The role of secondary hemorheological disorders in the pathophysiology of leg ulcers in several clinical conditions

In this brief review, the authors have examined the clinical disorders that may be associated to a condition of secondary hyperviscosity, observed in laboratory, and at times accompanied by the presence of leg ulcers. These leg lesions in fact, besides to be found in patients with primary hyperviscosity disorders (polycythemic, plasma and sclerocythemic), may be present in patients with secondary hyperviscosity, such as diabetes mellitus, arterial hypertension, critical limb ischaemia and chronic venous insufficiency. The leg ulcers are often chronic and refractory, and a marked alteration of the major components of the hemorheological pattern may contribute to aggravating and delaying the healing of these skin complications.

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Previously, the authors examined the clinical conditions responsible for primary and secondary hyperviscosity, and skin ulcers complications (Caimi et al, 2017a), while afterwards, the role of the primary plasma hyperviscosity in the pathophysiology of skin complications was examined (Caimi et al, 2017b), as well as the role of primary sclerocythemic hyperviscosity in skin ulcers (Caimi et al, 2017c).

The aim of this article was to establish the influence of the secondary hyperviscosity disorder, usually found in diabetes mellitus (DM), arterial hypertension, critical limb ischaemia and chronic venous disease which, during their clinical course, can complicate with leg ulcers.

Rheological alterations play a specific role in microcirculation when a potentially ischaemic condition emerges. Some changes develop in microcirculation in relation to the diameter and the wall permeability of microvessels, to the cell metabolism and to the haemorheological profile. Physiologically, the blood flow is related to blood velocity, vessel diameter and structure, and whole blood viscosity; the latter is determined by haematocrit, plasma viscosity, red blood cell (RBC) aggregation and deformability.

Clinical and experimental data have demonstrated that while the erythrocyte aggregation occurs at low shear flow, red cell deformability and plasma viscosity are all significant at high shear flow.

Determinants of blood of rheology

Considering that the values of hematocrit, found in the clinical conditions associated to the secondary hemorheological disorder, are normal, ergo the other hemorheological determinants that must be considered are red cell aggregation, which influences the flow dynamics and, especially, the resistance of blood *in vivo* (Cabel et al, 1997). *In vivo* erythrocyte aggregation arises at low shear forces or stasis and it is the principal determinant of low shear rate blood viscosity (Cabel et al, 1997). Erythrocyte aggregation is a reversible process; the aggregates are dispersed by mechanical or fluid flow forces, however, they reform when the forces are removed. Erythrocyte aggregation is primarily regulated by the intrinsic cell characteristics of RBCs and by the concentration of the macromolecules or of the plasma level of some proteins (Rampling et al, 2004). In blood, fibrinogen is one of the most important component of blood viscosity referable to its effective tendency to increase both plasma viscosity and erythrocyte aggregation (Lee, 1997).

There are two models for erythrocyte aggregation (Neu and Meiselman, 2007): bridging and depletion. In the first, erythrocyte aggregation occurs when the bridging forces, due to the adsorption of macromolecules onto adjoining cell surfaces, exceed the

disaggregating forces (Snabre and Mills, 1985; Brooks, 1988). In the second model, erythrocyte aggregation occurs because of a lower localised protein or polymer near the erythrocyte surface compared to the suspending medium (Bäumler et al, 1996). In both models, disaggregation forces are, respectively, electrostatic repulsion, membrane force and mechanical shearing. To distinguish between the effects of suspending media properties (e.g. protein type and concentration) from those intrinsic to the red blood cell, the term 'aggregability' was suggested to indicate the intrinsic tendency of erythrocyte to undergo aggregation while the aggregation refers to the rate, extent or strength of erythrocyte aggregation in any medium.

Albumin affects 36% of the difference between water and plasma viscosity (both Newtonian fluids), while its participation to the total plasmatic proteins is 60%. Fibrinogen corresponds to only about 4% of the total plasma proteins, while its participation to plasma viscosity is about 22%. The different addition given by several protein fractions to the plasma viscosity may be explained by their molecular size and shape. In fact, the fibrinogen is more asymmetric in comparison with other proteins, as well as the immunoglobulins and the globulins contribute to plasma viscosity to a greater degree than albumin with reference to higher molecular weight (Baskurt, 2007).

Erythrocyte deformability that is dependent on surface/volume ratio, cytosolic viscosity and membrane dynamic properties of RBCs. The human RBC during its 120-day lifespan in the circulation undergoes continuous passive shape changes. In arteries, it responds to shear stress becoming an ellipsoid; in the microcirculation, it has to go through capillaries, which have a transversal diameter that is a third of its own. The RBC has exclusive mechanical properties that make it elastic, able to respond to applied stresses and to undergo wide and reversible linear deformation maintaining constant the area of its membrane surface. An increase of 4% is enough to cause the cellular lysis. This aspect depends on its peculiar structure: its elasticity is the predisposition to maintain its own shape, which depends on the protein composition, while its viscosity is determined by the properties of the lipid structure.

Leukocyte deformability that depends on cytoskeleton and intracellular fluid; the cytoskeleton is composed of three main classes of proteins: actin, microtubules and intermediate filaments. These proteins are involved in the way a cell responds to deformation. It must be underlined that the actin cytoskeleton is not a constant structure and bears sudden rearrangement are pressed for by chemotactic stimuli. These stimuli, *in vivo* and *in vitro*, require a constant and directed cycling between monomeric globular (G-) actin and polymerised filamentous (F-) actin. In polimorphonuclear (PMN) cells, after stimulation there is an early increase in cortical F-actin and this latter causes significant changes in resistance to deformation (Tran-Son-Tay and Nash, 2007). The intracellular fluid is a water solution of enzymes and organic molecules and cellular organelles plus the nucleus. The role of the nucleus in leukocyte deformation has been previously described (Tran-Son-Tsay et al, 1994; Tseng et al, 2004).

Primary hyperviscosity disorders

Primary hyperviscosity may be subdivided into polycythemic, plasma and sclerocythemic (Lowe et al, 1981; Di Perri et al, 1983; Chien and Lang, 1987). The clinical conditions that may be part of polycythemic hyperviscosity are those caused by the bone marrow proliferative states, such as polycythemia, thrombocytemia and leukemia. Those that may be responsible for plasma hyperviscosity are plasma cell disorders (multiple myeloma, Waldenstrom macroglobulinemia, monoclonal gammopathy of undetermined significance-MGUS), cryoglobulinemia, cryofibrinogenemia, dysfibrinogenemia and the connective tissue diseases (systemic sclerosis, rheumatoid arthritis, systemic lupus erythematosus, Sjogren's syndrome). The clinical conditions that may be part of sclerocythemic hyperviscosity are hereditary spherocytosis, thalassemia and sickle cell disease.

Secondary hyperviscosity disorders

DM, arterial hypertension, critical limb ischaemia and chronic venous insufficiency are among the diseases that may cause a secondary hyperviscosity disorder associated to the presence of leg ulcers.

Diabetes mellitus

The authors' previous studies (Caimi et al, 1993a; Hopps et al, 2008; Caimi, 2013) related to this metabolic disease have discovered the presence of an alteration of the haemorheological profile. Increased whole-blood, plasma and serum

viscosity, increased RBC aggregation, and decreased RBC deformability. In addition, investigation using the spectroscopic fluorescence and employing fluorescent probes has found evident alterations of the erythrocytes and the polymorphonuclear PMN membrane rheology (Caimi et al, 1993a; Hopps et al, 2008; Caimi, 2013). The PMN rheology alteration seems to be related to the reduced activity of phosphofructokinase, to the increase in the hexose monophosphate shunt and to the activation of the poliol pathway, which characterises the PMN metabolism of people with diabetes.

Previously, many authors have examined the behaviour of the haemorheological profile in diabetic foot (Karandikar et al, 1994), in diabetic patients with lower-limb arterial ischaemia (Le Devehat et al, 2001) and in people with diabetes of both types with foot gangrene (Mantskava et al, 2006). Some authors believe also that the haemorheological alteration might be a marker of diabetic foot syndrome deterioration (Khodabandehlou et al, 2004b).

The clinical course and the treatment of diabetic foot syndrome (ischaemia, ulcers, gangrene), obtained with fibrinogen adsorption (Koll et al, 2002; Klingel et al, 2003) or with heparin-induced extracorporal LDL precipitation (HELP), improves the prognosis of the diabetic ulcers (Richter et al, 2001; Rietzsch et al, 2008; Weiss, 2012). Diabetic foot syndrome is a complication of long-standing DM; the combination of macro and microvascular disease associated with neuropathy leads to the development of leg ulcers.

A microvascular disease worsens with the increase in plasma viscosity and the decrease in erythrocyte deformability, as observed in DM; the plasma viscosity increase may be explained by the presence of hyperfibrinogenemia.

With DM, the factors influencing RBC deformability are a decreased surface/volume ratio (Jin et al, 2010) related to the sorbitol cytosolic accumulation and to the membrane lipid alterations, increased cytosolic viscosity related to the reduction in the organic phosphates, increased calcium and glycated haemoglobin, and alteration of the membrane dynamic properties related to the qualitative and/or quantitative membrane alterations of lipids and proteins.

In the erythrocyte membrane of diabetic subjects an increase in total cholesterol (Nayak et al, 2008), a decrease in phospholpids (Nayak et al, 2008), an increase in total saturated fatty acids (TS), a decrease in unsaturated fatty acids (TU) and then an increase in TS/TU ratio (Bakan et al, 2006) have been found. The red cell membrane of diabetic subjects shows an increase in protein glycation (Bryszewska and Szosland, 1988), an increase in band 8 and in membrane-bound haemoglobin, such as the presence of aberrant sialoglycoproteins bands (Petropoulos et al, 2007) and a decrease of ankirin concentration (Adak et al, 2008).

Recently, some authors (Rivelli et al, 2012; Nigra et al, 2016) have underlined how in diabetic subjects the reduction in erythrocyte deformability may be explained by the acetylation and translocation of the membrane tubulin (Mem-Tub). Tubulin is an erythrocyte protein that plays a significant structural role and that regulates the functional activity of Na+/K+- ATPase (Amaiden et al, 2012) and Ca2+-ATPase (Monesterolo et al, 2015).

DM is often associated with a thrombocytopathy related to the increased platelet adhesiveness and aggregability. These laboratory findings are present in DM before the development of vascular lesions and depend on poor metabolic control. The increase in platelet aggregation found in people with diabetes worsens the microcirculatory blood flow and slows down the healing of leg ulcers. All these haemorheological and coagulative alterations observed in DM are determining factors of the microcirculatory disorders.

Moreover, it must be underlined that the increase in glycated haemoglobin, besides reducing the erythrocyte deformability, shifts the haemoglobin dissociation curve and diminishes the values of P50; the latter acts negatively on the oxygen transport and contributes to the worsening of the leg ulcers.

Arterial hypertension

Arterial hypertension may be associated with skin ulcers (Martorell's ulcers), that are frequently symmetric and located in the distal third and anterolateral surface of the lower limbs (Graves et al, 2001; Vuerstaek et al, 2010; Franklin and Dissemond, 2011; Alavi et al, 2012; Lima Pinto et al, 2015; Hafner, 2016). Martorell's ulcers are noticeable for their painful red blisters, which soon become blue, purpuric and finally ulcerate. These ulcers may be preceded by pigmented pretibial patches. Pain, relented healing and poor clinical response to standard therapy are a specific sign of Martorell's ulcers. The study of microcirculation shows an increase in resistance of the arterioles associated to a limited compensatory mechanism. Other causal factors in the genesis of these ulcers

may be the alterations in the sympathetic innervation, a persistent arteriolar hypertonia and an abnormal arteriolar vascular response to vasoactive substances.

The impaired haemorheological profile in arterial hypertension observed by our group regards respectively RBCs and circulating PMNs, the latter examined at baseline and after *in vitro* activation with PMA and fMLP (Caimi et al, 1993b, 1997, 2000a; Hopps et al, 2009; Lo Presti et al, 2014), and in the past few years by other authors (Sloop et al, 2015; Radiosinska et al, 2016), may have a role in the clinical course of these ulcers. The same hemorheological alteration may contribute to the organic complications of arterial hypertension, such as left ventricular hypertrophy and hypertensive retinopathy. Another interesting point regards that the abnormalities in hemorheological profile seem more significant in the high-renin than in low-renin hypertensive subjects.

In arterial hypertension, the impairment of tissue oxygenation that accompanies this clinical condition, may have a role in the pathogenesis of leg ulcers (Cicco and Pirrelli, 1999). The impaired tissue oxygenation may depend on the reduction of the erythrocyte deformability found in this clinical condition (Odashiro et al, 2015; Fu et al, 2016). The alteration of this erythrocyte deformability is related to the RBC membrane abnormalities (Pytel et al, 2012; Rodrigo et al, 2014) observed in arterial hypertension even if other authors, recently, retain this alteration referable to the increase of the detyrosinated tubulin observed in the erythrocyte membrane (Amaiden et al, 2014).

Critical limb ischaemia

Some authors (Koenig et al, 1988) describe a positive association between peripheral arterial disease (PAD) and increased plasma viscosity. Ricci et al (2013) found a relationship between plasma viscosity and the increased risk of PAD, while Poredos and Zizek (1996) noted a relationship between the increase in plasma viscosity and the progression of PAD. Other authors (Woodburn et al, 1995) observed a correlation between the fibrinogen level and the presence of symptomatic PAD. In non-diabetic subjects suffering from PAD, whole-blood and plasma viscosity, such as RBC rigidity and aggregation, were significantly higher than in controls (Dupuy-Fons et al, 1995). The same parameters have been evaluated in diabetic subjects with PAD, resulting in significant increases in those who needed major amputation (Dupuy-Fons et al, 1996). Smith et al (1998) noted that the increase in fibrinogen, plasma and whole-blood viscosity in PAD patients resulted in risk factors for a later vascular intervention during a 6-year follow-up. Others (Vigilance and Reid, 2008) have suggested that increased plasma fibrinogen and plasma viscosity might impair the vasodilatation that occurs as a compensatory mechanism in case of hyperviscosity, resulting in a decreased peripheral blood flow.

Some authors studying PAD subjects have described a decrease in whole-blood filterability even if the washed RBCs of these patients did not differ from normal controls. However, in patients with chronic atheromatous ischaemia of the legs, a reduction of the red cell deformability, explored by using the elongation index, has been demonstrated by others (Drodz et al, 2001).

In the authors' haemorheological laboratory, in patients with vascular atherosclerotic disease, an impairment of the rheological parameters of the PMNs has been described (Caimi et al 1996, 1997). The role of the rheological properties of resting and activated leukocytes in the pathophysiology of the microcirculation is well known. Several papers underline that the flow and the rheological properties of circulating PMNs are altered in patients with intermittent claudication (Neumann et al, 1990), in those with critical limb ischaemia (Nash et al, 1988) and patients with acute myocardial infarction (Nash et al, 1989; Caimi et al, 2004), as well as in patients with acute ischaemic stroke (Ciuffetti et al, 1989; Caimi et al, 2000b).

In subjects with non-diabetic and non-hypertensive critical limb ischaemia, the haemorheological alteration seemed to play a pivotal role. However, after arterial reconstruction, no improvement in the haemorheological profile has been observed by some authors (Holmberg et al, 2000). The importance of the hemorheological profile in critical leg ischaemia is determined by the negative effects that blood viscosity and fibrinogen levels have on the intermittent claudication, as well as the negative prognostic significance of haemoglobin levels and of fibrinogen in the healing process of amputated limbs due to critical leg ischaemia. In the latter, there is a break between the microvascular flow and the microvascular defence systems.

A more rapid and evident haemorheological effect may be obtained by the employment of normovolemic hemodilution, pharmacological

defibrinogenation, plasma exchange and rheopheresis (Klingel et al, 2005). In critical leg ischaemia, the pathogenesis of leg ulcers is complex: endothelial injury and PMN and platelet activation, that act on the haemorheological pattern, might be responsible for the further damage in the microcirculation (Danielsson et al 2004; 2006).

Chronic venous insufficiency

In chronic venous disease (CVD), there is an evident alteration of the haemorheological profile. Some authors (Le Devehat et al 1989; 1990; Boisseau et al, 1995; Chabanel et al 1995; Khodabandehlou et al, 2004a) have found a marked increase in fibrinogen, plasma viscosity and erythrocyte aggregation. These anomalies (Le Devehat et al, 1989) were even more marked for the samples drawn from leg veins. During stasis, plasma viscosity, RBC aggregation and haematocrit increased, while RBC deformability decreased; no variation, instead, was observed in normal controls.

Other authors (Chwała et al, 2009) have examined the behaviour of erythrocyte elongation and aggregation in CVD patients, noting that both haemorheological parameters were no different to the control group. In relation to the obtained data, the authors believed that this datum may be explained considering that in the blood circulation of CVD patients, there is an increase of 'young erythrocytes' compared to the 'old' non-elastic ones.

The progression of chronic venous insufficiency can result in venous leg ulcers. (Comerota and Lurie, 2015). Persistent venous stasis associated with increased venous pressure causes leg ulcers. The increase in capillary permeability leads to the extravasation of proteins and fibrinogen from the capillaries. High fibrinogen concentration causes a fibrin cuff composition, blocking the diffusion of nutrients with a microcirculation impairment and subsequent skin necrosis.

In the pathophysiology of venous ulceration, the trapping and activation of PMNs and, in particular, their activation, play a principal role in the development of leg ulcer. Even if PMNs are present in the circulation in only relatively low numbers, they are 2–3 times larger and their deformability is 1,000-times lower than erythrocytes.

In patients with venous ulcers, authors have observed a decreased PMN membrane fluidity and an increased PMN cytosolic Ca++ concentration, especially after *in vitro* activation with PMA and fMLP (Hopps et al, 2014). The same finding has been found by the authors in patients with deep venous thrombosis (DVT) and in patients with post-phlebitic syndrome (Caimi et al, 1999). In patients with venous ulcers, an abnormal response of the PMN beta2-integrins (CD11b, CD11c, CD18), in particular after *in vitro* activation (Lo Presti et al, 2006), as well as in a group of patients with DVT, has also been noted (Caimi et al, 2005). The leukocyte-endothelium interactions, mediated by adhesion molecules, may play a key role in the pathogenesis of chronic venous ulcers. The evolution of chronic venous insufficiency in leg ulcers may be explained by the marked microcirculatory disorder and the functional alterations observed in polymorphonuclear cells (Smith, 2006; McDaniel et al, 2013).

To date, several approaches for the treatment of the venous leg ulcers are employed: electric and electromagnetic stimulation, compression, hyperbaric oxygen, drugs and surgical therapy. Previously, the fibrinogen adsorption was also suggested (Stucker et al, 2003).

Conclusions

Several clinical conditions responsible for the secondary hyperviscosity disorder may be associated with leg ulcers. However, a clear impact of the hemorheological alteration on these ulcers cannot be directly demonstrated. An acceleration of the healing process of leg ulcers, however, has been obtained through pharmacological treatment, plasma exchange or fibrinogen adsorption (rheosorb), which significantly improves the hemorheological profile. WINT

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