEGBUNA, Stavros K. KAKKOS, Robert KISTNER, Tilo KÖLBEL, Ralph L.M. URSTJENS, Nicos LABROPOULOS, James LAREDO, Christopher R. LATT IMER, Marzia LUGLI, Fedor LURIE,
Oscar MALETI, Jovan MARKOVIC, Erika MENDOZA, Javier L. MONEDERO, Gregory MONETA , Hayley MOORE, Nick MORRISON, Giovanni MOSTI, Olle NELZÉN, Alfred OBERMAYER , Tomohiro OGAWA, Kurosh PARSI, Hugo PART SCH, Fausto PASSARIELLO, Michel R. PERRIN, Paul PITTAL UGA, Seshadri RAJU, Stefano RICCI, Antonio ROSALES, Angelo SCUDERI, Carl-Erik SLAGSVOLD, Anders THURIN, Tomasz URBANEK, Andre VAN RIJ, Michael VASQUEZ, Cees H. A. WITTENS, Paolo ZAMBONI, Steven ZIMMET, Santiago Zubicoa EZPELETA
Venous hemodynamic changes in lower limb venous disease: the UIP consensus according to scientific evidence
International Angiology 2016 June;35 (3):236-352

2. Saphenous sparing options

Author: S. Gianesini
Tips & Tricks in Angiology.
Minerva 2016, in press

3. Phlebo-lymphedema ulcer management

Author. S. Gianesini, E. Menegatti, P. Zamboni
Lymphedema compendium
Springer 2016 in press

Appendix III

Presentations at societies as PhD candidate

January 2014 –December 2016

1. ***"Short endovenous laser ablation of the great saphenous vein in a modified CHIVA strategy"***: Innovation and excellence in phlebology, Albarella Island (ROVIGO), ITALY, 16-17 may 2014.
2. ***"Perioperative venous thrombo-embolism prophylaxis in general and orthopaedic surgery"***. ARCA meeting, Mestre (VENEZIA), ITALY, october 3, 2014.
3. ***"Laser-assisted saphenous-sparing strategy for reflux abolition"*** American College of Phlebology Annual Meeting, Phoenix, Arizona (USA), 7 novembre 2014.
4. ***"Echo-color-Doppler haemodynamic venous mapping"*** AFI regional meeting, Vicenza, Italy, jan 17, 2015.
5. ***"Lower limbs venous kinetics and consequnt impact on drainage direction"*** American Venous Forum, Palm Springs, California (USA), february 25, 2015
6. ***"Segmental shrinkage for sapheno-femoral reflux suppression"*** Italian Society of Phlebology (SIF), Piacenza (ITALY), march 6 2015
7. "***SFJ laser shrinkage for hemodynamic effect***". Sedona Days Meeting, Sedona (Arizona), USA, march 28, 2015.
8. "***Game Over to Leg Failure: venous hemodynamics in golf players***" DVT awareness days, University of Ferrara, Ferrara, april 15, 2015, ITALY
9. "***DVT: what it is, what it does, what to do***" DVT awareness days, University of Ferrara, Albarella Island, june 13, 2015, ITALY
10. "***Game Over to Leg Failure: venous hemodynamics in golf players***" DVT awareness days, University of Ferrara, Albarella Island, june 13, 2015, ITALY
11. **"*Iliac valve incompetence as a risk factor for sapheno-femoral reflux recurrence".*** XVI European Venous Forum, jul 2-4, 2015, St. Petersburg, RUSSIA.
12. "***Mini-invasive high tie vs traditional high ligation in sapheno-femoral junction refluxes***". International Union of Phlebology, Aug 27-29, 2015, Seoul, SOUTH KOREA.

13. "**Saphenous Sparing Techniques**", American College of Phlebology, Nov 13, 2015, Orlando, Florida, USA.
14. "**High Ligation**", American College of Phlebology, Nov 15, 2015, Orlando, Florida, USA.
15. "**Holmium laser histology**" Sclerotherapy meeting, Feb, 26, 2016, Bologna, Italy.
16. "**Holmium laser histology**" American Venous Forum, Feb, 27th, 2016, Orlando, Florida, USA
17. "**Lower limbs venous scanning**", invited lesson, march 29, 2016, University of Washington, Seattle, USA
18. "***Saphenous sparing techniques***", Venous Updates from the World, may 12th, 2016, University of Ferrara, Italy
19. ***"Varicose veins recurrences"***, Giornate Scannesi, jun 12th, 2016, Scanno (AQ), Italy
20. ***"Innovative surgical technique for lower limbs deep venous insufficiency treatment"*** European Venous Forum, jul 8th 2016, London (UK)
21. ***"Holmium laser for chronic venous disease"*** 3rd Venous Updates from the World, Ferrara, Italy.
22. ***"A specifically designed acquatic protocol for lower limb lymphedema management"*** European Venous Forum, jul 8th 2016, London (UK)
23. ***"Hemodynamic rationale in chronic venous disease treatment"*** Hydrotherapy meeting 2016, Ferrara (Italy)
24. ***"Venous thrombo-embolism guidelines: is it so difficult?"*** University of Ferrara updates, september 26th 2016, Ferrara (Italy).
25. ***"Foam sclerotherapy: tips & tricks"*** Phlebosophy meeting, September 26th, 2016, Venice (Italy).
26. **"Comparison among 18 mmHg vs 23 mmHg graduated elastic stockings in intermittent walking".** International days, University of Ferrara, sept 30th, 2016, Ferrara , Italy.

27. **“Pelvic flow, practical aspects”.** XIV Brazialian Association of Phlebology and Lymphology annual meeting, Sao Paulo, Brazil, oct 12-14, 2016.
28. **“Foam sclerotherapy safety”.** XIV Brazialian Association of Phlebology and Lymphology annual meeting, Sao Paulo, Brazil, oct 12-14, 2016.
29. **“Pelvic reflux treatment”.** XIV Brazialian Association of Phlebology and Lymphology annual meeting, Sao Paulo, Brazil, oct 12-14, 2016.
30. **“Flebogrif and Saphenous sparing: the difference among strategy and technique”.** XIV Brazialian Association of Phlebology and Lymphology annual meeting, Sao Paulo, Brazil, oct 12-14, 2016.
31. **“Saphenous sparing: the difference among strategy and technique”.** V International Inter-university meeting, Buenos Aires, Argentina, oct 20-22, 2016.
32. **“The first standardized acquatic protocol for phlebolymphedema”.** V International Inter-university meeting, Buenos Aires, Argentina, oct 20-22, 2016.
33. **“Venous World Inter-university Network project”.** V International Inter-university meeting, Buenos Aires, Argentina, oct 20-22, 2016.
34. **“Lower limbs venous kinetics impact on drainage direction”.** V International Inter-university meeting, Buenos Aires, Argentina, oct 20-22, 2016.
35. **“Comparison among 18 mmHg vs 23 mmHg graduated elastic stockings in intermittent walking”.** V International Inter-university meeting, Buenos Aires, Argentina, oct 20-22, 2016.
36. **“Holmium laser: histology and ultrasound evaluation”.** V International Inter-university meeting, Buenos Aires, Argentina, oct 20-22, 2016.
37. **“Mini-invasive high-tie vs traditional saphenofemoral ligation”.** V International Inter-university meeting, Buenos Aires, Argentina, oct 20-22, 2016.
38. **“Laser assisted-CHIVA”.** V International Inter-university meeting, Buenos Aires, Argentina, oct

20-22, 2016.

39. **“Game Over to Leg Failure project”.** V International Inter-university meeting, Buenos Aires, Argentina, oct 20-22, 2016.
40. **“Laws of physics in venous drainage”.** American College of Phlebology annual meeting, Los Angeles, USA, nov 3-6, 2016
41. **“Saphenous sparing: technique vs strategy”.** American College of Phlebology annual meeting, Los Angeles, USA, nov 3-6, 2016
42. **“Perforators should be left alone”.** American College of Phlebology annual meeting, Los Angeles, USA, nov 3-6, 2016
43. **“Saphenous sparing: technique vs strategy”.** Day Surgery society meeting, Noventa Vicentina (VI), Dec 17, 2016.

Moderations at societies as PhD candidate

January 2014 –December 2016

1. American College of Phlebology 2015 Annual Meeting, Nov 14, Orlando, Florida, USA.
2. American Venous Forum, discussant, 2016 Annual Annual Meeting, Orlando, Florida, USA
3. V International Inter-university meeting, Buenos Aires, Argentina, oct 20-22, 2016.
4. American College of Phlebology 2016 Annual Meeting, Los Angeles, California, USA.

Appendix IV

Awards as PhD candidate

January 2014 –December 2016

1. ***Travel Grant Award –International Union of Phlebology***

   "Mini-invasive high tie vs traditional high ligation in sapheno-femoral junction refluxes",

   International Union of Phlebology (UIP) chapter meeting

   August 27-29, 2015, Seoul, SOUTH KOREA.

2. ***Best electronic presentation****, European Venous Forum*

   "A specifically designed acquatic protocol for lower limb lymphedema management"

   Royal Society of Medicine, Jul 8th, 2016, London (UK).

3. ***2016 Phlebosophy award****,*

   2016 Phlebosophy meeting, Venice, September 17th, 2016