Potential Prognostic Biomarkers

Methods: TAHOE I and TAHOE II were prospective, single-arm studies using the same PGA implant. Slight modifications were made to the TAHOE II protocol (eg, removed mandate for heparin use and post-procedural compression). TAHOE I was conducted at three sites in Europe and enrolled 51 patients. TAHOE II was conducted at one site in the Dominican Republic and enrolled 30 patients. After treatment patients returned at 1 day, 1, 2, and 6 weeks, and 3 and 6 months. Vein occlusion, reflux, postprocedure pain (0-10), quality-of-life (CIVIQ2), Venous Clinical Severity Score (VCSS), and adverse events were assessed at each visit.

Results: Occlusion and reflux-free rates are summarized in Table I. Initial occlusion and reflux-free rates were >90% in both studies. Change in CIVIQ2 scores and VCSS scores to 6 months are summarized in Table II. CIVIQ2 scores were elevated at 1 day but showed improvement at 6 weeks that was sustained through 6 months. VCSS improved after day 1 and through to 6 months. Pain (median [IQR]) at day 1 was 2.0 (0.3) in the TAHOE I study and 2.5 (0.5) in the TAHOE II study but decreased to 0.0 (0.0) from 2 weeks to 6 months for both groups. The most commonly reported adverse events were induration (20% TAHOE I, 41.4% TAHOE II), erythema (12% TAHOE I, 48.3% TAHOE II), fever (34.8% TAHOE II), nausea (34.5% TAHOE II), and phlebitis (8% TAHOE I); all resolved by 6 weeks. No patient experienced neuropaxia.

Conclusions: Tumescence-free PGA implantation resulted in high initial occlusion with recanalization appearing in some patients at 3 months postprocedure. PGA is promising, but requires modification to achieve higher long-term occlusion and reflux-free rates.

Midterm Results of a Randomized Controlled Trial Comparing Endovenous Laser Ablation and Surgery for the Treatment of Small Saphenous Insufficiency

S. Nandhra, J. El-Sheikha, N. Samuel, T. Wallace, D. Carradice, I. Chetter. Hull-York Medical School, Hull, United Kingdom

Background: Early results of a randomized control trial (RCT) comparing endovenous laser ablation (EVLA) with surgery for the treatment of small saphenous vein (SSV) insufficiency demonstrated a rapid recovery with lower periprocedural pain and fewer sensory complications in those patients treated with EVLA. Two-year RCT follow-up aims to affirm whether EVLA is as effective as surgery for the management of SSV insufficiency in the medium term.

Methods: Patients with primary sapheno-popliteal junction (SPJ) incompetence and/or SSV reflux were randomized to either EVLA or surgery (SFJ ligation and stripping/excision of the SSV). Follow-up at 1, 6, 12, and 52 weeks assessed clinical recurrence, postprocedural complications, and disease-specific quality of life (QoL; Aberdeen Varicose Veins Questionnaire, AVVQ).

Results: A total of 106 patients were equally randomized, and 88 patients (83%) were assessed at 2 years with equal losses (n = 9) to follow-up in each group. At 2 years, the surgery group consisted of 32 women and 12 men with a median (IQR) age of 48 years (37-57), and the EVLA group consisted of 20 women and 24 men with a median age of 45 (39-58) years.

Recurrence: There was no significant difference in clinical recurrence (surgery, 10/44 [23%] and EVLA, 7/44 [16%]; P = 0.74) or SSV incompetence on duplex (surgery, 7/44 [16%] and EVLA, 2/44 [5%]; P = 157) between the two groups.

Complications: The early significant difference in sensory disturbance became nonsignificant at 2 years (surgery, 3/44 and EVLA, 1/44; P = 1.000).

Conclusions: Two-year follow-up demonstrates that EVLA for SSV insufficiency offers highly efficacious midterm benefits equivalent to surgery, and given its early postoperative superiority, should be considered first-line treatment.

Differential Metabolic Phenotype of Human Varicose Veins Tissue and Their Utility in Understanding Disease Pathogenesis and Identifying Potential Prognostic Biomarkers

M.A. Anwar, P. Vorkas, J. Li, J. Shallow, C.S. Lim, E. Want, E. Holmes, A.H. Davies. Imperial College London, London, United Kingdom

Background: Human metabolic phenotype reflects the generic and environmental perturbations, both of which predispose to the development of varicose veins (VV). It is considered core to understanding disease pathogenesis, and identification of biomarkers and targets for preventative and therapeutic strategies. This study aims to investigate the key metabolic differences between human varicose and non-varicose veins (non-VV).

Methods: Varicose vein segments (n = 81) were retrieved from patients undergoing conventional vein surgery. Non-VV segments (n = 35) were residual conduit veins collected from patients undergoing arterial bypass surgery, carotid endarterectomy, and repair of inguinal hernia. Each vein segment underwent sequential aqueous and lipid metabolite extraction. Aqueous and lipid extracts were analysed using 600 MHz 1H Nuclear Magnetic Resonance (NMR; Bruker) spectroscopy and Ultra-Performance Liquid Chromatography-Q-TOF Mass Spectrometer (UPLC-MS; Waters). Multivariate statistical analyses were performed using SIMCA 13 and MATLAB R2009b.

Results: The mean age of VV and non-VV patients were 45 years (range, 18-82 years) and 62 years (range, 32-85 years), respectively. Higher concentration of glutamate, taurine, and myo-inositol in aqueous extracts and phosphatidylcholine (PC) and sphingomyeline (SM) in lipid extracts were observed in VV group compared with non-VV. These differential metabolites were not correlated with age, gender, body mass index, current smoking, peripheral arterial disease, peripheral arterial disease, and aspirin and statin usage based on Pearson’s chi-square tests.

Conclusions: This study reports higher concentrations of SM, PC, myo-inositol, glutamate, and taurine metabolites in VV tissue extracts. Furthermore, this study proposes PC, SM, and myo-inositol metabolites as potential biomarkers associated with inflammatory and proliferative responses in varicose veins. Future studies should evaluate these metabolites as potential biomarkers for disease prognosis and progression.

Vascular Clinical Severity Score (VCSS), and adverse events were assessed at each visit.

Background: The need for more effective methods in the diagnosis of varicose veins is significant, with patients undergoing repeated imaging of veins, thus leading to increased costs to the healthcare system. Our aim was to investigate the prognostic effect of metabolites in human varicose veins. This analysis was performed using multiple vibrational spectroscopy and nuclear magnetic resonance (NMR) techniques.

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Morphological Changes in the Vein After Different Numbers of Radiofrequency Ablation Cycles

E.V. Shaidakov1, A.G. Grigoryan1, E.A. Ivlykhin2, D.A. Rosukhovskiy3, V.I. Rulatov4. 1Institute of Experimental Medicine of the Russian Academy of Medical Sciences, St Petersburg, Russia; 2Private surgical clinic “Medalp”, St Petersburg, Russia; 3Institute of Bioregulation and Gerontology of the Russian Academy of Medical Sciences, St. Petersburg, Russia

Background: It has not been clarified yet whether it is possible to decrease the percentage of recurrences after radiofrequency ablation (RFA) by way of increasing the number of the RFA cycles. The aim of this study is to assess the morphological changes in excised vein fragments after performing different durations of RFA exposure.

Methods: The study was performed on ten patients having a suprafascial segment of the great saphenous vein (GSV) with more than 22 cm in length and a minimum 5 mm in diameter, who had given their consent to intraoperative excision of suprafascial GSV segments after RFA-treatment through four 1-cm long diametrical wounds. Usually thermal ablation in suprafascial segment results in postoperative phlebitis. Therefore, in every day practice, we try to excise the suprafascial vein segment even if it was initially RFA-treated. Prior ultrasound analysis had shown an average 6.9 mm diameter of the suprafascial segments. The segment was divided into three 7-cm long sub-segments and one control segment of a minimum length of 1 cm. The first, second, and third segment were treated with three, two, and one RFA (ClosureFast) cycles, respectively, while the control segment was not exposed to RFA at all. After the treatment, the segments were excised, placed into a test-tube containing a fixer, and were then morphologically analyzed. The specimens were dyed using Hematoylin and Orcein. The ensuing analysis was performed by an experienced expert using the blind study method (the specimens were numbered without any hint as to the quantity of RFA cycles). The third RFA treatment cycle of the vein wall left all vein wall layers completely homogenized. We also revealed basophil of the intercellular substance and structural changes in the vein wall in the form of cracks all along the vein.
Conclusions: (1) The number of cycles has an impact on the depth of the vein wall damage. (2) One treatment cycle does not cause damage to all layers of the vein wall. (3) Three treatment cycles cause damage to all vein wall layers.

P-Selectin Inhibition Therapeutically Promotes Thrombus Resolution and Prevents Vein Wall Fibrosis Better than Enoxaparin and an Inhibitor to von Willebrand Factor

J.A. Diaz1, S.K. Wrobleksa2, A.R. Pechota3, A.E. Hawley1, K.J. Roscoff1, N.K. Doornbos1, J.E. Gabriel1, G. Reynolds1, P. Lester1, P. Londo1, S. Lowe1, P.K. Henke4, R.G. Schaub2, T.W. Wakefield1, D.D. Myers1
1University of Michigan, Ann Arbor, Mich; 24NKT Therapeutics, Inc, Waltham, Mass

Background: P-selectin (P-sel) and von Willebrand factor (VWF) promote venous thrombosis (VT). Aprompts are oligonucleotides targeting protein/protein interactions like P-sel, VWF, and their ligands. This study tested the therapeutic effects of aptamers against P-sel and VWF compared with a low molecular weight heparin, enoxaparin, on experimental VT.

Methods: Male juvenile baboons underwent experimental iliac VT. Occlusive thrombus was created and confirmed on day 0, and treatment initiated 2 days post-VT. Treatment groups included: controls with no treatment (n = 3); anti-P-sel aptamer ARC5692 (2 mg/kg intravenously [IV] + 1 mg/kg subcutaneously [SQ]); then 1 mg/kg SQ twice a day until euthanasia on day 21 (n = 3); anti-VWF Aptamer ARC15105 (250 μg/kg IV), then single doses of 250 μg/kg IV on days 6, 10, and 14 (n = 3); and enoxaparin 1.5 mg/kg SQ daily until day 21 post-VT. Coagulation tests, hematology, magnetic resonance venography, contrast venography, and ultrasonography were performed on days 0, 2, 6, 14, and 21. At 21 days, inferior venous cava and iliac veins were harvested for histology. Therapeutic levels of drugs were performed: thrombin-antithrombin (TAT) ng/mL, anti-thrombin III (ATIII) % activity, microparticles (MP) nm, fibrinogen mg/dL, prothrombin fragments 1.2 (F1.2) pmol, P-selectin ng/mL, and dilute Russell’s viper venom time (DRVVT) sec. The data were analyzed using the Wilcoxon test (same subject) and the Mann-Whitney test (different subjects).

Results: Significant differences (**) were observed between patients and controls as well as between arm and leg samples in all of the hemostatic markers, except fibrinogen (Table). As depicted in the Fig, TAT levels differed significantly from the control arm sample when compared against patients with varicose veins or leg samples. Evidence to support an increase in thrombotic activity in varicose vein patients is from statistically elevated TAT, ATIII, and F1.2. However, the relationship was inverse with MP and DRVVT. Evidence to support an increase in thrombotic activity in legs > arms is from statistically elevated TAT levels. However, the relationship was significantly inverse with ATIII and F1.2.

Conclusions: There is conflicting evidence for thrombosis risk assessment by elevated venous biomarkers in patients with varicose veins or leg samples. However, the differences observed between arm and leg samples require explanation. Venous leg sampling opens up a new anatomical site of investigation which may have future clinical value.

Table. P values of hemostatic markers comparing patients with controls and arms with legs

<table>
<thead>
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<td>DRVVT (seconds)</td>
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aPTT, Activated partial thromboplastin time; P-sel, P-selectin; VWF, von Willebrand factor. Arrows indicate significant increase or decrease (P ≤ .05).

Regional and Systemic Prothrombotic Biomarkers in Varicose Vein Patients and Healthy Controls

C.R. Lattimer1, E. Kalodiki1, M. Azzam1, G. Geroulakos1, J. Fareed2, D. Hoppernsteadt3, 1Ealing Hospital & Imperial College, London; 2United Kingdom; 3Lovisa University Medical Centre, Maywood, Ill

Background: The relationship between thrombosis and varicose veins is poorly understood. Varicose veins are seen in approximately 50% of patients with post-thrombotic syndrome and may be a risk factor for thrombosis. Furthermore, hemostatic markers are assessed usually from arm blood. Recirculating leg blood may be different in varicose veins. The aim of this study was to determine whether prothrombotic biomarkers varied between patients with varicose veins and healthy controls and whether standard venous samples from the arm differed from leg samples.

Methods: This was a prospective study on 24 patients (17 male; median age, 45 years [range, 25-91 years]) awaiting saphenous laser ablation and 24 healthy volunteers (17 male; median age, 42 years [range, 24-89 years]) without venous disease. The clinical CEAP distribution was: C2, 6; C3, 4; C4s, 1; C4s, 6; C5, 5; C6, 2; with a median Venous Clinical Severity Score and refluxing saphenous vein diameter of 6 mm (range, 4-10 mm) and 8.2 mm (range, 6-12 mm), respectively. Five mL of venous blood was taken from the antecubital fossa, with a concurrent sample from a varicose tributary (patients) or foot vein (volunteers). The following tests were performed: thrombin-antithrombin (TAT) ng/mL, anti-thrombin III (ATIII) % activity, microspheres (MP) nm, fibrinogen mg/dL, prothrombin fragments 1.2 (F1.2) pmol, P-selectin ng/mL, and dilute Russell’s viper venom time (DRVVT) sec. The data were analyzed using the Wilcoxon test (same subject) and the Mann-Whitney test (different subjects).

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Fig. The thrombin-antithrombin (TAT) levels in the normal control subjects were significantly lower than the levels in varicose vein patients and in the control legs.