Does Endovenous Laser Ablation Induce Endothelial Damage at the Saphenofemoral Junction?

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BACKGROUND AND OBJECTIVE One of the possible complications of endovenous laser ablation (EVLA) is thrombus progression into the common femoral vein or popliteal vein with the potential risk of pulmonary embolism or stroke. We set out to investigate the effect of laser energy applied under standardized treatment conditions on biomarkers of platelet and endothelial activation and on the hemostatic system.

METHODS Twenty patients with incompetence of the great saphenous vein were included in this prospective study. Blood samples of the iliofemoral and anticubital veins were collected before, during, and after EVLA. Plasma levels of soluble (s) P-selectin, soluble thrombomodulin (sTM), prothrombin fragment F1 + 2 (F1 + 2), and D-dimer were measured. (s) P-selectin and sTM were analyzed as surrogate markers of endothelial and platelet activation. F1 + 2 and D-dimer were monitored to quantify the degree of surgical trauma.

RESULTS Whereas there was no immediate rise of (s) P-selectin and sTM plasma concentrations in iliofemoral or anticubital blood, plasma levels of F1 + 2 and D-dimer increased significantly after EVLA.

CONCLUSION Pulsed mode laser ablation with an 810-nm fiber does not induce measurable platelet and endothelium activation in the iliofemoral or systemic blood. Furthermore, the immediate surgical trauma associated with EVLA appears to be modest.

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these steam bubbles transmit enough energy to induce vascular damage at the saphenous femoral junction (SFJ) or femoral vein, which might explain thrombus extension.

The adhesion molecule P-selectin is present in the alpha granules of platelets and the Weibel-Palade bodies of endothelial cells. Activated platelets expressing P-selectin on the surface release their granule contents, which facilitates the adhesion of platelets and neutrophils to the endothelium and causes platelet aggregation and thrombus growth.\textsuperscript{12,13} Thrombomodulin (TM) is an endothelial cell membrane protein that is rapidly released into blood upon endothelial cell activation.\textsuperscript{14} TM suppresses blood coagulation and is an important component of the protein C anticoagulant pathway.\textsuperscript{15} P-selectin and TM can be measured in plasma in their soluble (s) forms and have proved to be reliable indicators of platelet and endothelial activation.\textsuperscript{16–19} It has been demonstrated that (s) P-selectin is released in unstable angina within the first 3 hours,\textsuperscript{20} and (s) P-selectin is measurable within 30 minutes after percutaneous transluminal angioplasty (PTA).\textsuperscript{21} In trauma patients, sTM has been found after 10 minutes.\textsuperscript{17} An experimental study with endothelial cells showed that heat, caused by high-power ultrasound, activated the release of soluble TM within 2 minutes.\textsuperscript{22} F1 + 2 is a coagulation marker that is released when activated factor X cleaves prothrombin to produce thrombin. \textit{d}-dimer, a plasmin product of insoluble cross-linked fibrin, is a marker of fibrinolytic activity. In surgical patients, the increase in F1 + 2 and \textit{d}-dimer reflects the degree of tissue trauma associated with the surgical procedure.\textsuperscript{23–25}

In this study, (s) P-selectin and sTM were monitored as markers of endothelial and platelet activation and F1 + 2 and \textit{d}-dimer as surrogate markers for surgical trauma.

The aim of the study was to investigate whether an immediate rise of these markers can be observed when laser ablation of the GSV is performed under standardized treatment conditions, leaving the most proximal 2 cm untreated.

**Patients and Methods**

**Patients**

This prospectively designed, nonrandomized study enrolled 20 patients with primary GSV incompetence scheduled for EVLA performed under general or regional anesthesia. All patients had refused laser ablation to be performed under tumescent local anesthesia. Consenting patients had an average age of 47.5 ± 12.6 (7 male, 13 female).

All patients were examined using duplex scanning in an upright position (Logiq 7 General Electric Austria GmbH). GSV incompetence was ascertained according to reflux longer than 0.5 seconds after Valsalva maneuver or manual compression and decompression of the calf. Exclusion criteria were history or duplex signs of thromboembolic events, systemic antiplatelet agents or anticoagulant therapy within the preceding 14 days, ankle–brachial index less than 0.9, and tortuous GSV rendering the vein unsuitable for laser treatment. Because general anesthesia was used and because of the risk of postoperative hematoma from femoral vein puncture, all procedures were performed in a clinical in-patient setting. Ethical approval for the study was obtained from the Ethics Committee of the Medical University of Vienna. All patients gave written informed consent before treatment.

**Methods**

Central Venous Catheter Before EVLA, a 7-Fr, 16-cm central venous catheter (CVC) (Arrow International CS 17702, Erding, Germany) was inserted using the Seldinger technique into the iliofemoral vein of the leg to be treated. The position of the tip of the catheter in the iliofemoral transition area 5 to 8 cm proximal to the SFJ was confirmed using duplex scanning. The CVC was removed after completion of the procedure. Local compression was applied at the puncture site, and patients were
instructed to reduce physical strain until the first postoperative day to obviate hematoma due to femoral vein puncture.

**Procedure**

Because there was no indication or contraindication for general anesthesia versus regional anesthesia because all patients were in good health, with an average age of 47.5, patients’ requests for type of anesthesia could be respected; 15 patients underwent general anesthesia and five regional anesthesia.

EVLA was performed using the Diomed 810-nm-diode system (Diomed, Inc., Andover, MA). The GSV was cannulated adjacent to the knee under ultrasound (US) guidance using an 18-G needle. A 5-Fr introducer sheath was inserted over a guide wire, and after removal of the guide wire and the dilator, a sterile 600-μm bare laser fiber (KHP-GmbH, Grieskirchen, Austria) was introduced into the vein. In all patients, a distance of 1 to 2 cm from the SFJ to the laser tip was confirmed using US. Energy was delivered in a 13-W pulsed mode (1-second pulse duration, 1-second interval),

drawing the laser fiber manually at a speed of 2 mm/s until a distance of 2 cm proximal the skin entry site was reached.

After completion of EVLA and blood sampling, major varicose side branches were removed by phlebectomy in four patients and reticular veins by foam sclerotherapy in 16 patients. A class II (35 mmHg) thigh-length graduated compression stocking was applied postoperatively and worn for 2 to 3 weeks. According to the routine guidelines in our hospital, low-molecular-weight heparin (40 mg of enoxaparin, Lovenox, Sanofi-Aventis, Vienna, Austria) was administered postoperatively for 7 days as thrombosis prophylaxis.

**Follow Up**

US scanning was scheduled on days 1 and 7 after the procedure. Full-length scanning of the treated vein was included as a means of demonstrating vessel occlusion. The junction area was investigated for thrombus propagation. We did not do a full DVT scan. Patients were clinically examined for occurrence of bruising, paresthesia, and sensation of pain. Duplex US was repeated after 3 and 6 months to look for occlusion or obliteration of the treated vein.

**Blood Samples**

Blood samples for determination of F1 + 2, d-dimer, (s)P-selectin, and sTM were collected using a CVC from the iliofemoral vein segment at baseline at three different phases: before starting laser ablation of the GSV (f0), after having treated a section of approximately 10 cm (f1), and at the end of laser ablation (f2). Simultaneously, corresponding peripheral blood samples were drawn from an antecubital vein (b0, b1, b2). After the first 2 mL of blood was discarded, two samples of 4.5 mL of blood was collected into a citrate-containing tube (0.129M 3.8%, Vacutainer system, Becton Dickinson, San Jose, CA) and centrifuged at 2500 revolutions per minute for 10 minutes at 4°C. Plasma was then aliquoted (0.25 mL) and frozen at −70°C for subsequent batch analysis.

**Biomarkers and Assay Methods**

Commercially available enzyme-linked immunosorbent assay kits were employed to measure plasma antigen levels of (s) P-selectin (QuantikineSTM, R&D Systems, Minneapolis, MN; normal range 18–20 ng/mL; intra- and interassay coefficients of variation (CV) 6.8% and 8.9%, respectively.), sTM (Asserachrom thrombomodulin, Stago, Asnieres, France, normal range not given; intra- and interassay CV 8.1% and 9.8%, respectively). F1 + 2 (Enzygnost, Dade Behring, Schwalbach, Germany; normal range 26–226 pmol/L; intra- and interassay CV 8.6% and 9.1%, respectively), and d-dimer (Asserachrom D-dimer, Roche-Diagnostics, Mannheim, Germany; normal range <400 ng/mL; intra- and interassay CV 2.8% and 6.8%, respectively) were measured according to the manufacturers’ instructions. All analyses were done in duplicate, and all samples were thawed and analyzed at the same time.
Statistics

All data are presented as means ± standard deviations (SDs).

Statistical significance of differences between treatment groups was calculated using an analysis of variance for repeated measures (SPSS 15.0, SPSS, Inc., Chicago, IL). P < .05 was considered statistically significant.

Results

General Data

According to clinical, etiologic, anatomic, and pathophysiologic classification, there were 15 limbs in the C2 and five limbs in the C3 class. The mean length of GSV treated was 35.8 ± 6.8 cm. Linear endovenous energy density ranged between 68 and 97 J/cm. Miniphlebectomies of side branches or foam sclerotherapy were performed in four and 16 patients, respectively. US scanning performed on days 1 and 7 confirmed immediate occlusion of the GSV in all patients without DVT or protrusion of GSV thrombus into the common femoral vein (CFV). The closure rate after 6 months was 100%. Fourteen patients reported moderate pain, which was successfully treated using nonsteroidal antiinflammatory drugs. Bruising, which was noted in 16 patients, mainly at the sites of phlebectomies or foam sclerotherapy, had dissolved at the 3-month follow-up. No paresthesia was observed in any patient.

EVLA Did Not Change Endothelial and Platelet Activation Biomarkers

At baseline, there were no significant differences between the iliofemoral and anticubital samples in levels of (s) P-selectin and sTM (Table 1). After having treated a section of approximately 10 cm using EVLA (f1), the mean ± SD plasma levels of (s) P-selectin increased slightly during EVLA (f1) from 16 ± 5.8 ng/mL to the end point of EVLA (f0) 16.8 ± 7.6 ng/mL in iliofemoral plasma and from 16.3 ± 7.4 ng/mL to 17.1 ± 8.1 ng/mL in anticubital plasma, although these differences were not statistically significant (femoral p = .39; anticubital p = .71) and remained within normal range during EVLA (Table 1).

Likewise, the mean plasma levels of sTM showed no significant changes from baseline to end point in iliofemoral (from 6.8 ± 5.3 ng/mL to 8.0 ± 7.4 ng/mL) or anticubital blood (from 8.2 ± 7.8 ng/mL to 8.0 ± 7.4 ng/mL) (Table 1).

<table>
<thead>
<tr>
<th>TABLE 1. Outcome Parameters in Iliofemoral and Anticubital Venous Blood During Endovenous Laser Ablation (EVTA) (N=20)</th>
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<tr>
<td><strong>Mean ± Standard Deviation</strong></td>
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<tr>
<td><strong>D-dimer (ng/mL)</strong></td>
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<tr>
<td>Iliofemoral: Baseline(f0)</td>
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<td>During EVLA(f1)</td>
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*p-value of variance for repeated measurements, baseline versus endpoint of EVLT.
No Difference in Levels of Biomarkers in Iliofemoral and Anticubital Blood

Levels of the investigated markers (d-dimer, F1 + 2, (s) P-selectin, and sTM) did not differ significantly between iliofemoral and anticubital blood at any measurement.

EVL A Induced Increase in Surgical Damage Biomarkers

In contrast to the biomarkers of platelet and endothelial activation, the mean plasma levels of F1 + 2 continuously increased in femoral blood during EVLA, from 251.6 ± 97.0 to 415.3 ± 255.0 and in anticubital blood from 215.9 ± 98.4 to 370.2 ± 222.9. This increase was statistically significant for both locations (iliofemoral p = .03; anticubital p = .02). The pattern of changes of F1 + 2 plasma levels is shown in Figure 1 and Table 1. Comparing the baseline levels with the endpoint levels, iliofemoral blood increased 1.7 ± 1.0 times and anticubital blood 1.4 ± 0.9 times.

During EVLA, mean ± SD plasma levels of d-dimer increased in iliofemoral blood from 53.8 ± 42.1 to 74.9 ± 67.6 and in anticubital blood from 57.4 ± 52.3 to 69.7 ± 59.1 (Figure 2, Table 1). The rise from baseline to endpoint of EVLA was statistically significant for the iliofemoral (p = .04) and anticubital veins (p = .03), although all baseline and final values of iliofemoral and anticubital blood remained within the normal reference range of the laboratory. The increase from baseline to endpoint was 1.4 ± 1.2 times in iliofemoral blood and 1.2 ± 1.1 times in anticubital blood.

Discussion

The aim of this study was to investigate the immediate endothelial alteration of the most proximal part of the GSV induced by laser ablation using an 810-nm laser. Taking into account axial heat transmission at the laser tip, it is recommended that laser ablation of the GSV be started 1 to 2 cm distal to the SFJ. Even then, steam bubbles can spread toward the proximal GSV. (s) P-selectin and sTM, reliable markers of platelet and endothelial activation, were measured in blood samples collected from the iliofemoral segment during the procedure. F1 + 2 and d-dimer were also monitored to quantify the surgical trauma associated with laser ablation of the GSV.
No significant change in plasma levels of (s) P-selectin and sTM was observed. Soluble markers of endothelial activation were traceable from the beginning of the procedure, as soon as intact endothelium was exposed to a heat stimulus. Because destroyed endothelium along the GSV does not release any marker of endothelial activation,28 we suppose that (s) P-selectin and sTM levels mainly reflect heat-induced activation of the most-proximal part of the GSV and the SFJ area, which are not directly exposed to the laser beam and of surviving islets of endothelium within the treated vein. (s) P-selectin is not specific for endothelial cells, but as an activation marker for platelets, it is of particular importance for thrombus formation.29 Because no significant change in plasma levels of (s) P-selectin and sTM were observed, our findings indicate that EVLA does not induce immediate endothelial or platelet activation. Although (s) P-selectin and sTM have repeatedly been acknowledged as sensitive biomarkers,16,17,20,21 we cannot exclude that minimal activation was missed because of timing of sampling, lack of assay sensitivity, or dilution effects. We do not think that the laser protocol used influenced our results, because the average energy delivered was in accordance with recommendations for successful saphenous treatment.30

In outpatients, the increase in F1+2 and D-dimer, both activation markers of coagulation and fibrinolysis, supports the diagnosis of DVT.31 In surgical patients, hemostatic markers are poor predictors of DVT but are estimators of the degree of surgical trauma.23–25,32–34 In this study, F1+2 and D-dimer increased continuously during EVLA, reaching statistical significance for both parameters at the endpoint of laser ablation. Mean baseline levels of F1+2 were within normal range in antecubital blood but were high in iliofemoral blood, most probably due to insertion of the CVC. It has been shown that various conditions of blood sampling affect plasma levels of F1+2 but not D-dimer.35 Throughout the observation period, absolute values for D-dimer remained within the reference range despite increasing significantly. Pröbstle and colleagues also reported a D-dimer increase within normal limits during laser ablation. During this study, seven of 20 patients showed D-dimer levels exceeding the upper limit after the first postoperative day.5 In highly traumatizing surgery such as knee replacement surgery, hemostatic markers show a clear increase of their baseline levels during the surgical procedure.36 In less-traumatizing surgery such as laparoscopic cholecystectomy, perioperative increase of coagulation markers is less pronounced.24,32 In this study, the limited increase in hemostatic biomarkers supports the hypothesis that surgical trauma caused by EVLA is modest.

EVLA, as with many other new surgical techniques, has a defined learning curve requiring new operator skills such as targeted duplex scanning, wire and catheter handling, and in the case of local procedures, accurate placement of tumescent anesthesia. According to Mackenzie and colleagues, approximately 200 procedures are required to achieve satisfactory technical competence.37 If one is not used to performing EVLA under tumescent local anesthesia, it might be more difficult to position the laser tip exactly 1 to 2 cm below the SFJ. Surgeons still in their learning curve treated all patients with thrombus extension reported by Mozes and colleagues10 and Gibson and colleagues9 under tumescent local anesthesia. Therefore, it is worthwhile to consider whether thrombotic complications are more likely to represent inadvertent application errors than the thrombotic burden of the procedure itself. Some limitations of this study should be addressed. Because of the iliofemoral position of the CVC, blood samples only partially reflect the SFJ. Considerable dilution by femoral blood might have obscured marginal endothelial and platelet activation. Although the amount of energy administered in our study was comparable with that used in successful GSV occlusion with other laser systems, our results apply only to EVLA with an 810-nm laser using pulsed mode at 13 W.

Blood sampling can induce a rise in hemostatic markers. Therefore, we cannot definitely exclude
that the CVC itself caused a time-dependant elevation of hemostatic markers even in the absence of laser ablation, although if this had been the case, we would expect a decrease over the observation period because the insult to the vein is worst when the CVC is inserted.

In conclusion, pulsed mode laser ablation with an 810-nm fiber starting 1 to 2 cm distal to the SFJ does not induce measurable platelet and endothelium activation in the iliofemoral or systemic blood. Furthermore, the immediate surgical trauma associated with EVLA appears to be modest.

References


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