Research Article

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Exploring A Novel Approach Using Red and Violet Laser Therapy For Carotid Artery Stenosis - A Case Series

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Keywords

Carotid artery stenosis, Violet laser therapy, Ischemic stroke, Novel therapeutic approaches.

Introduction

Carotid artery stenosis (CS), characterized by atherosclerotic plaque accumulation within the carotid arteries, is a prominent risk factor for ischemic stroke [1]. The World Health Organization recognizes ischemic stroke as the second leading cause of mortality worldwide [2]. Complications arising from unstable atherosclerotic plaque, primarily through embolization (blood clot formation and travel), are responsible for approximately 75-80% of ischemic strokes [3]. However, reduced blood flow (hypoperfusion) due to severe stenosis also plays a role, potentially contributing to an estimated 20-25% of cases. This established link between CS and stroke necessitates the exploration of novel therapeutic approaches.

Current management strategies for CS primarily focus on lifestyle modifications, such as a healthy diet, exercise, and smoking cessation, which aim to improve overall cardiovascular health and potentially slow atherosclerotic plaque progression [4]. Antiplatelet medications are a mainstay of medical therapy [5], while these drugs cannot reverse arterial narrowing, they play a crucial role in reducing platelet activity and consequently the risk of stroke. However, long-term use of antiplatelet drugs comes with its own set of side effects, including stomach ulcers, kidney problems, high blood pressure, and tinnitus. For symptomatic patients with severe stenosis (70% to 99%), carotid endarterectomy, a surgical procedure to remove plaque, is often recommended [6].

Recent studies investigating the effects of low-level laser therapy (LLLT) on vascular health have shown promising results, and may provide a non-invasive, and non- pharmacological therapeutic option for CS. LLLT appears to influence factors like vascular endothelial growth factor (VEGF) and may potentially reduce inflammation within the vessel wall and plaque structure [7,8]. This study aims to contribute to the expanding body of research by evaluating the potential therapeutic effects of LLLT on both plaque size and morphology in patients diagnosed with CS.

Material and Methods

This study enrolled a total of 30 patients (17 male, 13 female) receiving treatment at a vascular surgery Femmed clinic in Warsaw, Poland. All participants were on established pharmacological therapy regimens, adhering to the 2023 Clinical Practice Guidelines on the Management of Atherosclerotic Carotid and Vertebral Artery Disease set forth by the European Society for Vascular Surgery. Throughout the 12-week study period, each patient underwent bi-weekly ultrasound examinations to monitor their condition. Both the North American Symptomatic Carotid Endarterectomy Trial (NASCET) criteria and the Gray-Scale Median (GSM) scale were employed to assess the severity of carotid artery stenosis and atherosclerotic plaque instability, respectively. The GSM value is one of the most widespread methods of studying atherosclerotic plaques and it is important to comprehensively standardize image post-processing. Higher GSM values indicate denser, more calcified plaques, which are generally considered more stable. A lower GSM value suggest softer plaques with a higher lipid or fluid content, potentially indicating a more unstable plaque and a worse prognosis for CS.

All participants received laser therapy using the Erchonia EVRL device, which emits a combination of red (635 nm) and blue-violet (405 nm) lasers, each delivering a power output of 7.5

mW. Treatment sessions were conducted bi-weekly for a duration of 15 minutes. Following ultrasound-guided localization of the atherosclerotic plaque within the carotid artery, the treatment was applied to the neck region. Written informed consent was obtained from each participant prior to their involvement in any study procedures.

Ultrasonographic analysis

All carotid artery examinations were conducted utilizing the Aloka ProSound F75 ultrasound system. A linear 7.5 MHz probe was employed to acquire longitudinal and transverse images of atherosclerotic plaques within the carotid arteries. These acquired images were digitally stored on a USB flash drive for subsequent transfer to CD-ROM discs using a personal computer. The stored images were maintained in the Tagged Image File Format (TIFF) for further computer-based analysis.

GSM analysis

Ultrasound image analysis employed the Gray-Scale Median (GSM) method outlined by Nicolaides et al. [9]. This method has been chosen due to multiple studies demonstrating a positive correlation between GSM values and the presence of unstable atherosclerotic plaques within the carotid arteries. Notably, the multicenter ICAROS study served as the primary foundation for our study design, as it was the first to utilize GSM values for stroke prevention during carotid artery stenting [10]. Consistent with the ICAROS study, a GSM score exceeding 25 was employed as the criterion for plaque instability in our investigation. Patients with GSM values exceeding 25 were categorized into the unstable plaque group. The aim of the study was to assess the effect of low-level laser on atherosclerotic plaque, both its size and its structure.

Results

A total of 30 participants (n = 30) successfully completed the study. The age range spanned from 41 to 80 years old. The participant demographics included 17 males (56.7%) and 13 females (43.3%). Among the participants, 18 individuals (60.0%) identified as current smokers, while 12 individuals (40.0%) reported having diabetes. Notably, 8 participants (26.7%) reported having both smoking and diabetes diagnoses.

Gender: n (%)		Smoking: n (%)		Diabetes: n (%)	
Male	17 (57%)	Smoker	18 (60%)	Diabetic	12 (40%)
Female	13 (43%)	Non-Smoker	12 (40%)	Non-Diabetic	18 (60%)

Change in CAS % Constriction: Baseline to 12 Weeks (Endpoint) Assessment

The study concluded with a statistically significant reduction in the degree of stenotic lesions within the irradiated carotid arteries. This reduction in mean percent stenosis was quantified as -3.8% compared to baseline values. A t-test for two correlated samples was performed to evaluate the significance of the change in mean % constriction value from baseline to endpoint, and it was found that the -3.80 % constriction change was statistically significant at $p<0.0001$ ($t=+6.69$, $df=29$). Table 1 below shows the mean, standard deviation, and range of the % constriction values across all subjects as measured at Baseline and at 12 Weeks (Endpoint), and the change between the two values.

Table 1: CAS: % Constriction: Baseline to 12 Weeks

	Baseline	12 Weeks	Change
Mean	54.97	51.17	-3.80
Std. Dev.	9.36	8.27	3.11
Range	36 - 69	38 – 63	-8 - +7

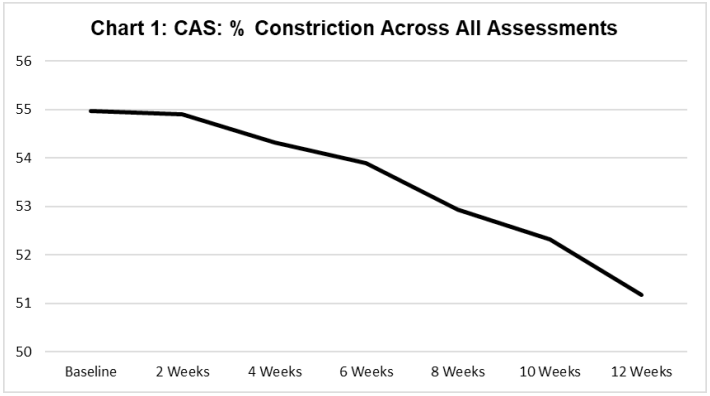
Change in CAS % Constriction: Across All Assessments

From Chart 1 above that there was a consistent progressive decrease in mean % constriction value across study progression that did culminate in a statistically significant decrease at study endpoint (12 weeks). Table 2 below shows the mean, and standard deviation of the % constriction values across all subjects as measured at each assessment point.

Table 2: CAS: % Constriction: All Assessment Points

	Baseline	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks	12 Weeks
Mean	54.97	54.90	54.33	53.90	52.93	52.33	51.17
Std. Dev.	9.36	9.18	8.76	8.95	8.67	8.13	8.27

Chart 1 below shows the trajectory of the % constriction values across the 12 weeks of the study according to the values listed in Table 2 above.



A One-Way ANOVA for 7 correlated samples was performed to evaluate the significance of the changes in values across study duration, which was found to be not statistically significant, at $p>0.05$. ($F=0.786$, $p=0.582$). However, despite the lack of statistical significance, it can be seen from Chart 1 above that there was a consistent progressive decrease in mean % constriction value across study progression that did culminate in a statistically significant decrease at study endpoint (12 weeks) as demonstrated by the baseline to endpoint t-test analysis.

Change in CAS % Constriction: Baseline to 12 Weeks: Analysis by Gender

Gender did not influence the treatment effect of the EVRL laser on CAS % constriction in carotid artery stenosis patients. Table 3 below shows the mean, standard deviation, and range of the % constriction values as measured at Baseline and at 12 Weeks, and the change between the two values, by gender.

Table 3: CAS: % Constriction: Baseline to 12 Weeks: Gender Analysis

	Male (n=17)			Female (n=13)		
	Baseline	12 Weeks	Change	Baseline	12 Weeks	Change
Mean	57.53	53.65	-3.88	51.62	47.92	-3.69
Std. Dev.	9.70	8.04	3.33	8.05	7.69	2.93
Range	36 - 69	39 – 63	-8 - +7	42 - 65	38 – 62	-8 - +3

The difference in the change in mean % constriction value from baseline to endpoint between male and female subjects is minimal at -0.19%, in favor of female subjects. A t-test for two independent samples found this -0.19% difference to be not statistically significant, at $p>0.05$ ($t=+0.16$, $df=28$, $p=0.874$). Therefore, gender did not influence the treatment effect of the EVRL laser on CAS % constriction in carotid artery stenosis patients.

Change in CAS % Constriction: Baseline to 12 Weeks: Analysis by Smoking Status

Although smoking did not affect the efficacy of EVRL laser treatment for CAS % stenosis in patients with carotid stenosis, a change in % concentration from baseline was observed in non-smokers compared to smokers by (-1.30%). Table 4 below shows the mean, standard deviation, and range of the % constriction values as measured at Baseline and at 12 Weeks, and the change between the two values, by smoking status.

Table 4: CAS: % Constriction: Baseline to 12 Weeks: Smoking Status

	Smoker (n=18)			Non-Smoker (n=12)		
	Baseline	12 Weeks	Change	Baseline	12 Weeks	Change
Mean	55.67	52.39	-3.28	53.92	49.33	-4.58
Std. Dev.	10.41	8.85	3.41	7.82	7.30	2.54
Range	36 - 69	38 – 63	-7 - +7	43 - 65	39 – 61	-8 – -1

The mean change in % constriction value from baseline to endpoint is slightly greater for subjects who are non-smokers than for subjects who are smokers, a difference of -1.30%. A t-test for two independent samples found this -1.30% difference to be not statistically significant, at $p>0.05$ ($t=+1.13$, $df=28$, $p=0.268$). Therefore, smoking status did not influence the treatment effect of the EVRL laser on CAS % constriction in carotid artery stenosis patients.

Change in CAS % Constriction: Baseline to 12 Weeks: Analysis by Diabetic Status

Diabetic status did not influence the treatment effect of the EVRL laser on CAS % constriction in carotid artery stenosis patients. Table 5 below shows the mean, standard deviation, and range of the % constriction values as measured at Baseline and at 12 Weeks, and the change between the two values, by diabetic status.

Table 5: CAS: % Constriction: Baseline to 12 Weeks: Diabetic Status

	Diabetic (n=12)			Non-Diabetic (n=18)		
	Baseline	12 Weeks	Change	Baseline	12 Weeks	Change
Mean	55.17	51.67	-3.50	54.83	50.83	-4.00
Std. Dev.	10.73	8.57	3.71	8.65	8.30	2.74
Range	36 - 67	40 – 63	-7 - +7	43 - 69	39 – 63	-8 – +3

The mean change in % constriction value from baseline to endpoint is slightly greater for subjects who are non-diabetic than for subjects who are diabetic, a difference of -0.50%. A t-test for two independent samples found this -0.50% difference to be not statistically significant, at $p>0.05$ ($t=+0.43$, $df=28$, $p=0.67$). Therefore, diabetic status did not influence the treatment effect of the EVRL laser on CAS % constriction in carotid artery stenosis patients.

Change in GSM Score: Baseline to 12 Weeks (Endpoint) Assessment

The mean change in GSM score over a period of 12 weeks was 34.07 and was statistically significant, which supports an increase in the stability of atherosclerotic plaque and a reduction and limitation of the inflammatory process. Table 6 below shows the mean, standard deviation, and range of GSM scores across all subjects as measured at Baseline and at 12 Weeks (Endpoint), and the change between the two values.

Table 6: GSM Score: Baseline to 12 Weeks.

	Baseline	12 Weeks	Change
Mean	65.33	99.40	34.07
Std. Dev.	19.97	7.41	16.74
Range	32 - 102	87 - 115	13 - 55

A t-test for two correlated samples was performed to evaluate the significance of the change in mean GSM Score from baseline to endpoint, and it was found that the mean 34.07 change in GSM Score was statistically significant at $p<0.0001$ ($t=-14.24$, $df=29$).

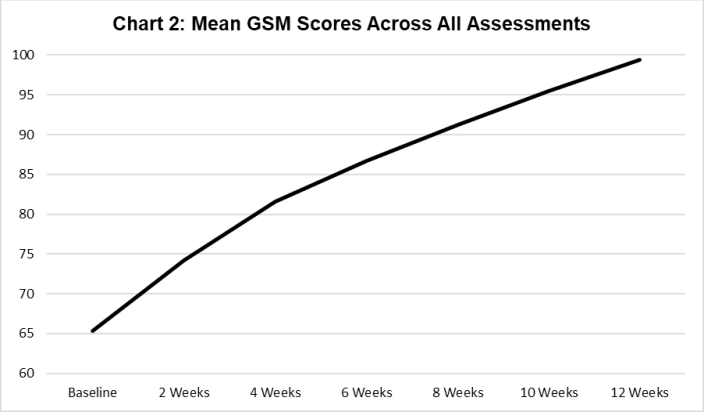
Change in GSM Score: Across All Assessments

Table 7 below shows the mean, and standard deviation of GSM scores across all subjects as measured at each assessment point.

Table 7: GSM Score: All Assessment Points.

	Baseline	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks	12 Weeks
Mean	65.33	74.20	81.60	86.70	91.23	95.43	99.40
Std. Dev.	19.97	16.71	12.99	12.19	9.99	8.19	7.41

Chart 2 below shows the trajectory of the mean GSM scores across the 12 weeks of the study according to the values listed in Table 7 above.



A One-Way ANOVA for 7 correlated samples was performed to evaluate the significance of the changes in mean GSM score across study duration, which was found to be statistically significant, at $p<0.0001$. ($F=25.098$). This indicates that the consistently progressive increase in mean GSM score that occurred across study progression is statistically significant.

Mean GSM Scores: Baseline to 12 Weeks: Analysis by Gender

Gender did not influence the treatment effect of the EVRL laser on GSM score in carotid artery stenosis patients. Table 8 below shows the mean, standard deviation, and range of mean GSM scores as measured at Baseline and at 12 Weeks, and the change between the two values, by gender.

Table 8: GSM Scores: Baseline to 12 Weeks: Gender Analysis

	Male (n=17)			Female (n=13)		
	Baseline	12 Weeks	Change	Baseline	12 Weeks	Change
Mean	67.24	98.76	31.53	62.85	100.23	37.38
Std. Dev.	20.47	7.31	16.58	19.82	7.76	17.00
Range	32 - 92	89 - 112	9 - 58	43 - 102	87 - 115	4 - 67

The difference in the change in mean GSM score from baseline to endpoint (12 Weeks) between male and female subjects is 5.86, in favor of female subjects. A t-test for two independent samples found this difference to be not statistically significant, at $p>0.05$ ($t=+0.95$, $df=28$, $p=0.35$). Therefore, gender did not influence the treatment effect of the EVRL laser on GSM score in carotid artery stenosis patients.

Change in Mean GSM Scores: Baseline to 12 Weeks: Analysis by Smoking Status

Smoking status did not influence the treatment effect of the EVRL laser on GSM score in carotid artery stenosis patients. Table 9 below shows the mean, standard deviation, and range of mean GSM scores as measured at Baseline and at 12 Weeks, and the change between the two values, by smoking status.

Table 9: GSM Scores: Baseline to 12 Weeks: Smoking Status.

	Smoker (n=18)			Non-Smoker (n=12)		
	Baseline	12 Weeks	Change	Baseline	12 Weeks	Change
Mean	61.67	97.78	36.11	70.83	101.83	31.00
Std. Dev.	21.55	7.53	17.71	16.69	6.81	15.37
Range	32 - 92	87 - 110	9 - 67	55 - 102	92 - 115	4 - 56

The mean change in GSM score from baseline to endpoint (12 Weeks) is 5.11, in favor of subjects who are smokers. A t-test for two independent samples found this difference to be not statistically significant, at $p>0.05$ ($t=+0.81$, $df=28$, $p=0.425$). Therefore, smoking status did not influence the treatment effect of the EVRL laser on GSM score in carotid artery stenosis patients.

GSM Scores: Baseline to 12 Weeks: Analysis by Diabetic Status

Diabetic status did not influence the treatment effect of the EVRL laser on GSM score in carotid artery stenosis patients. Table 10 below shows the mean, standard deviation, and range of mean GSM scores as measured at Baseline and at 12 Weeks, and the change between the two values, by diabetic status.

Table 10: GSM Scores: Baseline to 12 Weeks: Diabetic Status.

	Diabetic (n=12)			Non-Diabetic (n=18)		
	Baseline	12 Weeks	Change	Baseline	12 Weeks	Change
Mean	69.83	100.08	30.25	62.33	98.94	36.61
Std. Dev.	23.24	8.23	17.33	17.52	7.02	16.32
Range	32 - 102	87 - 115	4 - 58	43 - 92	87 - 112	9 - 67

The mean change in GSM scores from baseline to endpoint is 6.36, in favor of subjects who do not have diabetes. A t-test for two independent samples found this difference to be not statistically significant, at $p>0.05$ ($t=-1.02$, $df=28$, $p=0.316$). Therefore, diabetic status did not influence the treatment effect of the EVRL laser on GSM score in carotid artery stenosis patients.

Discussion

Mitochondrial Dysfunction and Atherosclerosis

Mitochondrial dysfunction is a well-established hallmark of CS and can be triggered by various factors, including cholesterol, hyperlipidemia, and oxidized LDL [11]. Damage to the mitochondrial protein complexes within the electron transport chain (ETC) disrupts cellular homeostasis and leads to excessive production of reactive oxygen species (ROS) [12]. While basal ROS levels are crucial for cellular signaling, excessive ROS generation overwhelms antioxidant defenses and contributes to oxidative stress, a key driver of atherogenesis [13]. Respiratory complex I (NADH:ubiquinone oxidoreductase) is considered the primary source of mitochondrial ROS (mtROS) [14]. When malfunctioning, electrons may leak during NADH oxidation at the flavin mononucleotide site, generating mtROS. Similarly, dysfunctional respiratory complex complex IV (Cytochrome c Oxidase) can also contribute to increased ROS production due to inefficiencies in electron transfer. This excessive ROS can cause mutations in mitochondrial DNA (mtDNA), ultimately weakening the expression of respiratory protein complexes and resulting in the release of damage-associated molecular patterns and apoptosis-triggering molecules. These events culminate in cell death and inflammation, thereby perpetuating atherogenesis [15].

LLLT: Targeting the Mitochondria for Therapeutic Benefit

LLLT administration with visible wavelengths offers a promising therapeutic approach for targeting mitochondrial dysfunction that may contribute to atherosclerosis. LLLT exerts its effects through a photochemical process involving the absorption of specific wavelengths by mitochondria ETC protein complexes. Red wavelengths are primarily absorbed by complex IV [16], while violet/blue wavelengths are absorbed by complex I [17]. This targeted absorption can enhance the activity of these protein complexes, leading to increased proton gradients and ultimately boosting adenosine triphosphate (ATP) production, a critical energy source for proper endothelial cell function [7]. ATP production can activate transcription factors, such as NF-κB, to induce many gene transcript products [18]. Additionally, LLLT may potentially reduce excessive ROS generation by improving the efficiency of the ETC, thereby mitigating the weakening

effect on respiratory complexes. This ability to modulate ROS production positions LLLT as a potential therapeutic strategy for atherosclerosis.

Potential Synergistic Effects of Red and Violet/Blue Wavelength

This novel study leverages a synergistic combination of red and violet/blue LLLT wavelengths, potentially targeting the mitochondrial ETC. Prior LLLT research by Stemer et al. investigated the efficacy of near-infrared 808nm LLLT for acute ischemic stroke within the first 24 hours of symptom onset [19]. While the primary outcome did not reach statistical significance, the study demonstrated a favorable safety profile yielding valuable insights into future studies.

This is the first known study assessing the combination of red and violet/blue wavelengths on CS degree of stenosis, however past *in-vitro* and animal studies have explored the potential mechanisms of these two wavelengths in arterial disease.

Promoting Cholesterol Efflux

Yin et al. reported that red laser (635nm) administration in atherosclerotic mice promoted macrophage cholesterol efflux, thereby inhibiting foam cell formation in plaques [20]. Their findings suggest that LLLT may upregulate ATP-binding cassette transporters A1 expression in macrophages, facilitating cholesterol efflux and reducing foam cell formation.

Influencing Vascular Tone

Plass et al. investigated the potential of LLLT to induce vasodilation in coronary arteries [21]. Their study employed red light sources of varying wavelengths and demonstrated a dose-dependent vasodilatory effect. The most pronounced vasodilation, reaching 56.8% increase, was observed at 10 J/cm^2. This effect highlights the potential of LLLT to improve blood flow to the heart muscle, potentially alleviating angina symptoms associated with coronary artery disease.

Enhancing Endothelial Cell Growth

In a study by Kipshidze et al., low-power red laser irradiation (632 nm) was found to significantly increase the production of VEGF by smooth muscle cells, fibroblasts, endothelial cell growth and cardiac myocytes [22]. These findings suggest a potentially significant role for LLLT in promoting vascular repair and myocardial photo angiogenesis.

Modulating Nitric Oxide Production

Pope et al. investigated the influence of laser wavelength and irradiance on a key signaling molecule in the vasculature, nitric oxide (NO) [23]. Their study revealed that the specific wavelength of light used significantly impacted intracellular NO levels. Exposure to blue light at 447nm triggered the most substantial increase (33.6%) in NO production compared to red or near-infrared wavelengths. Endothelial NO production plays a multifaceted role in CS. NO exerts well-established vasodilatory effects, but its influence extends beyond this function. NO possesses potent anti-inflammatory properties, critically important in CS, where chronic inflammation within arterial walls is a key driver of atherosclerotic plaque buildup [24]. Additionally, NO inhibits platelet aggregation, thereby mitigating blood clot formation [25].

Cytochrome P450 (CYP) Enzymes

The observed benefits in this study may also be attributable, in part, to the photoactivation of cytochrome P450 (CYP) enzymes by violet/blue light. CYP enzymes possess a heme prosthetic group, which incorporates a porphyrin molecule allowing for maximal light absorption within the violet/blue spectrum [26,27]. Research has demonstrated the ability of CYP enzymes to metabolize arachidonic acid into vasoactive metabolites [28]. Expanding on this concept, subsequent studies have highlighted the critical role of CYP enzymes expressed within the cardiovascular system in regulating vascular homeostasis. Notably, compelling evidence suggests that activation of a specific CYP epoxygenase in endothelial cells represents a vital step in NO and prostacyclin-independent vasodilation, particularly in the heart and kidney [28].

Lipid Reduction

Red light therapy with a wavelength of 635 nm has been demonstrated to stimulate mitochondria within adipocytes, leading to a reduction in adipocyte size [29]. The proposed mechanism for this effect is a multi-step process involving increased ATP synthesis, followed by subsequent upregulation of cAMP levels, activation of protein kinase A, and ultimately, stimulation of cytoplasmic lipase activity. Cytoplasmic lipase is an enzyme responsible for the hydrolysis of triglycerides into fatty acids and glycerol. These breakdown products can then be eliminated from the cell through pores formed in the cell membrane. If a similar mechanism can be induced within atherosclerotic plaques, it offers a potential therapeutic approach for reducing lipid content and plaque burden.

Conclusions

Our investigation explored the application of low-level laser therapy with red and violet/blue wavelengths in patients with carotid artery stenosis. The results demonstrated a significant reduction in stenotic changes, along with enhanced stability of atherosclerotic plaque and reduced inflammation. Notably, these positive outcomes were independent of factors such as gender, smoking history, or diabetic status. These findings suggest that low-level laser therapy holds promise as a therapeutic strategy to not only impede atherosclerotic progression but also potentially mitigate its complications, including ischemic stroke. Further exploration through multicenter trials is crucial to elucidate the underlying mechanisms of action and firmly establish this modality as a novel and efficacious treatment for vascular diseases.

Conflicts of Interest

Dr. Andrzej Eberhardt performed all duties in this case series (including laser procedures, doppler ultrasound and data capturing) at his respective independent site. Dr. Eberhardt received no financial support for the research and has no conflict of interest.

Travis Sammons is an employee of the manufacturer of the EVRL device (Erchonia Corporation) and co-authored the manuscript.

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