Ablation of the great saphenous vein with nontumescent n-butyl cyanoacrylate versus endovenous laser therapy

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ABSTRACT

Objective: The endovenous application of n-butyl cyanoacrylate (NBCA) is a new nontumescent ablation technique for the treatment of venous insufficiency. The aim of this study was to retrospectively compare an NBCA-based ablation method with endovenous laser ablation (EVLA) for the management of incompetent great saphenous veins.

Methods: Between May 2013 and August 2014, there were 339 patients with incompetent varicose veins who were treated with either the endovenous application of NBCA (VariClose Vein Sealing System [VVSS]; Biolas, Ankara, Turkey) or EVLA. The preprocedural, intraprocedural, postprocedural, and follow-up data of the patients were collected and retrospectively compared.

Results: The mean age was 45.09 ± 12 years in the VVSS group and 47.08 ± 11 years in the EVLA group (P = .113). The average ablated vein length was 31.97 ± 6.83 cm in the VVSS group and 31.65 ± 6.25 cm in the EVLA group (P = .97). The average tumescent anesthesia use was 300 mL (range, 60-600 mL) in the EVLA group. The average procedure time was 7 minutes (range, 4-11 minutes) in the VVSS group and 18 minutes (range, 14-25 minutes) in the EVLA group (P < .01). On the basis of ultrasound examinations performed at the end of the procedure, all procedures in both groups were successful, and the target vein segments were fully occluded. The 12-month total occlusion rates in the VVSS and EVLA groups were 98.6% and 97.3%, respectively (P = .65). In both the VVSS and EVLA groups, the Venous Clinical Severity Score declined significantly with no difference between groups. There were fewer adverse events after VVSS treatment compared with EVLA treatment (pigmentation, P ≤ .002; phlebitis, P ≤ .015). There was no need for tumescent anesthesia in the VVSS group.

Conclusions: The NBCA-based vein sealing system is a fast and effective treatment option for the management of incompetent saphenous veins that does not involve tumescent anesthesia, compression stockings, paresthesia, burn marks, or pigmentation. Further large-scale studies with long-term outcomes are required to identify the optimal treatment modalities for patients with saphenous vein insufficiency. (J Vasc Surg: Venous and Lym Dis 2017;5:210-5.)

Chronic venous insufficiency with the resultant varicose veins is an important entity that is responsible for substantial morbidity. Lower limbs can manifest a range of signs that include edema, pigmentation, and venous ulcers.1 The treatment of venous insufficiency has changed dramatically during the past decade. Conventional methods have been replaced by interventional modern methods, and new technologies are being introduced every year. Conventional methods, such as ligation and stripping, are associated with complications, including hematomata and paresthesia, and are perceived as risky and disfiguring.2 The long recovery times associated with conventional methods are decreasing the popularity of these methods.3

Foam sclerotherapy is the most commonly used minimally invasive technique for the treatment of varicose veins worldwide, but it is plagued with high recurrence rates.4 Adverse effects, such as air embolism, headache, pulmonary embolism, and deep venous thrombosis (DVT), are substantial disadvantages of this treatment.5 Endovascular ablation techniques, such as laser and radiofrequency ablation, are widely used and proven treatments. The procedural techniques and methodologies differ between these two techniques, but the results are fundamentally similar, and both achieve nearly 90% long-term success rates.5,6 Although thermal ablation represents a breakthrough that is associated with fewer complications, the need for tumescent anesthesia and the occurrence of adverse events such as burns, pigmentation, and paresthesia are unavoidable downsides.5-7

Although the current techniques are successful, the search for new innovative techniques is rapidly intensifying with the goals of increasing the success rate, improving the patient’s quality of life, and reducing the potential complications. A new technique for vein ablation has recently been developed that involves the endovenous application of n-butyl cyanoacrylate...
(NBCA) and does not require the use of tumescent anesthesia. Although approved in the European Union, it is not approved for use in the United States. In addition, the results of a 2-year trial of the cyanoacrylate embolization of incompetent great saphenous veins (GSVs) were published.8 During endovenous application, NBCA rapidly solidifies and creates a rapid polymerization reaction. This reaction creates an inflammatory effect on the vein wall that induces an ablative reaction. On injection into a vein, a compress over the vein seals the vessel by polymerization.8-10

The aims of this study were to present our experience with this new nontumescent ablation method, to compare this experience with laser ablation, and to present both anatomic and clinical results of our 12-month follow-up.

METHODS

Study design

The study was a purely retrospective review of the record. Here we present the results of 150 patients treated with the endovenous application of NBCA (VariClose Vein Sealing System [VVSS]; Biolas, Ankara, Turkey) and 189 patients treated with 1470-nm endovenous laser ablation (EVLA: Evlas Circular Fiber, Biolas). Between May 2013 and May 2014, there were 202 patients initially treated with EVLA; then between April 2014 and August 2014, there were 166 patients treated with VVSS. All patients who had full follow-up were selected for comparison. The full follow-up groups consisted of 150 patients in the VVSS group and 189 patients in the EVLA group. The follow-up interval was 1 year.

The primary indications for the procedures were a GSV diameter >5.5 mm (2-3 cm distal to the saphenofemoral junction [SFJ]) with venous reflux lasting for >0.5 second. All patients were symptomatic. All patients were treated with either ablation of the GSV or endovenous application of NBCA. The remaining refluxing tributaries were treated with microphlebectomy at the same session. There was no concurrent treatment of the small saphenous vein or anterior accessory saphenous vein. The inclusion and exclusion criteria are listed in Table I. The patients’ histories and physical examination findings and color Doppler ultrasound (CDUS) results were noted in the primary evaluation. The preoperative clinical disease severity was graded according to the Clinical, Etiology, Anatomy, and Pathophysiology (CEAP) classification, and the clinical findings were assessed with the Venous Clinical Severity Score (VCSS). As a retrospective study, consent of the patients and Institutional Review Board approval were waived by our institution.

Procedural protocols

Endovenous application of NBCA. The disposable VVSS includes 3 mL of VariClose NBCA and the VariClose Delivery System (VDS). The GSV was accessed with a 6F introducer set with the assistance of CDUS. A 0.035-inch × 150-cm guidewire was sent through the 6F introducer sheath to the SFJ. Once the guidewire was confirmed to be in the SFJ by CDUS, a 5F long introducer sheath was advanced to the SFJ over the guidewire. After confirmation of the position of the long introducer sheath at the beginning of the SFJ, the long introducer sheath was pulled back from the SFJ by 6 cm. The sheath was pulled back by 6 cm because the 4F delivery catheter’s tip comes out of the long introducer sheath by 3 cm. Thus, the 4F delivery catheter had to be positioned 3 cm distal to the SFJ. Once the position of the 4F delivery catheter was confirmed, the NBCA injection setup was complete. A VDS gun and adaptor were connected to each other, and 2 mL of the 3 mL of the NBCA were aspirated into the injector. The injector was connected to the gun adaptor and the end of the delivery catheter by a spin lock mechanism.

After setup and positioning of the VDS were complete, the procedure began. First, pressure over the SFJ was applied with CDUS, and closure of the SFJ was confirmed. Before the injection of NBCA inside the vein lumen, the delivery catheter was primed. One trigger push was applied for 1 second to prime the delivery catheter. After priming, the trigger was pushed again for 5 seconds, and this time, the delivery catheter was pulled back at 2 cm/s. The VDS was set up for the injection of 0.03 mL of NBCA per centimeter. Continuous pressure was applied over the target vein segment simultaneously with the pulling back of the delivery catheter by the CDUS probe without releasing the pressure from the SFJ. Every 5 seconds or 10 cm, the trigger had to be pressed. This trigger-pressing and pressure application routine was performed continuously until the target vein segment was fully sealed. Once the entire vein
Table I. Study eligibility criteria

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
<th>Exclusion criteria</th>
</tr>
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<tbody>
<tr>
<td>1. Age ≥20 years and ≤70 years</td>
<td>1. Tortuous GSV</td>
</tr>
<tr>
<td>2. Vein diameter at the GSV ≥5.5 mm and ≤15 mm</td>
<td>2. Symptomatic peripheral arterial disease history or an ABI &lt;0.9</td>
</tr>
<tr>
<td>3. Reflux in GSV &gt;0.5 second</td>
<td>3. History of DVT or PE</td>
</tr>
<tr>
<td>4. CEAP classification between C2 and C5</td>
<td>4. Life expectancy &lt;2 years</td>
</tr>
<tr>
<td>5. Patients attended the follow-up examinations</td>
<td>5. Active thrombophlebitis in the deep or superficial veins</td>
</tr>
<tr>
<td>6. Patients were sufficiently mentally healthy to consent to the operation</td>
<td>6. Significant femoral or popliteal venous insufficiency and perforator vein insufficiency</td>
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</table>

Exclusion criteria

1. Tortuous GSV
2. Symptomatic peripheral arterial disease history or an ABI <0.9
3. History of DVT or PE
4. Life expectancy <2 years
5. Active thrombophlebitis in the deep or superficial veins
6. Significant femoral or popliteal venous insufficiency and perforator vein insufficiency
7. Known sensitivity to cyanoacrylate adhesives
8. Aneurysm >15 mm in the target vein
9. Previously treated GSV
10. Existence of malignant disease
11. Pregnancy
12. Immobilization

ABI, Ankle-brachial index; CEAP, Clinical, Etiology, Anatomy, and Pathophysiology classification; DVT, deep venous thrombosis; GSV, great saphenous vein; PE, pulmonary embolism.

segment was injected with NBCA with the continued application of pressure during the procedure, a final pressure was applied for 30 seconds over the entire target vein segment.

**EVLA.** The suitability for EVLA was assessed in the preoperative evaluation. The disposable Bioslas Evlas Circular Fiber EVLA kit consisted of 600 μm of radially extending fiber that functioned at a wavelength of 1470 nm with the 6F introducer kit. The GSV access was achieved with the 6F introducer kit under the assistance of CDUS. The laser fiber was advanced through the sheath to 0.5 cm distal to the superficial epigastric vein. Confirmation of the fiber’s position was achieved on the basis of both CDUS and the laser light beam. After confirmation, tumescent anesthesia was applied around the tissue surrounding the GSV. Thermal laser energy was applied from the SF3 to the access point at 10 J/mm (ie, 10 J of energy per millimeter of the diameter), and the apparatus was slowly pulled back. During the pullback, pressure was applied over the vein to increase the obliteration of the vein.

**Definitions of adverse events**

Patients with reddening of the skin area involving 20% or more of the treated part or blistered skin involving 20% or less of the treated area were regarded as having skin burn. Patients without pain and with tolerable pain requiring no additional analgesics were regarded as having no pain, and those requiring additional analgesics or topical cooling or those in whom pain affected their activities of daily life were regarded as having pain. Patients with specific color or darker bruising involving 20% or more of the treated area or those with markedly dark bruising involving 20% or less of the treated area were regarded as having bruising. Patients with numbness or tingling sensation of the treated area were regarded as having paresthesia.

**Postprocedural management**

The NBCA patients were mobilized after the treatment without any prescription of compression stockings. The laser group patients were prescribed class II compression stockings (thigh high) for 2 weeks after the treatment. All patients underwent physical examinations and CDUS control before and after the procedure and at the first week and 6 and 12 months after treatment. CDUS controls at 6 and 12 months after treatment were done by the radiology department, whereas first-week controls were performed in our clinic with CDUS by surgeons who had CDUS course certification.

Procedural success was defined as complete occlusion of the treated vein segment or a partial recanalization of <5 cm. Clinical recovery was assessed by comparing the VCSS values before and after the procedures.

**Statistics**

The analyses were performed using the SPSS Statistics for Windows, version 22.0 (IBM Corp, Armonk, NY). The continuous data are presented as the mean ± standard deviation and median (minimum-maximum) values. The comparisons between groups were performed with χ² and Fisher exact tests for categorical variables. As the results of the Shapiro-Wilk test for normality showed that the continuous variables were not distributed normally, the Mann-Whitney U test was used to compare the groups and Wilcoxon rank sum test was performed for the comparison within the groups. The P value < .05 was considered statistically significant.

**RESULTS**

There were 339 successfully performed ablation procedures for GSV insufficiency. There was no bilaterally handled patient in the same session. The VVSS group consisted of 74 men and 76 women (150 patients in total), and the EVLA group consisted of 95 men and 94 women (189 patients in total). The mean ages were 45.09 ± 12 years in the VVSS group and 47.08 ± 11 years in the EVLA group (P = .11). The average preprocedural GSV diameters were 6.88 ± 1.80 mm (range, 5.5-15 mm) in the VVSS group and 7.15 ± 1.77 mm (range, 5.5-14 mm) in the EVLA group (P = .06). All patients were symptomatic.

The preprocedural CEAP classifications and demographic and baseline characteristics are illustrated in Table II.
The average ablated vein lengths were 31.97 ± 6.84 cm in the VVSS group and 31.65 ± 6.25 cm in the EVLA group. The average tumescent anesthesia use was 300 mL (range, 60-600 mL) in the EVLA group. The average procedure times were 7 minutes (range, 4-11 minutes) in the VVSS group and 18 minutes (range, 14-25 minutes) in the EVLA group (P < .001). The procedural characteristics are summarized in Table III. There were fewer adverse events (Table IV) after VVSS treatment compared with EVLA treatment (pigmentation, P ≤ .002; phlebitis, P ≤ .015). There was no need for tumescent anesthesia in the VVSS group.

**Anatomic success.** On the basis of the ultrasound examinations performed at the end of the procedures, all of the procedures in both groups were successful, and the target vein segments were fully occluded. In the first week, one patient in the VVSS group experienced a partial recanalization > 5 cm at the SFJ level because of the lack of experience with the use of the dispensing gun. (This patient was our second patient, so we thought that might be because of lack of experience or the learning curve at that time.) In the EVLA group, two patients exhibited partial recanalizations > 5 cm at the SFJ level. At 6 months, one patient in the VVSS group presented with partial recanalization > 5 cm at the mid-GSV level, whereas two patients presented with total recanalization and one patient presented with partial recanalization > 5 cm in the EVLA group. No additional recanalizations were observed at the 12-month follow-up. Thus, the total 12-month occlusion rates were 98.6% and 97.3% in the VVSS and EVLA groups, respectively (P = .65).

**VCSS.** The VCSS values were recorded at each follow-up and compared with the baseline values. The VCSS declined from 7.53 ± 1.03 to 2.79 ± 1.05 (P < .001) in the VVSS group and from 7.73 ± 1.58 to 2.83 ± 1.21 (P < .001) in the EVLA group, but there was no difference between groups (Table III).

**Adverse events.** Adverse events (Table IV) were less frequent after VVSS treatment compared with EVLA treatment for pigmentation (P = .002) and phlebitis (P = .015). The most important adverse events were pain and phlebitis in the VVSS group, which occurred at rates

### Table III. Procedure characteristics

<table>
<thead>
<tr>
<th></th>
<th>VVSS</th>
<th>EVLA</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSV diameter, mm</td>
<td>6.88 ± 1.80 (range, 15-5.5)</td>
<td>7.15 ± 1.77 (range, 14-5.5)</td>
<td>.065</td>
</tr>
<tr>
<td></td>
<td>(6.05 [4.6-16.0])</td>
<td>(6.70 [4.5-14.0])</td>
<td></td>
</tr>
<tr>
<td>Length of the ablated GVS, cm</td>
<td>31.97 ± 6.84 (range, 15-5.5)</td>
<td>31.64 ± 6.26 (range, 14-5.5)</td>
<td>.974</td>
</tr>
<tr>
<td></td>
<td>(30 [23-70])</td>
<td>(30 [23-70])</td>
<td></td>
</tr>
<tr>
<td>Amount of tumescent anesthesia, mL</td>
<td>—</td>
<td>300 (range, 60-600)</td>
<td></td>
</tr>
<tr>
<td>Procedure duration, minutes</td>
<td>7 (range, 4-11)</td>
<td>18 (range, 14-25)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Occlusion rate</td>
<td>148 (98.6)</td>
<td>184 (97.3)</td>
<td>.659</td>
</tr>
<tr>
<td>Pretreatment VCSS</td>
<td>7.53 ± 1.03 (range, 15-5.5)</td>
<td>7.73 ± 1.58 (range, 14-5.5)</td>
<td>4.95</td>
</tr>
<tr>
<td></td>
<td>(7 [7-13])</td>
<td>(7 [7-13])</td>
<td></td>
</tr>
<tr>
<td>Post-treatment VCSS</td>
<td>2.79 ± 1.05 (range, 15-5.5)</td>
<td>2.83 ± 1.21 (range, 14-5.5)</td>
<td>.882</td>
</tr>
<tr>
<td></td>
<td>(2 [1-6])</td>
<td>(2 [2-6])</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td></td>
</tr>
</tbody>
</table>

**Notes:**
- EVL, Endovenous laser ablation; GSV, great saphenous vein; VCSS, Venous Clinical Severity Score; VVSS, VariClose Vein Sealing System.
- Categorical variables are presented as number (%). Continuous variables are presented as mean ± standard deviation (median [minimum-maximum]) unless otherwise indicated.
- aC2 test.
- bMann-Whitney U test.
- cWilcoxon signed rank test.
Regarding the use of NBCA in chronic venous disease.7-9 Paresthesia was observed and resolved within the first week. In 1.59% of the patients, paresthesia was observed and resolved within the 6-month follow-up. In 2.65% of the patients experienced burning, pigmentation, and bruising, respectively, but these conditions resolved by the 6-month follow-up. In the EVLA group, 8.99% of the patients experienced pain in the first week and 5 days (4-7), and 2.12%, 5.82%, and 2.65% of the patients experienced burning, pigmentation, and bruising, respectively, but these conditions resolved by the 6-month follow-up. In 1.59% of the patients, paresthesia was observed and resolved within 6 months. Phlebitis was observed and treated with 14 days of antibiotic (sulfactam-ampicillin) and nonsteroidal anti-inflammatory medications in 7.94% of the patients. DVT was diagnosed on the patient’s visit to our outpatient clinic and radiologic CDUS confirmation. DVT was treated with anticoagulant medication (low-molecular-weight heparin) in 1.6% of the patients. These DVTs dissolved in an average of 10 days (7-15 days). All of them were endovenous heat-induced thrombosis class 1. The adverse events are summarized in Table IV. This study revealed that the rate of complications after NBCA use was significantly lower than that of EVLA, which are summarized in Table IV (pigmentation, phlebitis: P ≤ .05). EVLA complications are generally caused by thermal effects, which supports the use of NBCA because of the lower frequency of complications associated with this procedure. The closure rate after NBCA use was as high as that of EVLA. Recent studies compared EVLA, ultrasound-guided foam sclerotherapy, and conventional surgery for great saphenous varicose veins and found that after 1 year, the anatomic success rate was highest after EVLA (88.5%), followed by conventional surgery (88.2%) and ultrasound-guided foam sclerotherapy (72.2%).15 Rasmussen et al reported that 5-year follow-up of a randomized controlled trial comparing EVLA with open surgery in patients with GSV incompetence did not show any significant difference between the two groups in primary or secondary end points. EVLA seems to be a valid alternative to open surgery.11 Both treatment methods showed good safety profiles.

In a European multicenter cohort study, Proebstle et al reported a 98.6% immediate success rate.9 In a randomized trial comparing NBCA and radiofrequency ablation, the NBCA closure rate was reported to be 100%,10 whereas we observed a closure rate of 98.6% at 12 months of follow-up. In the former report, the application and type of NBCA differed from those used in our present study. The differences between these studies included viscosity, application type, and initial positioning 5 cm away from the junction. The product that we used in this study was a low-viscosity NBCA that provided immediate polymerization and a sealing effect in <5 seconds. This polymerization enabled rapid procedures (the mean NBCA application time was 20 seconds). Because the delivery of NBCA was continuous and without any pulsations or stops, each millimeter of the venous lumen was injected with a thin layer of NBCA. The combination of this application method and the low viscosity of the NBCA significantly increased the success rate (98.6%) and decreased the phlebitis rate (2%) compared with the results from earlier NBCA studies.

The rapid closure and minimal procedural time prevented DVT and pulmonary embolism. Because the NBCA polymerized so quickly, the SFJ portion of the GSV was rapidly closed, and the application of the correct amount of pressure over the SFJ reduced the risk of media flow into the deep vein. Moreover, the absence of heat ensured that burn marks, pigmentation, and paresthesia did not occur. The fact that tumescent anesthesia is not required largely accounts for the improved procedure time for EVLA compared with EVLA (7 minutes for VVSS and 18 minutes for EVLA [P ≤ .01] in our study) and the patient’s comfort after the procedure.5 The postoperative pain was slightly decreased compared with EVLA. Moreover, the absence of thermal effects ensured that burn marks, pigmentation, and paresthesia did not occur. This polymerization creates an inflammatory effect over the vein wall. This inflammatory effect creates an immediate tensile force that is the first stage of the polymerization process. The second stage consists of a stable tensile force, and the third stage is the final polymerization during which bonding to the endothelium occurs.3,14

Recent studies have reported promising results associated with the use of NBCA for the treatment of venous insufficiency.6 Our results also confirm earlier findings regarding the use of NBCA in chronic venous disease.7-9

<table>
<thead>
<tr>
<th>Table IV. Adverse events</th>
<th>Group</th>
<th>VVSS (n = 150)</th>
<th>EVLA (n = 189)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain (first week)</td>
<td>7 (4.7)</td>
<td>17 (9.0)</td>
<td>.123a</td>
<td></td>
</tr>
<tr>
<td>Burns</td>
<td>—</td>
<td>4 (2.1)</td>
<td>.133a</td>
<td></td>
</tr>
<tr>
<td>Pigmentation</td>
<td>—</td>
<td>11 (5.9)</td>
<td>.002a</td>
<td></td>
</tr>
<tr>
<td>Bruising</td>
<td>—</td>
<td>5 (2.6)</td>
<td>.069a</td>
<td></td>
</tr>
<tr>
<td>Paresthesia</td>
<td>—</td>
<td>3 (1.6)</td>
<td>.258a</td>
<td></td>
</tr>
<tr>
<td>Phlebitis</td>
<td>3 (2.1)</td>
<td>15 (7.9)</td>
<td>.015a</td>
<td></td>
</tr>
<tr>
<td>DVT</td>
<td>—</td>
<td>3 (1.6)</td>
<td>.258a</td>
<td></td>
</tr>
</tbody>
</table>

DVT, Deep venous thrombosis; EVLA, endovenous laser ablation; VVSS, VariClose Vein Sealing System. Values are reported as number (%).

aP<.05 test.
bFisher exact test.
of a need for compression stocking use improved the patients’ quality of life.

Morrison et al reported that adverse events were similar between these groups. No severe procedure- or device-related adverse events occurred in either group. Device-related adverse events with cyanoacrylate embolization were mostly cases of phlebitis of the treated GSV. The difference might reflect the mechanism of action of the adhesive.10

This study has several limitations, including the retrospective nature of the analysis of a single database, the relatively small number of patients, and the lack of objective measures for evaluating the postinterventional pain and discomfort. We also failed to stratify the patients on the basis of objective measures of reported varicose veins or the quality of life before and after the procedures. We did not record data regarding the disappearance rates of varicose veins after the procedures. We focused only on the closure rates of the techniques. In addition to these limitations, our follow-up period was too short, and data regarding the long-term efficacy of NBCA use are currently lacking.

Thus, the long-term success compared with other treatment options, such as surgery, remains to be assessed by future studies. Furthermore, our study has the limitation that the patients undergoing EVLA and VVSS were not treated concurrently. Therefore, it is possible that the differences noted between the procedures (or lack of difference) may be confounded by unmeasured changes in technique or follow-up care as the result of an evolution of our experience in treating chronic venous insufficiency. In addition, the study was not sufficiently powered to be classified as a noninferiority study. This means that the nonsignificant differences between treatment groups cannot be interpreted to mean that the treatments are equivalent.

CONCLUSIONS
Current minimally invasive methods of ablating the saphenous vein involve the use of thermal energy and require the use of tumescent anesthesia and postoperative compression stockings. The NBCA-based vein-sealing system has been suggested to be a viable alternative method that does not involve the use of tumescent anesthesia or require the postoperative use of compression stockings and has a shorter procedure time. Vein ablation rates and complication rates are comparable to those of EVLA.

AUTHOR CONTRIBUTIONS
Conception and design: IK, HE, FG, MB, NB, EE, AB
Analysis and interpretation: IK, HE, FG, MB, NB, EE, AB
Data collection: IK, HE, FG, MB, NB, EE, AB
Writing the article: IK, HE, FG, MB, NB, EE, AB
Critical revision of the article: IK, HE, FG, MB, NB, EE, AB
Final approval of the article: IK, HE, FG, MB, NB, EE, ME, AB

Statistical analysis: ME
Obtained funding: Not applicable
Overall responsibility: MB

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