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Sclero-Resistance, Sclero-Sensibility and Sclero-Sensitization.

Sclérorésistance, sclérosensibilité et sclérosensibilisation.

Ferrara F.

Summary

Aim: The aim of this study was to assess the importance of the tunica adventitia in sclerotherapy, measuring sclero-resistance, sclero-sensitivity and sclero-sensitization parameters.

Materials and Methods: 900 cases of Great Saphenous Vein (GSV) varicosity treated with Sigg-method sclerotherapy were examined for sclero-resistance as indicated by an ultrasound sclero-resistance marker (SRM+). Sclero-sensitivity was assessed by contact thermography in 356 cases of GSV varicosity with collagen alterations treated with Sigg sclerotherapy. Sclero-sensitization and treatment efficacy were assessed in 50 cases of non-reflux related telangiectasia recurring after a first course of foam microsclerotherapy and retreated with a second sclerotherapy regimen administered together with intradermal glycosaminoglycans (GAG).

Results: Indicative of incomplete parietal fibrosis, the ultrasound marker SRM+ was most frequently found among patients with recurrence. The degree of sclero-sensibility was found to be inversely proportional to proteoglycan concentrations in the Ground Substance (GS), which in turn was seen to present in differing amounts in the various connective tissue diseases. Sclerotherapy combined with the administration of GAGs proved significantly effective in the treatment of recurrent telangiectasia, indicating GAG sclero-sensitizing properties.

Conclusions: The efficacy of sclerotherapy depends on eliciting a regular fibrotic reaction starting from the connective tissue of the adventitia, which is modulated by ground substance GAG metabolism.

Keywords: *sclerotherapy, proteoglycans, thermography, ultrasound.*

Résumé

Objectif : *Le but du travail a été d'étudier le rôle de la tunique adventice dans l'évolution de la sclérothérapie, en évaluant les trois anomalies de la réactivité veineuse: la sclérorésistance, la sclérosensibilité et la sclérosensibilisation.*

Matériel et méthode : *Sur un collectif de 2520 membres (cas), présentant des varices primitives, associées à un reflux tronculaire long et soumis à sclérothérapie, selon la méthode de Sigg, ont été évaluées soit la sclérorésistance, en recherchant la marque échographique MSR, soit la sclérosensibilité en utilisant la thermographie de contact. La sclérosensibilisation, au contraire, a été évaluée par analyse photographique de 50 membres, présentant des télangiectasies récidives, après un premier cycle de microsclérose, et soumis au protocole MTT (microsclérothérapie + injections intradermiques d'un mélange de glycosaminoglycanes GAG)*

Résultats : *Parmi les varicoses saphéniennes sclérosées, l'incidence d'échecs, la plus haute, était significativement à la charge des cas associés à la présence de la marque échographique MSR de sclérorésistance. En ce qui concerne les variations de la sclérosensibilité, ont été relevées des différences significatives de la réactivité à la sclérose, selon la typologie des altérations conjonctivales associées. La sclérosensibilité était élevée dans les patients atteints d'affections, caractérisées par une réduction des protéo-glycanes (GAG + protéines) du tissu conjonctif, comme dans le diabète, la vieillesse et les connectivites ; sa réduction, au contraire, était associée à une augmentation des protéoglycanes, comme dans l'hypothyroïdie. L'injection de GAG (Protocole MTT) a augmenté la sclérosensibilisation des télangiectasies récidivées à la première sclérose.*

Conclusions : *L'efficacité de la sclérose est liée à une fibrose de la paroi veineuse, assurée par le tissu conjonctif de la tunique adventice, son évolution est modulée par la concentration des GAG de la substance fondamentale.*

Mots-clés : sclérothérapie, protéoglycanes, thermographie, écho-Doppler.

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Aim of the study

The aim of the study was to assess the role of the tunica adventitia in sclerotherapy.

Introduction

The Author uses the following definitions [1]:

a. **Sclero-resistance**: the condition whereby the venous wall is refractory to all sclerosants;
b. **Sclero-sensitivity**: the individual reaction to a particular sclerosant of identical concentration and physical form injected using the same method into same-size veins;
c. **Sclero-sensitization**: the variable response of a vein to further sclerotherapy employing the same method of injection and an identical sclerosant of the same physical form and concentration.

Materials and methods

This observational, retrospective study was conducted on two groups of patients:

- 2520 limbs (cases) with primary saphenous varicose veins (C_{2-6}, Ep, As_{2-4}, ± p, Pr), sclerosed with iodate solution according to Sigg technique;
- 50 limbs with telangiectasias not associated with long or short reflux (C1, Ep, $As_{1\pm5}$, Pr) resistant to an initial sclerotherapy course with 0.25-0.5% Polidocanol foam.

Methods

a. ***Sclero-resistance*** was assessed by Echo-Doppler.
At ultrasound the walls of the largest veins (termination of the large and short saphenous) and arteries display a thin line or track at the level of the tunica media. This line is wider than normal (> 0.4 mm) in varicose veins **(Figure 1)**.

In a previous study (2005), the Author suggested that the persistence after sclerotherapy of this anechoic intra-parietal space could be used as a sclero-resistance marker (SRM+) for the persistence of the "medio-intimal line".

This is borne out by the fact that the anechoic line is constantly associated with cases resistant to subsequent sclerotherapy, and is absent in the event of successful treatment (SRM-) [2, 3] **(Figure 2)**.

b. ***Contact thermography*** [3, 4] (a set of plates sensitive to temperatures ranging between 27.5° and 35°C) was used to assess **sclero-sensitivity**.

Thermography provides important information on the function and anatomy of the superficial venous circulation, thanks to the local rise in temperature generated, not only by the presence of blood in the vessels, but also by metabolic activity in the tissues.

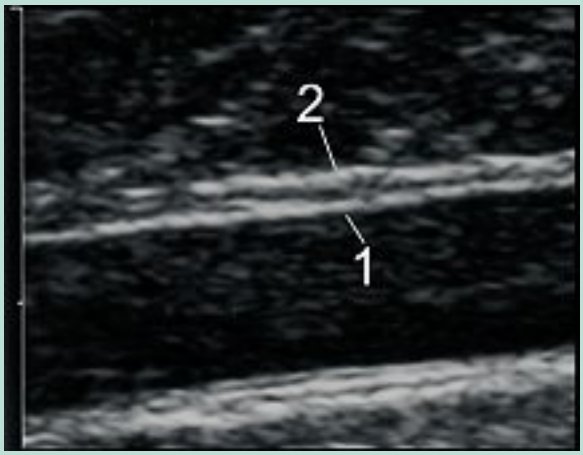


FIGURE 1 : Ultrasound picture of termination of refluxing GSV with evident SRM+ sign: 1 intima, 2 adventitia; the anechoic space between the two is indicative of the media.

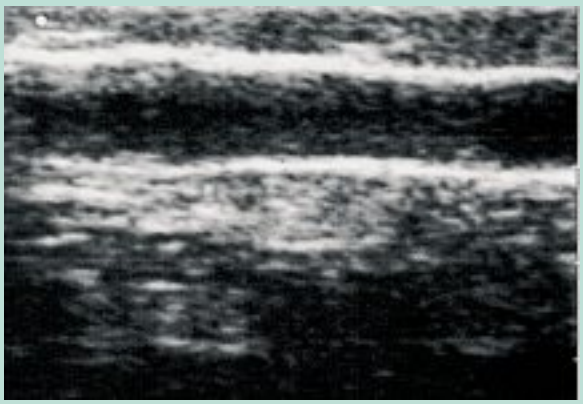


FIGURE 2 : Same case as Figure 1. Ultrasound picture after successful sclerotherapy: venous wall comprises one single echodense thickness (absence of intima and media).

		Success	Failures	Total
MSR+	Count.	102	70	172
	% of Total	11.33%	7.77%	19.11%
MSR-	Count.	690	38	728
	% of Total	76.66%	4.22%	80.88%
Total	Count.	792	108	900
	% of Total	88%	12%	100.0%

TABLE 1 : Sclero-resistance Marker (SRM+) Distribution in Successful and Recurring Sclerotherapy Cases.

Thermography is therefore an important diagnostic tool for phlebitis [5].

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Sclerosensitivity		Groups A	B	C	D	Control	Total
Normal	Count	12	15	8	74	148	257
	% of Total	2.2%	2.7%	1.4%	13.3%	26.6%	46.2%
High	Count	33	56	16	10	45	160
	% of Total	5.9%	10.1%	2.9%	1.8%	8.1%	28.8%
Low	Count	6	4	6	116	7	139
	% of Total	1.1%	0.7%	1.1%	20.9%	1.3%	25.0%
Total	Count	51	75	30	200	200	556
	% of Total	9.2%	13.5%	5.4%	36.0%	36.0%	100.0%

TABLE 2 : Distribution into 4 groups of the 556 sclerosed limbs with different sclero-sensitivity assessed with thermography.

The temperature rises recorded by thermography are indicative of the vein's reaction to a damage-inducing agent that triggers platelet aggregation, inducing the release of vasoactive amines, which in turn cause hyperthermia.

Vasoactive amine production is directly proportional to the inflammatory reaction.

By reducing vein lumen and content, effective sclerotherapy should reduce the HOT-SPOT (H-S) surface area of initial hyperthermia, indicative of varices and/or the thermal gradient with nearby tissue by at least 1.5°C [3, 4].

Heightened sclero-sensitivity will cause an increase of both H-S surface area and temperature. Conversely, unchanged H-S values are indicative of lower sclero-sensitivity.

c. ***Photographs of limbs pre- and post telangiectasia treatment*** were computer processed to assess the degree of **sclero-sensitization** *via* the **1-10 Effectiveness Score (ES)** that had proved useful in a previous study by the Author (2012), providing objective numerical indication of treatment efficacy [6].

Materials

a. 900 of the 2520 cases (C_{2-6}, Ep, As_{2-4}, ± p, Pr) were examined for the prevalence of the SRM+ **sclero-resistance** marker among successful treatment outcomes and patients with recurrence. The statistical analysis was carried out using the chi-square method.

b. 556 of the 2520 cases (C_{2-6}, Ep, As_{2-4}, ± p, Pr) were assessed with thermography, 356 of whom presented severe connective tissue alterations:
- old age > 70 years in 51 cases (**Group A**);
- diabetes in 75 cases (**Group B**);
- collagen disease in 30 cases (**Group C**);
- hypothyroidism in 200 cases (**Group D**).
- The remaining 200 disease-free cases made up the **Control Group**.

Three levels of **sclero-sensitivity** were defined:
- low - characterized by unchanged H-S dt;
- high - increased dt;
- and normal - lower dt.

Table 2 shows the sensitivity attributed to all study groups.

Statistical results were obtained with a contingency table categorical bivariate analysis **(Table 3)**.

c. 50 cases of micro-sclerotherapy resistant telangiectasias (C1, Ep, $As_{1±5}$, Pr) were retreated with the multi-therapy treatment protocol (MTT): 0.25-0.5% Polidocanol foam micro-sclerotherapy combined with intradermal mesoglycan [6].

Effectiveness scores (ES) were calculated for each of the two treatment courses **(Table 4** and **Table 5**; **Figure 3** and **Figure 4)**.

The degree of **sclero-sensitization** induced by the MTT protocol was calculated using Student's t-test for independent data **(Table 6)**.

Photographs were taken using a digital camera with a 50 mm lens mounted on a tripod at always the same height and distance from the patient, who was always standing for the photographs.

Sclerosensitivity inferential statistics		Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square		322.042	8	.000
Likelihood Ratio		322.713	8	.000
Linear-by-Linear Association		25.301	1	.000
Symm. Meas. (approx. Sig.)	Phi	.761		.000
	Cramer's V	.538		.000
	Contingency Coefficient	.606		
N of Valid Cases		556		

TABLE 3 : Inferential statistics of thermography results: statistically significant difference for all sclero-sensitivity levels among Groups A, B, C, D and controls.

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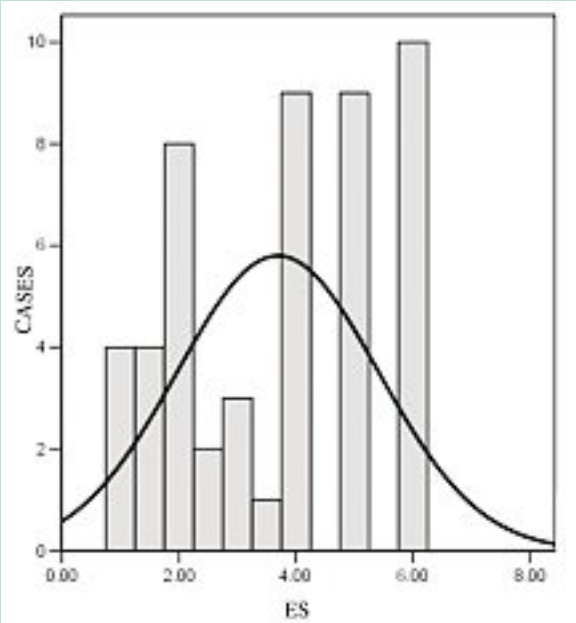


FIGURE 3 : ES scores for sclerotherapy for telangiectasias: frequency histograms on a normal distribution curve (X axis classes; Y axis frequency).

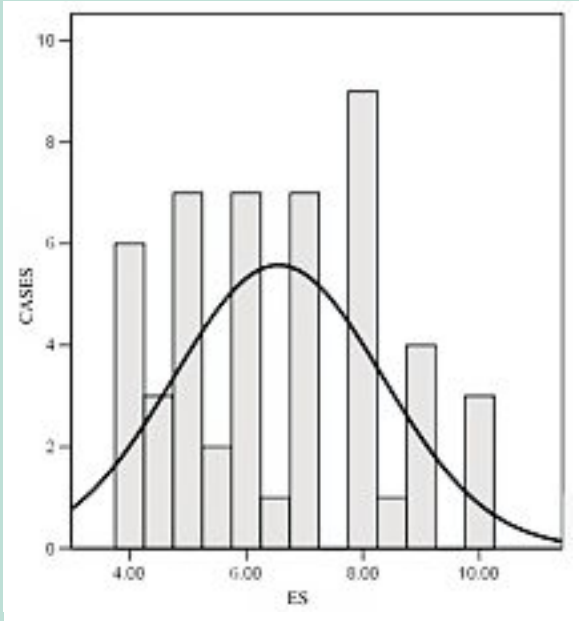


FIGURE 4 : ES scores for MTT for telangiectasias: frequency histograms on a normal distribution curve (X axis classes; Y axis frequency).

Statistics of ES values (sclerotherapy)		Cases 50
ES value min		1
ES value max		6
Mean		3.6900
Median		4.0000
Mode		6.00
Std. Deviation		1.71990
Skewness		–.080
Std. Error of Skewness		.337
Kurtosis		–1.393
Std. Error of Kurtosis		.662
Percentiles	25	2.0000
	50	4.0000
	75	5.0000

TABLE 4 : Descriptive statistics of Effectiveness Score (ES) distribution after first sclerotherapy course for telangiectasias.

Statistics of ES values (MTT)		Cases 50
ES value min		4
ES value max		10
Mean		6.5500
Median		6.2500
Mode		8.00
Std. Deviation		1.78786
Skewness		.233
Std. Error of Skewness		.337
Kurtosis		-.994
Std. Error of Kurtosis		.662
Percentiles	25	5.0000
	50	6.2500
	75	8.0000

TABLE 5 : Descriptive statistics of Effectiveness Score (ES) distribution after MTT for telangiectasias.

Photographs were in black and white and taken under indirect lighting from a 650W / 30000K halogen lamp whose beam was directed at a 450 angle against a light-colored gray wall.

The aim was to ensure that all photographs, whether before or after treatment, were always taken under exactly the same conditions.

4 photographs were taken for each limb: front, back and the two sides, and processed.

Median overall luminosity for each limb silhouette was obtained by means of the Histogram function of Photoshop software.

Increased luminosity values following treatment were quantified on the above-mentioned visual analogue scale from 0 (no response) to 10 (absence of telangiectasia) and considering each point on the score as corresponding to 2 points on the median luminosity reading.

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One-Sample Test	Test Value = 3.69					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
ES of MTT	11.311	49	.000	2.86000	2.3519	3.3681

TABLE 6 : One-simple Test. Statistically significant difference between median ES values after the first sclerotherapy course and after MTT treatment of telangiectasias.

The average score for the 4 photographs per limb was taken as the overall effectiveness score (ES) for each limb **(Table 6)**. Statistical analysis was carried using Student's t test for independent data **(Table 6)**.

Results

a. The **sclero-resistance** ultrasound marker (SRM+) was higher in cases of recurrence compared to successfully treated patients, with a statistically significant difference of $\chi 2$ 162.49 (corr. Yates); $p < 0{,}001$; odds ratio : 12.46.

b. **Sclero-sensitivity** was seen to be higher in Groups: A (Elderly), B (Diabetes) and C (Collagen Disease) compared to the control group, the difference being statistically significant ($p < 0.001$), but lower in Group D (Hypothyroidism) **(Table 3)**.

c. MTT (sclerotherapy combined with intradermal mesoglycan) was seen to be significantly ($p < 0.001$), more effective than sclerotherapy alone in treating recurrent telangiectasias **(Table 6)**. MTT was therefore seen to have a **sclero-sensitizing** effect.

Comment

a. The statistically significant association between recurrent telangiectasias and persistence of the anechoic "medio-intimal line" at ultrasound confirms the reliability of this marker (SRM+).

On successful sclerosis, the vein no longer displays this anechoic sign between the intima and the adventitia but rather appears as a single ecodense structure **(Figure 2)**.

The vein wall reaction to sclerosis is excellent when the tunica media is also involved in the fibrotic reaction. If, on the other hand, it remains unaffected, preserving its elastic muscular elements, recurrence is likely.

Histological studies have shown that the only tunica still recognizable after successful vein sclerosis is the adventitia, which is hyperthermic and vascularized with connective tissue offshoots reaching into the treatment-induced thrombus [7].

b. **Sclero-sensitivity** is significantly influenced by connective tissue pathophysiology.

Venous connective tissue comprises three components:
- cells,
- fibers,
- and **amorphous ground substance (GS)**.

GS seems to play a preponderant role in sclero-sensitivity on account of its three functions:
- trophic (allowing nutrient exchange between blood and cells);
- defensive (its viscosity hinders the spread of bacteria);
- and mechanical (GS cements collagen and elastic fibers) [8].

GS is a multiple-phase colloidal system formed by a dispersing aqueous phase in which various electrolytes are dissolved, and a dispersed phase comprising enzymes, non-structured glycoproteins and proteoglycans.

Proteoglycans are made of a protein axis enveloped by negatively charged **glycosaminoglycan (GAG)** ramifications that attract the positive pole of the dipolar water molecule (Van der Waals forces).

Their ability to fix water confers compactness and elasticity to connective tissue [9]. Increased sclero-sensitivity is associated with diseases entailing lower proteoglycans counts, such as diabetes [10] and old age [11], but also collagen diseases.

It has been suggested that collagen conditions are triggered by underlying abnormal cell homeostasis caused by non-enzymatic glycosylation of proteoglycans [12], a metabolic failing causing lowered proteoglycans counts frequently found in aging and diabetes [13].

Conversely, hypothyroidism, which is associated with lower sclero-sensitivity, has been shown to present increased concentrations of proteoglycans in the ground substance [14, 15, 16, 17].

c. The greater efficacy of MTT treatment in treating telangiectasias is related to the sclero-sensitizing effect of mesoglycan, a mix of sulfate GAGs (Chondroitin sulphate 8.5%, Dermatan sulphate 35.5%, Heparan sulphate 47.5% and Heparin slow 8.5%).

In a previous study (2013), the Author demonstrated that the connective tissue stimulation achieved with just intradermal injection of mesoglycan led to more effective sclerotherapy results when treating telangiectasias of a diameter of < 0.3 mm [6].

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Since these small veins have no tunica adventitia they are structurally similar to capillaries, the connective tissue of the dermis providing the support normally ensured by the tunica adventitia.

By strengthening the GS, mesoglycan restores the wall support function in telangiectatic vessels. GS chemical-physical properties depend on the degree of long chain GAG polymerization.

Moreover, fibroblasts are surrounded by a viscous gel environment comprising highly polymerized GAGs that affect both cell kinetics and metabolism [18].

Following sclerotherapy fibroblast reactivity, in both the inflammatory and proliferative phases, is therefore also impacted also, by the chemical-physical balance existing in the pericellular ground substance.

GAG administration modulates fibroblast inflammatory and proliferative response, increasing sclero-sensitization on account not only of the direct action on the interstitial component of the adventitia but also for its impact on the cell.

Conclusions

The pathophysiology of venous and arterial intima has been well documented.

However, unlike arteries, veins have a thinner media layer and a more developed tunica adventitia whose important nutritional and support functions are sustained not only by the vasa vasorum and its lymphatic system but also by its particular connective tissue that contains a prevalence of Type III collagen and ground substance [19].

Three key conclusions may be drawn from this study.

a. **Sclero-resistance** results show that unsuccessful sclerotherapy is associated with a failure of the intima to weld to the tunica adventitia, which, when sclerosis is successful, form a single vein wall thickness.

b. **Sclero-sensitivity** is considerably affected by the pathophysiology of the connective tissue of the tunica adventitia.

c. **Sclero-sensitization** is affected by GAG administration. It follows that GS is the most important connective tissue component, accounting for the role played by the adventitia in the effectiveness of sclerotherapy.

This claim is confirmed by the association of low-concentration proteoglycans with increased sclero-sensitivity (in diabetes, aging and collagen disease) and conversely by the lower sclero-sensitivity reported in the presence of higher proteoglycan counts (in hypothyroidism).

The tunica adventitia therefore has a major role in sclerotherapy whose effectiveness may be improved by modulating the amorphous ground substance with the administration of sulfate GAGs.

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