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Histological damage of saphenous venous wall treated *in vivo* with radial fiber and 1470 nm diode laser

G. SPREAFICO [1], R. GIORDANO [2], A. PICCIOLI [1], G. IADEROSA [2], P. PAVEI [1], E. GIRALDI [1], M. FERRINI [1], A. NOSADINI [1], U. BACCAGLINI [1]

[1]*Center of Multidisciplinary Day Surgery, University Hospital of Padua, Padua, Italy*
[2]*Unit of Pathological Anatomy, Local Health Unit N. 13, Dolo, Venice, Italy*

***Aim*. Aim of this study was to evaluate the histological damage to the saphenous trunk after a 1470nm diode laser treatment performed by using a radial fiber.**
***Methods*. At the end of an endovenous laser procedure, five patients underwent a biopsy of a segment of the saphenous trunk, including a treated as well as a non-treated part of the vein. Such segments were all colored with Hematoxylin-eosin, and some of them also with Azan Mallory, Trichrome Masson, Van Gieson, Weigert, Alcian and Alcian-PAS.**
***Results*. Through microscopic analysis, the intimal layer was recognizable around all the vessel circumference, though with clear signs of coagulative necrosis also detectable in the tunica media, at a progressively deeper location correlated with the amount of delivered energy. Inside the tunica media, cavities and fissures were present. No histological signs of carbonization, vaporization or perforation were observed either on the intimal or media layers. All the described damage was widespread, uniform and constant around the whole vein wall circumference.**
***Conclusion*. Endovenous treatment of the saphenous trunks with a 1470 nm laser and radial fiber causes no contact damage. Vice versa, this procedure results in deep and uniform coagulation injury getting deeper as the delivered energy increases. The presence of cavities and separations in the tunica media suggests that a significant dose of energy is absorbed by the water of this layer and transformed into vapour, hence creating both thermal and mechanical damage.**

KEY WORDS: Lasers - Histology - Optic fiber technology.

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Corresponding author: G. Spreafico, MD, Center of Multidisciplinary Day Surgery, University Hospital of Padua, Ospedale Giustinianeo, via Giustiniani 2, Padua, Italy. E-mail: spreaficog@libero.it

Since 2001, when endovenous laser started to be used in the treatment of lower limbs varices,[1] a lot of clinical experience has confirmed both efficacy and safety of the procedure.[2, 3]

Over these years materials employed in the laser field as well as optical fiber have increasingly been developed.

In addition to 810, 940 and 980 nm lasers, which have hemoglobin as chromophore, a 1470 nm laser with water as chromophore is now available. In the same way, besides flat fibers with frontal light emission, a radial fiber with 360° lateral light emission has been devised.[4]

The use of a 1470 nm laser with a radial fiber has significantly changed the postoperative course, resulting in less pain and less ecchymosis.[5, 6]

This evidence suggests that the mechanism of action and the type of damage to the venous wall are different from those obtained with a 980 nm laser and flat fibers, the patterns of which were detailed in an article published in 2006.[7]

Materials and methods

Five patients, two women and three men with a mean age of 47 years, presenting great saphenous vein insufficiency, CEAP C2 Ep, and without previous phlebitis or sclerotherapy, gave informed consent for the biopsy of a segment of the saphenous trunk at the end of the endovenous laser procedure carried out with radial fiber and a 1470 nm laser.

Endovenous laser ablation was performed on an ambulatory basis with tumescent anesthesia, with a 1470 nm diode laser (CERALAS E 15 Watt- Biolitec Ag, Germany) and a radial fiber (Elves PL – Biolitec Ag, Germany). Laser was set in a continuous mode with 5-8 watts of power and delivered energy between 50 and 100 J/cm.

At the end of the procedure, a segment of the vein containing a treated as well as a non-treated part was taken with the help of an exovenous Mayo stripper.

The taken vein was immediately fixed with formalin.

Multiple specimens were taken from both the treated and the non-treated segment.

Sections were colored with hematoxylin eosin, Azan-Mallory, trichrome Masson, Van Gieson, Weigert, Alcian and Alcian-PAS.

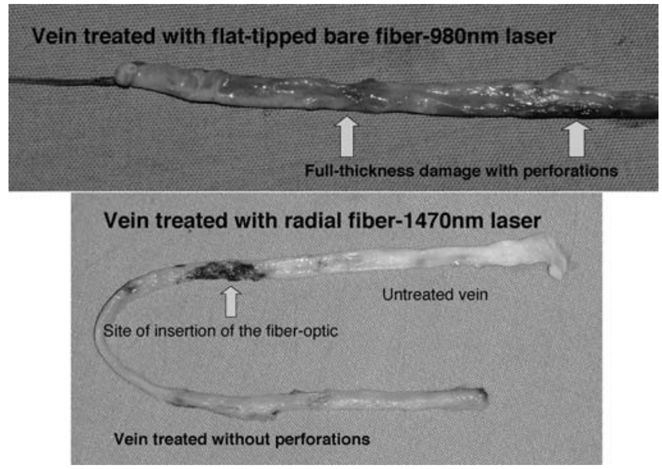

Figure 1.—Macroscopic appearance of a saphenous vein segment treated with flat-tipped bare fiber and 980nm laser showing perforations (top), and with radial fiber-1470nm laser, without any perforations (bottom).

Results

The macroscopic aspect of the treated segments was constant (Figure 1). The vein appeared as a not compressible, not collapsable solid cord, with a thickened wall, grey-colored, opaque and with a reduced caliper when compared with the non-treated wall and with a gummy consistency.

In the adventitial side the vein lost the typical pink color and appeared grayish-white (Figure 2). The endothelium was dull yellowish with some residual coagulated blood.

No signs of carbonization or perforation were observed, either on the endothelial or adventitial side.

Through microscopic analysis (Figure 3), the tunica intima was recognizable around all the vessel circumference, even though the cell nuclei were pyknotic with marked eosinophilia of the tissue. In the tunica intima, no signs of carbonization or vaporization were present. Between intima and media layers, fissures of separation were sometimes present.

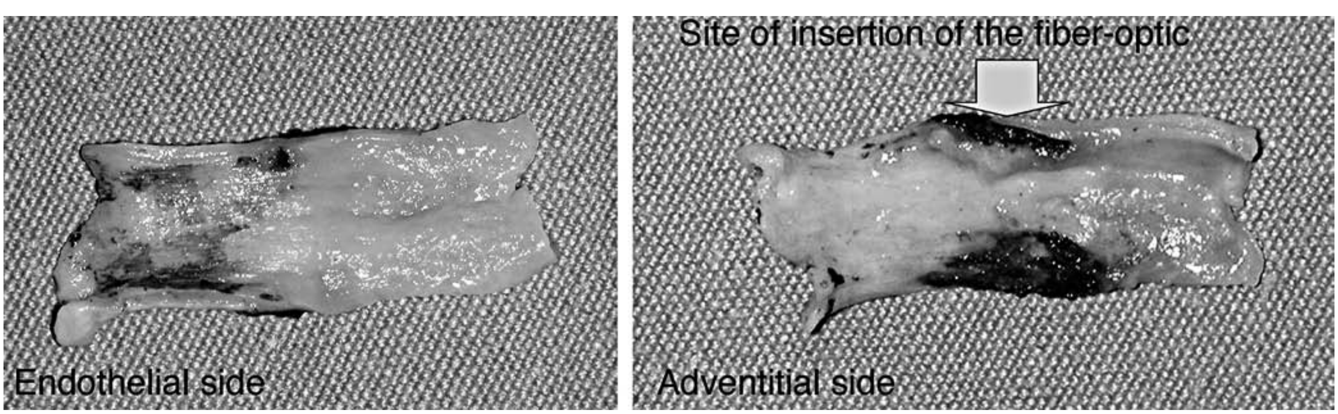

Figure 2.—Macroscopic appearance of a segment of a saphenous vein treated with radial fiber-1470 nm laser (left side of the specimen), untreated on the right side. Endothelial and adventitial views.

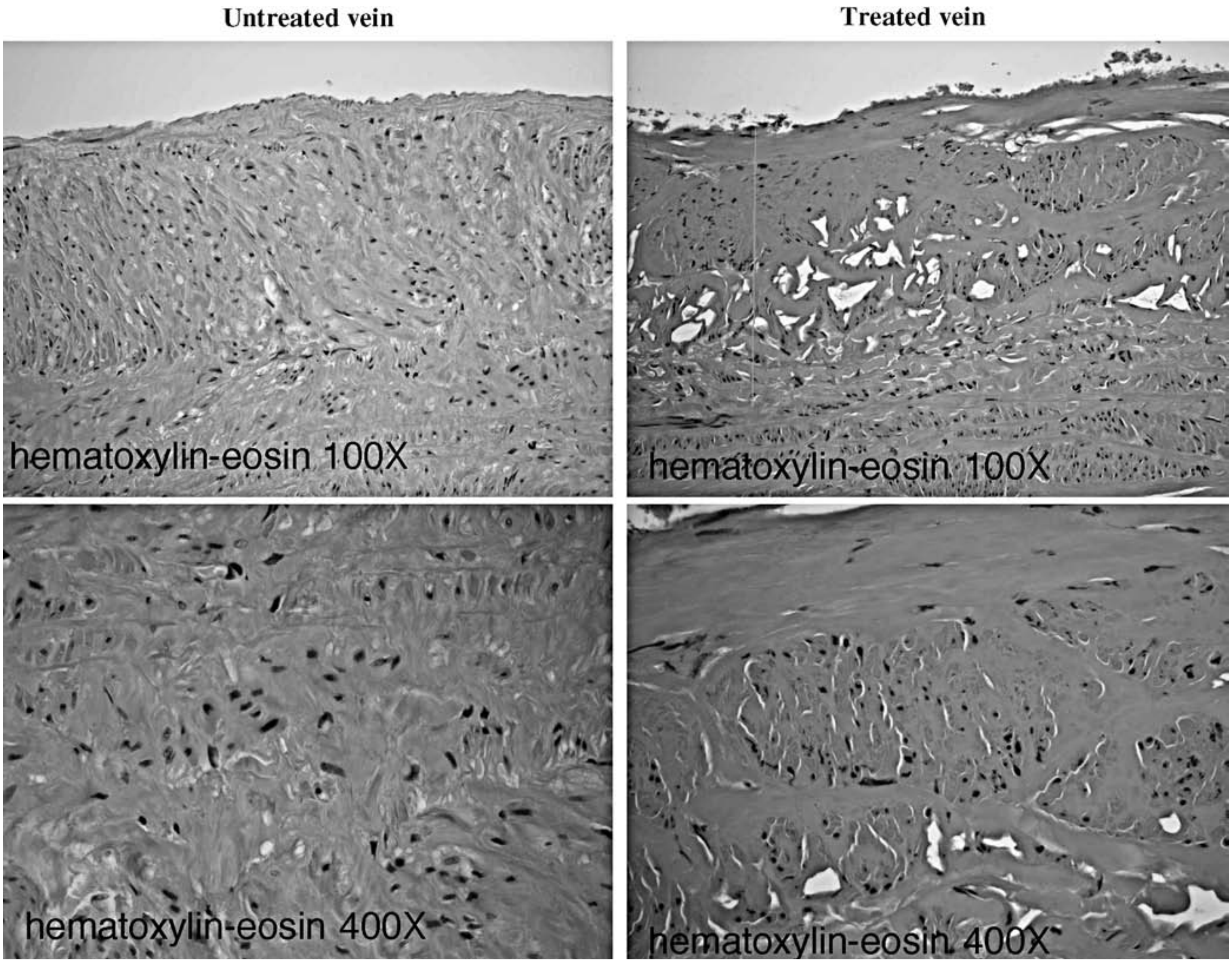

Figure 3.—Microscopic appearance of a vein treated with radial fiber-1470nm laser (right) and untreated vein (left). Hematoxylin-eosin 100-400X. No display of contact damage - reduction of venous wall thickness – increased eosinophilia of the citoplasma and intercellular substance – appearance of vacuolisations and fissures in subendothelial site and tunica media - nuclear pyknosis - karyorrhexis - collagen hyalinosis.

In the cases of hyperplasia of the intima, the intimal tunica was conserved, whereas the subendothelial layer had widespread damage, with lacunae and cavities, mostly with transversal comb-like direction, and a dissection between the intimal hyperplasia and the tunica media (Figure 5). The media layer was strongly eosinophilic, with a thickness reduction for a depth of 300 micron, with many empty cavities and fissures (Figure 3).

Within 300 micron of depth, cells presented with pyknotic nuclei, karyorrhexis or a corkscrew fashion of the nucleus; the cytoplasm looked strongly eosinophilic and slim fissures surrounding single cells were present. Collagen bundles were eosinophilic and hyaline. Specific staining for collagen fibers (trichrome Masson, Azan Mallory, Van Gieson) showed color changes in the segment between intima and the neoformed cavities of the media. These cavities were Azan-Mallory pink-red colored, indicating the presence of thermal damage (Figure 4). Elastin specific

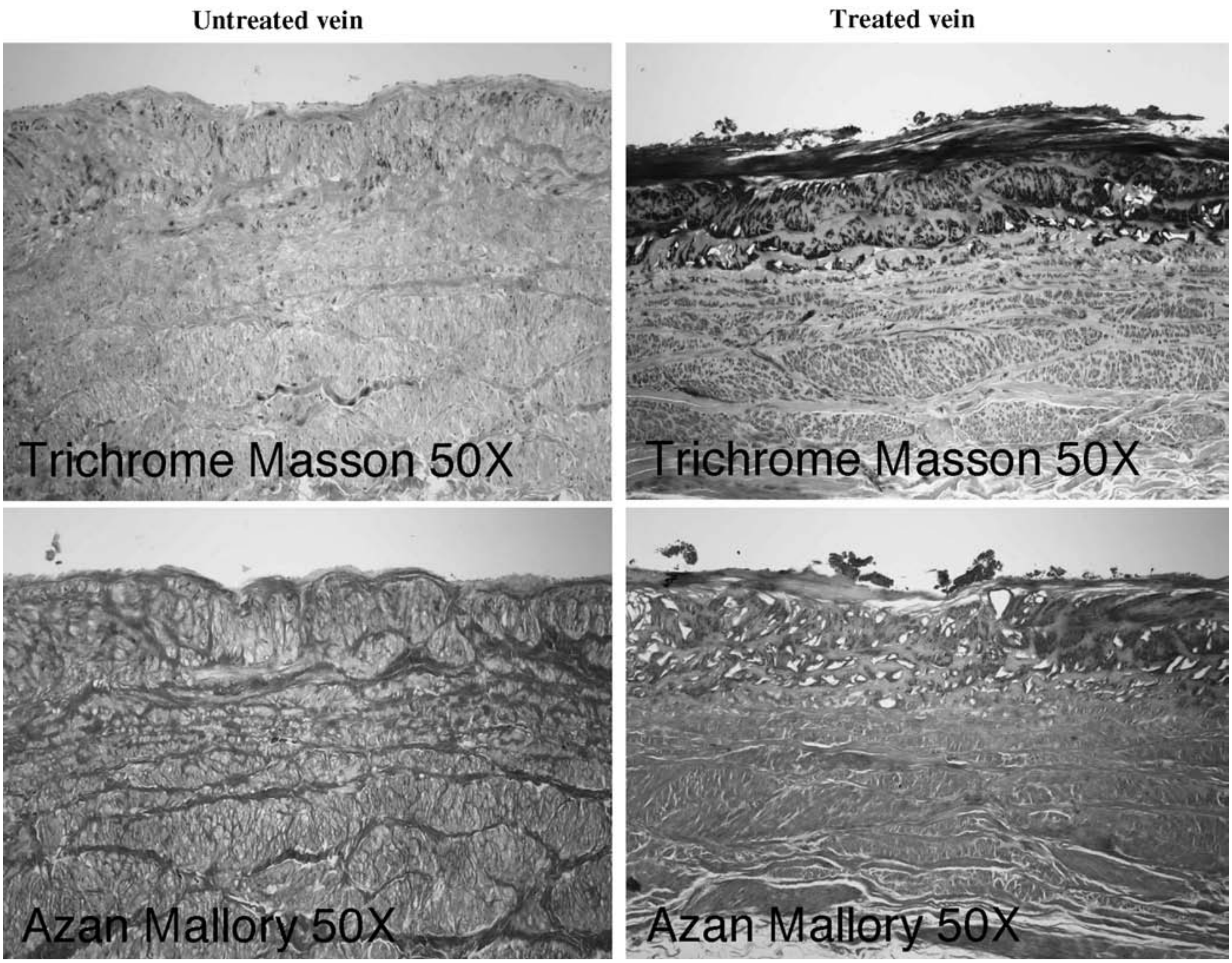

Figure 4.—Microscopic appearance of a vein treated with radial fiber-1470nm laser (right) and untreated vein (left). Trichrome Masson 50X, Azan Mallory 50X. The collagen is stained in green with Masson's trichrome; in blue/sky-blue with Azan-Mallory. The pink-red staining of collagen with Azan-Mallory in treated vein is a mark of thermal damage.

Weigert coloration highlighted serious damage to the internal elastic membrane.

Specific coloration for intercellular substance (Alcian, Alcian-Pas) showed, when compared with non-treated vein, a proteoglycan and glicosaminoglycan loss of colorability with Alcian and bright coloration of acid and neutral glycoproteins with Alcian-PAS coloration.

Veins treated with higher energy (80-100 J/cm) had diffuse circular fissures visible to the adventitial layer (Figure 5). The adventitial damage was not well definable since damage, mainly fissures, was also visible in non-treated segments and was probably due to the traumatic maneuver of the exovenous stripping.

Comparing the type of damage caused by flat fiber and 980 nm laser with radial fiber and 1470 nm laser (Figure 6), it has been noted that the first treatment modality caused limited damage to the area of contact of the fiber with the vein, resulting in charring, vaporization of the wall and consequent ablation at varied depth as far as perforation, whereas the wall extra contact points looked substantially uninjured.

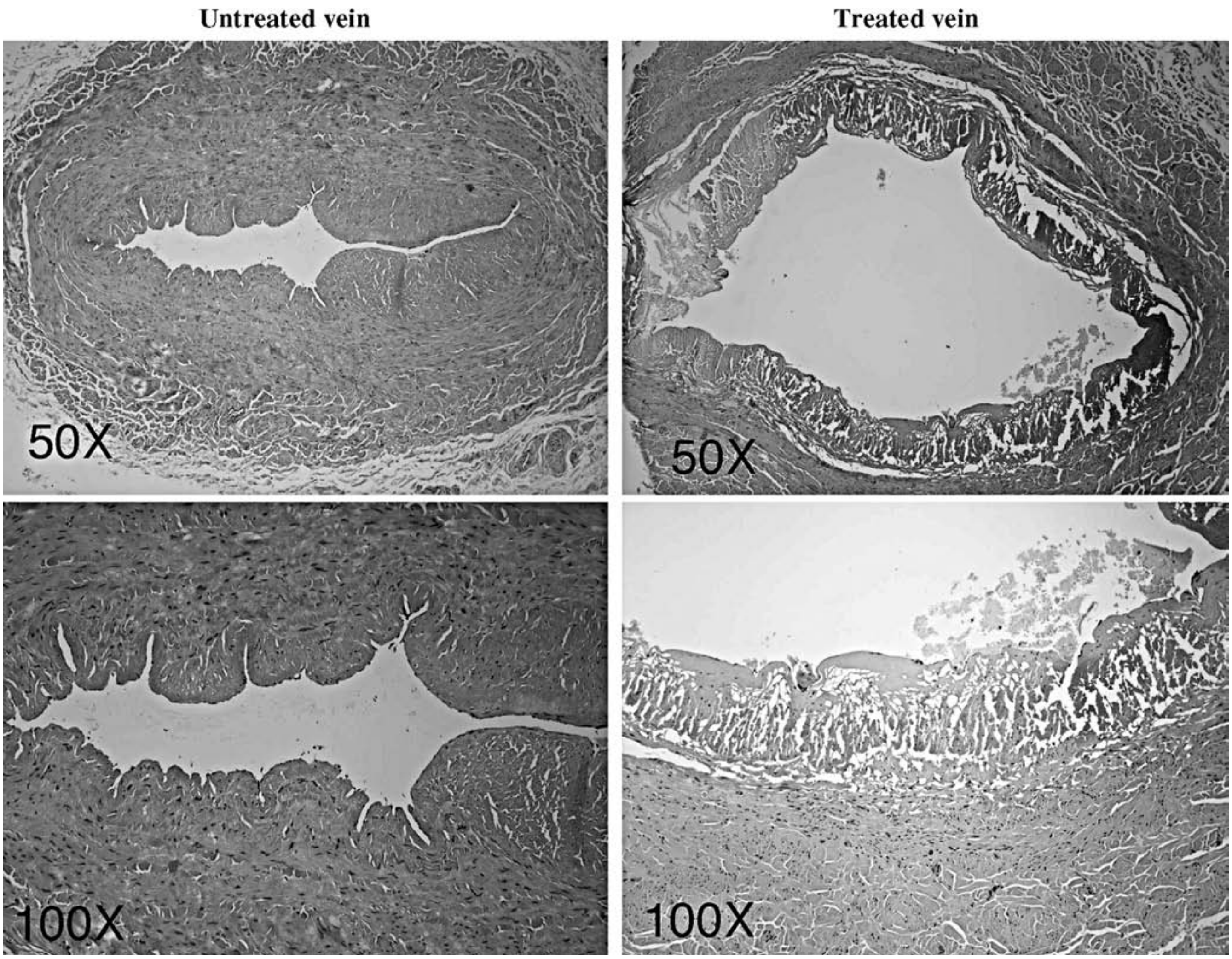

Figure 5.—Microscopic appearance of an untreated vein (left) and of a treated segment with radial fiber-1470 nm laser (right), continuous mode, 6 W, Linear Endovenous Energy Density: 80 J/cm. Hematoxylin-eosin 50-100X.

Vice versa the veins treated with radial fiber and 1470 nm laser did not show any sign of contact damage, while deep coagulative necrosis, vacuolizations and fissures at the subintimal site, at the intraintimal site of the intimal hyperplasia and in the media, at a progressively deeper location, directly correlated with the delivered amount of linear energy were present.

If the 1470 nm laser was used with a flat fiber, we noticed the reappearance of injury from contact with charring and ablation of the vessel wall, as it happened when using the 980 nm laser.

Discussion

To date no data on histological patterns of saphenous veins treated with radial fiber and 1470 nm laser are available, either in humans or in animals or *ex-vivo*.

The reduction of postoperative pain and ecchymoses,[4] the absence of smell of burned flesh during laser activity, the absence of charring, the emission of a 360 degrees light from the radial fiber, frontally directed on vein wall, and the affinity of 1470 nm light for water, suggest that the mechanism of ves-

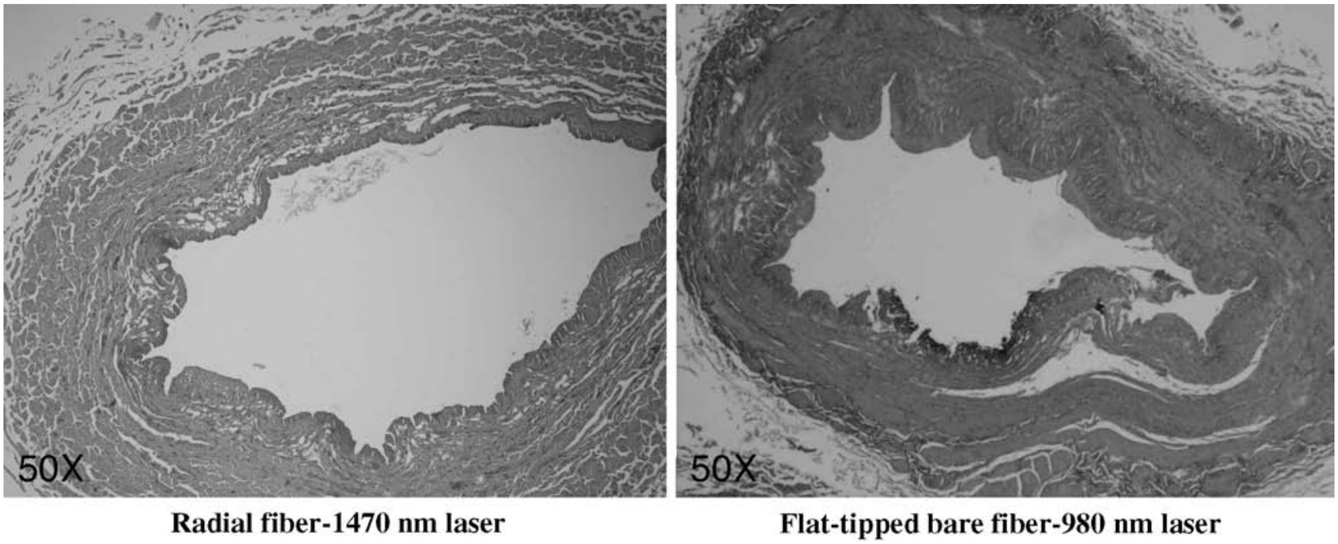

Figure 6.—Microscopic appearance of a vein treated with radial fiber-1470 nm laser (left), showing no contact lesions and diffuse and homogeneous damage of the tunica media, and a vein treated with flat-tipped bare fiber and 980 nm (right), showing a localized area of charring and ablation and a quite-normal appearance of the rest of venous wall.

sel wall damage due to these new devices is different from that caused by flat fiber and 980 nm laser. The absence of charring and vaporization might depend on the area of light emission of the radial fiber, which is about 10 times superior to that of a flat fiber, thus maintaining energy density under the threshold of ablation.[8]

The macroscopic pattern of the treated vein (grayish color, increased consistency, diameter reduction, increased wall thickness) is consistent with uniform and deep coagulation damage to the wall. This macroscopic aspect is similar to that achievable in vitro with the immersion of a vein in water at 85° for 30 seconds.[9]

Coagulative necrosis of the wall is demonstrable with early histology, *i.e.*, at the end of laser procedure, and involves the vein for a thickness of at least 300 micron with uniform and circumferential damage. Histological signs of coagulative necrosis are well demonstrable in the cells, collagen, intercellular substance and elastin.

This area of coagulation seems to correspond to the hyperechoic ring which is evident on ultrasound transversal images of treated trunk and creates a target aspect, distinctive of radial fiber and 1470 nm laser treatment.

Notably, the entity of thermal damage demonstrable with traditional histology is lower than the one highlighted by electron microscope scanning and immunohistochemistry (for example NBT coloration of alkaline phosphatase).[10-12]

Of interest for the comprehension of the mechanism of action of the radial fiber and 1470 nm laser is the formation of cavities and fissures in the subintima layer, in the intraintimal site of the intimal hyperplasia, and mainly in the media layer. Cavities are compatible with the explosion of water vapor bullae (popcorn effect) [13] and might correspond to the clinical data on the popcorn bursting noise, which can be heard during laser activity. The thin fissures surrounding single cellular elements or coagulated collagen fibers might derive from the coarctation. of these coagulated structures in the presence of modified intercellular substance. The largest fissures with circular aspect, especially those in the tunica media, might be the result of a lack of cohesion between muscular bundles of the media, due to a combined effect of collagen and intercellular substance alterations, together with the retraction of muscular elements.

The most evident damage to the intimal hyperplasia may derive from an elevated concentration of water in this tissue and/or from the closeness to the light source of the laser.

The cavities, in the tunica media, look like burst bubbles of vapor. This data could be confirmed by the Azan-Mallory coloration, where cavities are surrounded by a pink-red ring, typical of thermal damage.[14] The presence of these cavities, deep in the media, confirms that a significant dose of laser light reached this site and was able to cause water to evaporate. Moreover, the explosion of these cavities increases the damage to the venous wall adding a mechanical injury to a thermal one.

The localization of the fissures in the media might be a good index of the depth of the damage to the vessel wall and, in this respect, it seems interesting to note that, when increasing linear energy, fissures appear deeper in the media.

The importance of using both radial fiber and 1470 nm laser to obtain the described histological patterns is confirmed by examining the results achievable with the use of the same laser, but with a flat fiber. Similarly to the treatments with a 980 nm laser, contact lesions with charring, vaporization and the histological patterns already described in our article of 2006, reappear.[7]

Even if the number of histologically examined cases is modest, it is important to underline that the above mentioned lesions have been demonstrated with consistency and uniformity in all the examined histological specimens.

Conclusions

In conclusion, treatment of saphenous trunks with radial fiber and 1470 nm laser does not cause contact lesions. Conversely it determinates uniform and deep coagulation damage, with a graduation in depth in relation to the intensity of delivered energy.

The presence of vacuolizations or fissures inside the media indicates that a significant dose of energy is absorbed by the water contained in the media layer and turned into vapor, creating both mechanical and thermal damage.

This is the proof that venous wall itself is the target of 1470 nm laser light [15] and that the heat and the mechanical damage do originate inside the venous wall.

Clinical data (modest or absent post-treatment pain) seem to indicate that this type of coagulative and mechanical damage induce an inflammatory response which is modest and clearly inferior to that produced from flat fiber and 980 nm laser, but to date no histological confirmation is available.

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