



Histology of saphenous veins after treatment with the ClariVein® device – an ex-vivo experiment

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Summary

Background: Endovenous treatment modalities are used increasingly to treat varicose veins. The ClariVein® catheter is a new endoluminal mechanico-chemical obliteration technique which can be used without tumescent anesthesia. It is still unclear what changes the mechanical tip of the catheter has on the walls of the vein.

Patients and methods: Five great saphenous vein specimens were obtained atraumatically by crosssection. Then the veins were treated ex vivo with the ClariVein® catheter without sclerotherapy. The activated catheter rotating tip (3 500 U/min) was steadily withdrawn at 1–2 mm per second. Subsequently, histological and immunohistochemical investigations of treated (cv) and untreated specimens (plain) were performed. A 4-point score was calculated to compare the results.

Results: The mechanical part of the catheter caused a subtle incomplete destruction of the endothelium (endothelium cv: 2.2 vs. plain: 1, $p = 0.04$). Changes in the media or adventitia were not seen. Immunohistochemical presentation of the endothelium of the intima was demonstrated with antibodies against CD31 (cv: 3.4 vs. plain: 2.8), CD34 (cv: 3.8 vs. plain: 3.2) and factor VIII (cv: 2.2 vs. plain: 1, $p = 0.004$).

Conclusions: The mechanical part of the ClariVein® catheter caused a subtle incomplete destruction of endothelium, which was confirmed histologically and immunohistochemically. The reduced expression of factor VIII in the treated vein could be caused by the release of preformed factor VIII granules due to the minimal mechanical irritation.

Introduction

In recent decades, the spectrum for the treatment of insufficient great saphenous veins has broadened greatly [1]. Minimally invasive techniques are increasingly used to treat insufficiency of the great saphenous veins. Methods include thermal ablation techniques (laser or radiofrequency ablation) and chemical ablation such as liquid or foam sclerotherapy [2]. With endoluminal techniques, there is thrombosis and thus transformation of the treated vein into a fibrous cord.

Endothermal ablation techniques require considerable analgesia, usually in the form of tumescent anesthesia. In addition to the sometimes long learning curve for the surgeon, treatment takes a relatively long time. Also, for thermal methods a

generator must be used, adding to the cost of such procedures [3]. Foam sclerotherapy is more effective than liquid sclerotherapy in the treatment of venous insufficiency, but the occlusion rate is > 76 % after one year [4].

A new endoluminal mechano-chemical treatment option (ClariVein®, Vascular Insights, Madison USA; sales in Germany: ab medica Deutschland GmbH & Co. KG, Düsseldorf, Germany) has been developed to improve upon the negative aspects of thermal ablation (tumescent anesthesia, generator costs) and ultrasound-guided foam sclerotherapy (lower efficacy). Vein closure is accomplished with endovenous mechanical damage and then sclerotherapy. Preliminary clinical use in studies with 29 [5] and 25 [6] patients with varicosity of the great saphenous vein has shown occlusion rates of 97 % after 260 days.

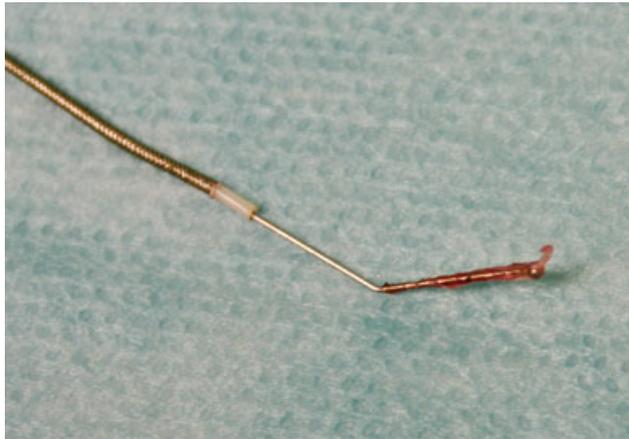


Figure 1 Leftover fibers on the tip of the catheter after treatment.

In our own experience with the ClariVein® technique, we found that at the end of treatment there was residual tissue on the catheter tip (Figure 1). Yet it is unclear what effects the rotating catheter (Figure 2) has on the vein. An understanding of the effects of intraluminal techniques, especially thermal techniques, has been increased significantly by morphological studies [7–10]. At present, there are no published morphological studies on the ClariVein® technique. The aim of this single center ex vivo study was to examine the histological changes caused by the mechanical portion of the ClariVein® system.

Materials and methods

Five patients (4 women, 1 man) underwent a cross-section and stripping operation for atraumatic removal near the junction of 8–10 cm of an insufficient great saphenous vein (VSM). Ultrasound studies measured a preoperative diameter 3 cm distal to the saphenofemoral junction of the insufficient veins of 8.2 mm (6.5–11 mm). The dissected portions of the VSM were stored in a physiological saline solution. After about 10 minutes, a 1 cm long portion of the vein was cut off (reference sample, referred to as “plain” in the remainder of the text) and sent for histological processing. The remainder of the vein (referred to as “cv”) was treated ex vivo with the mechanical portion of the ClariVein® catheter. The catheter was withdrawn at a rate of 1–2 mm per second.

The ClariVein® catheter is an intravascular infusion and catheter system for administering intravenous fluids which has a rotating catheter tip. The tip rotates at about 3,500 U/minute and causes irritation of the intima as well as vasospasms in vivo. The sclerosing agent is delivered below the rotating tip to the wall of the vein and dispersed evenly. After placing the catheter on the module, the wire is advanced out of the catheter sheath about 2 cm into the lumen. The rotating wire is flexible and is made of stainless steel (304 V). The module consists of a

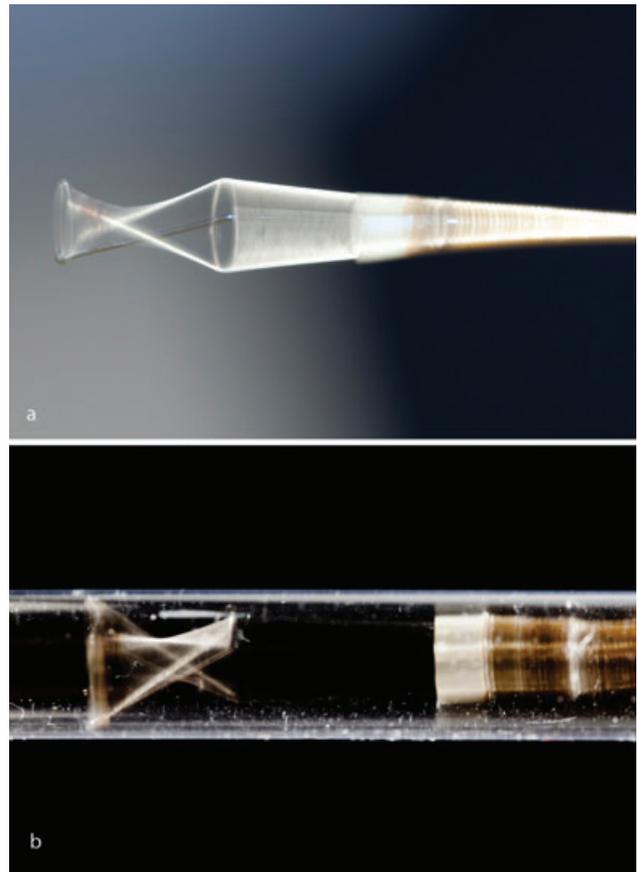


Figure 2 Ex vivo picture of the rotating tip of the catheter. The rotating wire demonstrates a “hourglass-like profile” in air (a) and also within the lumen of a synthetic tube (b).

battery pack (9 V DC) to provide the rotary action and a grip to hold the needle in place.

The 5 venous segments were treated with the rotating catheter tip at ambient temperature (21 °C). Each ex vivo treatment was performed once. After treatment, the veins were sent for histological processing. The treated and untreated vein segments, which were placed in 4 % buffered formalin, were embedded in paraffin and then evaluated as hematoxylin & eosin (H&E) stained tissue sections under microscopy. Also, immunohistochemical staining of the endothelium was performed with antibodies to CD31 (CD31 monoclonal antibody, SIG-3632, Covance Laboratories GmbH, Germany), CD34 (CD34 monoclonal antibody, PN IM0786 Beckman Coulter GmbH, Germany), and factor VIII (factor VIII-related antigen monoclonal antibody, SIG-3114, Covance Laboratories GmbH, Germany). CD31 is a marker for endothelial cells [11]. CD34 is a glycosylated transmembrane protein that is expressed by hematopoietic stem cells and vascular endothelial cells [12]. Factor VIII is a clotting factor and a glycoprotein. It

Table 1 Summary of the evaluation of H&E staining and immunohistochemical staining.

Score: Endothelium		
1	Normal complete endothelium	
2	>50 % <100 % depiction of endothelium	
3	>10 % <50 % depiction of endothelium	
4	<10 % depiction of endothelium, none	
Score: Immunohistochemical staining		
1	100 %	Very strong reactivity
2	>50 % <100 %	Strong reactivity
3	>10 % <50 %	Reduced reactivity
4	<10 %, absent	Absent, very low, or no reactivity

may be identified using immunohistochemistry in endothelial cells, tumor cells of endothelial origin, megakaryocytes and thrombocytes [13].

The histological sections were examined under light microscopy (Olympus BX41TF, Hamburg, Germany) using 10x, 40x and 200x objectives. The preparations were evaluated by an independent assessor. To measure the extent of the endothelial damage (Table 1) in a hematoxylin and eosin (H&E) section, we used the following classification: 1: complete depiction of the endothelium; 2: >50 % and <100 % depiction of the endothelium; 3: >10 % and <50 % depiction of the endothelium and 4: <10 % endothelium or none. Immunohistochemical reactivity (Table 1) was evaluated as follows: 1: very strong reactivity; 2: strong reactivity (>50 % and <100 %); 3:

reduced reactivity (>10 % and <50 %) and 4: very low or none (<10 %).

The study was approved by the local ethics commission (AZ 162-12).

The data were analyzed descriptively. Means and ranges were used. A t-test was used to calculate the differences between treated (cv) and untreated (plain) veins. The significance level was $p \leq 0.05$.

Results

The average patient age (4 women, 1 man) was 50.2 years (27–68 years). All of the plain preparations had a complete endothelium in the histological H&E sections; the total score was 1 (Table 2). The intima, media, and adventitia were completely intact.

The cv preparations had intermittent endothelial damage. A few sections had more damage while others had hardly any at all. The total score was 2.2. There was no evident damage to the media or adventitia by the catheter. There was a significant difference ($p = 0.004$) in endothelial damage between the two groups.

The immunohistochemical studies of endothelial cells using CD31 led to a total score of 3.4 in the cv group. For plain specimens, immunohistochemical staining for CD31 resulted in a total value of 2.8 and thus these were also not consistent ($p = 0.178$). Similar results were found for the intima of the cv group with staining for CD34, with an average total score of 3.8 and thus low reactivity. Among the plain specimens, the endothelial cells of the intima exhibited reduced reactivity (total score: 3.2, $p = 0.07$). There was strong immunohistochemical staining of the endothelial cells of the intima for

Table 2 Summary of the results of the evaluation of H&E staining and immunohistochemical (IHC) staining. Hematoxylin and eosin (H&E), vein segment with ex vivo treatment with the rotating wire (CV), vein segment without catheter treatment (plain).

Patient	Treatment	H&E	CD31 IHC	CD34 IHC	FVIII IHC
1	CV	2	4	4	2
	Plain	1	3	3	1
2	CV	2	3	4	3
	Plain	1	2	4	1
3	CV	3	4	4	2
	Plain	1	4	4	1
4	CV	2	3	3	2
	Plain	1	3	3	1
5	CV	2	3	3	2
	Plain	1	3	2	1
Mean total	CV	2.2	3.4	3.8	2.2
	Plain	1	2.8	3.2	1

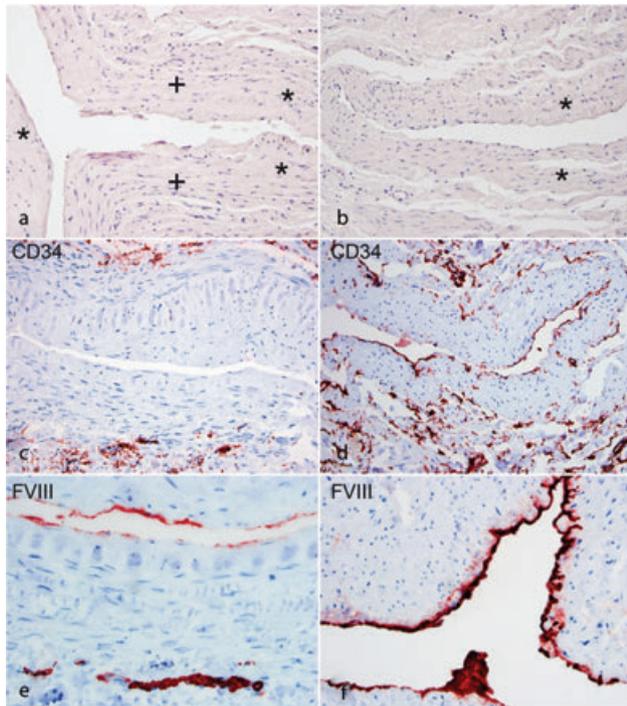


Figure 3 Illustration of hematoxylin and eosin (H&E) and immunohistochemical (IHC) staining. Vein segment after treatment with the ClariVein® catheter; (H&E staining, 1 : 200x) specific endothelial lesions (+) alternating with continuous endothelium (*) (a); vein segment without treatment (H&E staining, x 1 : 200x) continuous endothelium (*) (b); vein segment after treatment with the ClariVein® catheter (IHC anti-CD34 AK, 1 : 200x) (c); vein segment without treatment (IHC anti CD 34 AK, 1 : 200x) (d); vein segment after treatment with the ClariVein® catheter (IHC anti-factor VIII, 1 : 200x) (e); vein segment without treatment (anti-factor VIII IHC, 1 : 200x) (f).

factor VIII (score: 1) in the plain specimens. A significantly lower reactivity was seen in the cv group (total score: 2.2) ($p = 0.004$). In both groups, immunohistochemical staining of the endothelial cells in the intravascular and perivascular vessels showed marked reactivity to CD31, CD34, and factor VIII.

Discussion

The present study shows that the mechanical portion of the ClariVein® catheter causes subtle, incomplete destruction of the endothelium. No changes to the media or adventitia were observed as a result of mechanical catheter placement (Figure 3). In *ex vivo* studies, the mechanical portion of the catheter merely causes damage to the endothelium, unlike endoluminal thermal methods [14]. Use of the RFITT method (radiofrequency-induced thermotherapy) on histo-

logical sections has shown that comprehensive tissue destruction with wall defects and intramural hemorrhage can occur. With endoluminal laser treatment, especially with use of low-frequency lasers and a bare fiber, postoperative ecchymosis, pain, and phlebitis can occur. Given varying energy delivery, this may lead to transmural destruction of the vessel. Such lesions are visible on histology after *in vitro* therapy [9]. In a pilot study by Elias and colleagues [4] with the ClariVein® technique, 3 out of 29 patients developed ecchymosis. A possible explanation may be that the mechanical catheter tears out a valve in the vein, injuring the vessel wall, and ultimately leading to hemorrhage around the vessel.

With the induction of an endothelial injury by sclerosing agents (for example, polidocanol), there is activation of the clotting system with formation of thrombin and development of a thrombus with subsequent vessel occlusion [15].

The lower expression of factor VIII in the treated veins compared with untreated segments may be due to release or preformed factor VIII granules due to mechanical irritation. In an *in vivo* situation, a sclerosing agent is administered while the catheter is being withdrawn. In Germany, polidocanol (Aethoxysklerol®) is approved for use in the following concentrations: 0.25 %, 0.5 %, 1 %, 2 %, and 3 % for sclerotherapy of varices [16]. Early treatment failure after sclerotherapy is caused by recanalization of the treated vessel segment. An incomplete thrombus with residual islands of endothelial cells may cause recanalization. Mc Arree and colleagues [17] have shown that polidocanol causes endothelial damage, but that the damage is incomplete. Additional mechanical as well as chemical endothelial damage both could complete the endothelial damage and force the formation of a desired thrombus across the entire lumen. It is conceivable that the rotation could produce a more even distribution of the sclerosing agent on the vessel wall. Further studies, especially long-term investigations, are needed to confirm this *in vivo*.

Limitations: The present investigation was an *ex vivo* study with a relatively small number of vein specimens. Given the absence of perfusion, lacking surrounding tissue, and the absence of vasospasms, the *ex vivo* surroundings are vastly different from an *in vivo* situation. The preoperative diameters of the veins as well were of varying sizes. This makes the comparison of the *ex vivo* and *in vivo* situations difficult. In addition, there are also ethical limitations on endoluminal treatment with the mechanical portion of the ClariVein® catheter and subsequent vein stripping operations *in vivo*. An advantage of this *ex vivo* study is the simple assessment of new endoluminal techniques for the treatment of varices.

To summarize, the mechanical portion of the ClariVein® catheter has been shown to cause subtle incomplete destruction of the endothelium. No changes to the media or adventitia were observed due to the mechanical catheter placement.

The reduced expression of factor VIII in the treated veins may be caused by the release of preformed factor VIII granules as a result of mechanical irritation.

Conflict of interest

The study was initiated and performed at the Department of Dermatology at the University of Leipzig. A ClariVein® catheter was provided for the purposes of the study by ab medica Deutschland GmbH & Co. KG, Düsseldorf, Germany.

Part of the study was presented at the 54th annual conference of the German Society of Phlebology in Lübeck (21/09/12).

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References

- Rasmussen LH, Lawaetz M, Bjoern L, Vennits B, Blemings A, Eklof B. Randomized clinical trial comparing endovenous laser ablation, radiofrequency ablation, foam sclerotherapy and surgical stripping for great saphenous varicose veins. *Br J Surg* 2011; 98: 1079–87.
- Kandler M, Fellmer PT, Wetzig T. Varicose vein surgery. *J Dtsch Dermatol Ges* 2012; 10: 157–62.
- Almeida JJ, Kaufman J, Göckeritz O, Chopra P, Evans MT, Hoheim DF, Makhoul RG, Richards T, Wenzel C, Raines JK. Radiofrequency endovenous ClosureFAST versus laser ablation for the treatment of great saphenous reflux: a multicenter, single-blinded, randomized study (RECOVERY study). *J Vasc Interv Radiol* 2009; 20: 752–9.
- Hamel-Desnos C, Allaert FA. Liquid versus foam sclerotherapy. *Phlebologie* 2009; 24: 240–6.
- Elias S, Raines JK. Mechanochemical tumescentless endovenous ablation: final results of the initial clinical trial. *Phlebologie* 2012; 27: 67–72.
- van Eekeren RR, Boersma D, Elias S, Holewijn S, Werson DA, de Vries JP, Reijnen MM. Endovenous mechanochemical ablation of great saphenous vein incompetence using the ClariVein device: a safety study. *J Endovasc Ther* 2011; 18: 328–34.
- Kandler M, Helbig D, Averbek M, Simon JC, Wetzig T. Histologie einer Rezidivvarikose nach Behandlung mit dem Radiofrequenzverfahren. *Phlebologie* 2011; 40: 217–20.
- Reich-Schupke S, Mumme A, Stücker M. Histopathological findings in varicose veins following bipolar radiofrequency-induced thermotherapy – results of an ex vivo experiment. *Phlebologie* 2011; 26: 69–74.
- Proebstle TM, Sandhofer M, Kargl A, Gül D, Rother W, Knop J, Lehr HA. Thermal damage of the inner vein wall during endovenous laser treatment: key role of energy absorption by intravascular blood. *Dermatol Surg* 2002; 28: 596–600.
- Schmedt CG, Sroka R, Steckmeier S, Meissner OA, Babaryka G, Hunger K, Ruppert V, Sadeghi-Azandaryani M, Steckmeier BM. Investigation on radiofrequency and laser (980 nm) effects after endoluminal treatment of saphenous vein insufficiency in an ex-vivo model. *Eur J Vasc Endovasc Surg* 2006; 32: 318–25.
- Longacre T, Rouse RV. CD31. A new marker for vascular neoplasia. *Advances in Anatomic Pathology* 1994; 1, 16–20.
- Natkunam Y, Rouse RV, Zhu S, Fisher C, van De Rijn M. Immunoblot analysis of CD34 expression in histologically diverse neoplasms. *Am J Pathol* 2000; 156: 21–7.
- Sehested M, Hou-Jensen K. Factor VIII related antigen as an endothelial cell marker in benign and malignant diseases. *Virchows Arch A Pathol Anat Histol* 1981; 391: 217–25.
- Zierau U, Popp A, Lahl W, Sell M. Histology of a varicose great saphenous vein treated with RFITT. *Phlebologie* 2009; 38: 71–5.
- Wollmann JC. Schaum – Zwischen Vergangenheit und Zukunft. 8. Bonner Venentag 2002. *Vasomed* 2002; 16: 34–5.
- Rabe E, Gerlach H, Breu F. X, Guggenbichler S, Stücker M, Pannier F. Leitlinie: Sklerosierungsbehandlung der Varikose der Deutschen Gesellschaft für Phlebologie (ICD 10: I83.0, I83.1, I83.2, I83.9). *Phlebologie* 2012; 4: 206–13.
- McAree B, Ikponmwosa A, Brockbank K, Abbott C, Homer-Vanniasinkam S, Gough MJ. Comparative stability of sodium tetradecyl sulphate (STD) and polidocanol foam: impact on vein damage in an in-vitro model. *Eur J Vasc Endovasc Surg* 2012; 43: 721–5.