

# Biomarkers of low-grade inflammation in primary varicose veins of the lower limbs

R. FLORE<sup>1</sup>, F.R. PONZIANI<sup>2</sup>, L. GERARDINO<sup>2</sup>, A. SANTOLIQUIDO<sup>1</sup>,  
A. DI GIORGIO<sup>1</sup>, A. LUPASCU<sup>1</sup>, A. NESCI<sup>2</sup>, P. TONDI<sup>1</sup>

<sup>1</sup>Internal Medicine and Angiology, School of Medicine, Catholic University of the Sacred Heart, Rome, Italy

<sup>2</sup>Internal Medicine and Gastroenterology, School of Medicine, Catholic University of the Sacred Heart, Rome, Italy

**Abstract. – OBJECTIVE:** To analyze serum biomarkers of CVD in selected patients with primary axial reflux of great saphenous vein in one or both lower limbs.

**PATIENTS AND METHODS:** Ninety-six patients affected by uncomplicated varicose veins, were enrolled in the study. A unilateral, primary axial reflux in great saphenous veins was detected in 54 patients (U-CVD group) and a bilateral one in 42 (B-CVD group). Sixty-five age and sex-matched subjects without venous reflux were enrolled as controls. Mean venous pressure of both lower limbs at the distal great saphenous vein (mGSVP) and venous reflux were measured by continuous-wave Doppler ultrasound and echoduplex scanning, respectively. Reactive Oxygen Species (ROS), tissue Plasminogen Activator (t-PA) and its Inhibitor 1 (PAI-1) activities, Hematocrit (HTC), White Blood Cells (WBC), Neutrophils (NEU), Platelets (PLT), Fibrinogen (FIB) and Blood Viscosity (BV) were assessed in blood samples drawn from the antecubital vein.

**RESULTS:** B-CVD group showed higher fibrinogen values ( $p < 0.005$ ) and higher mean venous pressure ( $0 < 0.0001$ ) in comparison to controls, while U-CVD did not. No difference was found between both groups and controls for all the other parameters.

**CONCLUSIONS:** Increased fibrinogen levels in patients with bilateral varicose veins may represent an early warning signal, as it could be associated to the long-term progression of chronic venous disease.

*Key words:*

Inflammation, Varicose veins, CEAP classification, Fibrinogen.

## Introduction

Chronic venous disorders include the whole spectrum of morphological and functional abnormalities of the venous system, whose first steps are telangiectasias, reticular veins or malleolar flare. The occurrence of varicose veins represents the

next step, and is considered the initial manifestation of chronic venous disease (CVD) indicating the need for investigation and care. Varicose veins are the most common form of CVD of the lower limbs, affecting up to 30% of the population in Western countries with a slightly higher prevalence in women than in men (25-33% and 10-20%, respectively)<sup>1-4</sup>. Edema without skin changes, skin changes ascribed to venous disease (e.g., pigmentation, venous eczema, lipodermatosclerosis), with or without healed ulceration and, finally, skin changes with active ulceration are considered complication or further progression of CVD, now named chronic venous insufficiency (CVI). All of these conditions are precisely classified according to presence of clinical signs [C], causes [E], anatomic distribution [A] and pathophysiology [P] (CEAP classification)<sup>5-7</sup>. CVD results in considerable morbidity, with a heavy socioeconomic impact not only due to severe manifestations (CEAP classes C<sub>3</sub>-C<sub>6</sub>) but also due to cosmetic consequences (CEAP classes C<sub>1</sub>-C<sub>2</sub>) that may negatively affect patient's quality of life. Risk factors associated with CVD include age, female sex, family history of varicose veins, obesity, pregnancy, previous deep venous thrombosis, previous leg injury, prolonged standing or sitting posture at work<sup>8,9</sup>. However, many etiopathogenic aspects of CVD are still controversial, in particular in the earlier CEAP classes. Moreover, factors causing the natural progression of CVD are not fully understood.

Recently, CVD has been associated with enhanced oxidative stress, probably the culprit mechanism for vessel wall damage<sup>10</sup>. Our group showed a hyperproduction of reactive oxygen species (ROS) in blood collected from varicose great saphenous veins after one hour of upright position, while no difference was observed after one hour of a recumbent position<sup>11</sup>. ROS hyperproduction in varicose lower limbs could be corrected by great saphenous vein stripping<sup>12</sup>.

Other authors showed that ROS levels and redox state have a key role in controlling blood coagulation cascade and thrombosis<sup>13</sup>. Experimental data demonstrated that ROS can modify fibrinolytic response, affecting the production of tissue plasminogen activator (t-PA) and its inhibitor PAI-1, increasing PAI-1 expression by endothelial cells<sup>14-16</sup>. Finally, several hemorheological abnormalities such as increased blood cell count and aggregation, increased low-shear rate viscosity and elevation in plasma fibrinogen have been identified in venous blood collected from the feet of patients with CVD<sup>17,18</sup>.

In the present study we investigated a well selected cohort of working-age patients affected by primary, uncomplicated varicose veins of the lower limbs with axial great saphenous vein reflux ( $C_{2s}$ ,  $E_p$ ,  $A_s$ ,  $P_{r2,3}$ ) in one or both lower limbs<sup>7</sup>. First, we tested the hypothesis that oxidative stress, modifications of leukocyte and platelet count, impaired fibrinolysis and blood hyperviscosity are present; second, we investigated whether bilateral venous disease versus unilateral venous disease influences the above mentioned biomarkers of disease progression.

## Patients and Methods

The study population was selected among CVD patients of working age consecutively admitted to the Angiology Outpatient Service of the A. Gemelli Hospital, Catholic University of the Sacred Heart, Rome, Italy. They claimed symptoms and signs related to chronic venous disease of the lower limbs and all underwent medical history assessment, physical examination and high resolution B-mode Duplex ultrasonography (IU22 ultrasound machine, Philips Medical Systems, Monza, Italy) of the lower limbs. We selected patients affected by uncomplicated primary varicose great saphenous vein with axial reflux (above and below the knee), i.e.  $C_{2s}$ ,  $E_p$ ,  $A_s$ ,  $P_{r2,3}$  of the CEAP classification, in one or both lower limbs, a 3-5 *Venous Clinical Severity Score* (VCSS) for each limb and a 0-1 *Venous Disability Score* (VDS)<sup>7,19-21</sup>.

Exclusion criteria were edema, skin changes or ulcers of the leg, present or previous deep vein thrombosis, deep or perforating venous reflux. Moreover we excluded patients affected by obesity (BMI >30), peripheral arterial disease, recent infections, chronic alcoholism, habitual smoking, use of antioxidants, phlebotonic drugs or estrogenic progestinics in the last three months, diabetes

mellitus, arterial hypertension, renal failure or cancer. Non-smoking healthy individuals, matched for age and sex, comprised a control group. At the enrollment, none of the patients or controls assumed medications or wore compression socks. The study was approved by the Ethical University Committee, and informed consent was obtained from all patients and controls.

Subjects were examined in a constant temperature room (20°C) and underwent the following:

- Echoduplex examination of the lower limbs according to the Society for Vascular Surgery and the American Venous Forum Practice Guidelines<sup>22</sup>.
- Venous pressure (VP) measurement of the lower limbs: while the subject was standing we applied a sphygmomanometer tourniquet at the calf and measured the pressure at distal portion of the great saphenous vein with a CW Doppler 8 mHz probe in each limb according to a standardized method<sup>23</sup>. The value considered (mGSVP) was the mean of the pressures obtained in each limb, expressed in mmHg.
- Blood collection from the antecubital vein after fasting, by means of a 21-gauge syringe, after minimal stasis (less than a minute) to assess the following:
  - a) Reactive Oxygen Species (ROS).
  - b) Plasma tissue plasminogen activator activity (t-PAA) and plasminogen activator inhibitor-1 activity (PAI-1a).
  - c) Fibrinogen (FIB).
  - d) Hematocrit (HTC).
  - e) White blood cell (WBC) count.
  - f) Platelets (PLT) count.
  - g) Blood viscosity (BV).

For the measurement of Reactive Oxygen Species (ROS), blood was centrifuged at 3000 rpm (0.65 g) for 10 minutes; serum was separated and analyzed for the presence of ROS with the dROMs test (DIACRON, Grosseto, Italy). Briefly, for each patient 5 µl of serum or standard were added to a solution containing 1 ml of acetate buffer (pH 4.8) and 10 µl of chromogen. After incubation at 37°C for 75 minutes, samples were analyzed by spectrophotometry (Beckman DU640) at 505 nm wavelength. Results were calculating using the following formula:

$$\text{ROS} = \frac{\text{Sample absorbance}}{\text{Standard absorbance}} \times \text{Standard concentration}$$

Data were expressed in conventional units (U), 1 U corresponding to a concentration of 0.08

mg/dl hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). The normal range is 200-300 U, while values higher than 300 U indicate oxidative stress (oxidative stress threshold)<sup>11</sup>.

Plasma tissue plasminogen activator activity (t-PAa) and plasminogen activator inhibitor- 1 activity (PAI-1a) were measured by a technique based upon chromogen substrates with a spectrophotometric reading (Beckman DU640) at a 405 nm wavelength according to a previously described method<sup>24</sup>.

Fibrinogen values were obtained from plasma specimens analyzed by photometric testing (range 200-400 mg/dl).

Hematocrit, white blood cell and platelet counts were obtained by an ADVIA 120 Siemens instrument.

Blood viscosity at 230 sec<sup>-1</sup> and 23 sec<sup>-1</sup> was measured by a Brookfield Viscometer with a thermostatic pump at a constant temperature of 37°C.

All measurements were performed in a blinded fashion, were carried out twice on each sample and results were calculated as mean values with an acceptable difference of 10% within each pair.

### Statistical Analysis

Statistical analysis was performed by using the Statistical Package for Social Science (SPSS Inc., Chicago, IL, USA), release 15.0. Continuous variables were expressed as mean ± standard deviation, categorical variables were reported as frequencies and the appropriate parametric

(ANOVA) or non-parametric test (Mann-Whitney or  $\chi^2$  test for categorical variables) was used to assess significance of the differences between subgroups. Multivariate analysis was performed to evaluate the relationship between bilateral CVD and variables significantly correlated at the univariate analysis. The coefficients obtained from the logistic regression were expressed in terms of odds ratios with 95% confidence intervals. A *p* value below 0.05 indicated a significant difference.

### Results

Ninety-six patients were enrolled in the study. A unilateral axial reflux in the great saphenous vein was detected in 54 patients (U-CVD group); 42 subjects showed a bilateral disease (B-CVD group). Control group consisted of sixty-five individuals. The male/female ratio was 19/46 in controls, 15/39 in U-CVD group and 12/30 in B-CVD group, while the mean age was 43.97±7.79 in controls, 42.31±8.17 in U-CVD group and 45.33± 8.86 in B-CVD group. Body Mass Index was 23.63±3.32 for controls, 23.68±2.62 for U-CVD group and 24.71±2.96 for B-CVD group. No statistical difference was found regarding gender, age and BMI between the three groups.

At univariate analysis (Table I), the comparison between U-CVD group and controls was not significant for most of the considered parameters (ROS, t-PAa, PAI-1a, HTC, WBC, NEU, PLTS,

**Table I.** Population characteristics, univariate and multivariate analysis.

	Controls (65)	U-CVD (54)	B-CVD (42)	Univariate (p) <sup>*</sup>	Multivariate (p) <sup>*</sup>
mGSVP (mmHg)	54.27 ± 17.49	58.19 ± 15.55	69.29 ± 20.29	<b>&lt; 0.0001</b>	<b>&lt; 0.0001</b>
ROS (U)	258.45 ± 47.70	258.31 ± 81.02	257.19 ± 84.56	0.992	
t-PAa (AU/ml)	1.13 ± 0.65	1.29 ± 0.58	1.19 ± 0.60	0.402	
PAI-1° (AU/ml)	19.02 ± 10.52	18.38 ± 11.13	19.38 ± 10.81	0.898	
Htc (%)	41.25 ± 3.34	40.46 ± 3.76	40.31 ± 3.52	0.314	
WBC/mmc	5959.38 ± 1573.47	6167.41 ± 1341.21	6014.29 ± 1328.63	0.726	
NEU/mmc	3333.23 ± 1082.41	3609.09 ± 1130.76	3378.57 ± 1058.14	0.362	
PLT (×10 <sup>9</sup> /l)	222.12 ± 44.73	221.22 ± 47.24	218.26 ± 53.61	0.918	
FIB (mg/dl)	251.37 ± 46.73	260.31 ± 53.67	278.12 ± 48.26	<b>0.026</b>	<b>0.004</b>
BV (cp) 230 sec <sup>-1</sup>	4.07 ± 0.37	4.03 ± 0.46	4.07 ± 0.43	0.879	
BV 23sec <sup>-1</sup> (cp)	7.56 ± 0.93	7.49 ± 1.00	7.46 ± 0.85	0.831	

Statistically significant values are in bold. Ctrl = Controls; U-CVD = Unilateral Chronic Venous Disease; B-CVD = Unilateral Chronic Venous Disease; BMI = Body Mass Index; mGSVP = venous pressure at distal great saphenous vein (mean of two limbs); ROS= Reactive Oxygen Species; t-PAa = tissue Plasminogen Activator activity; PAI-1a= Plasminogen Activator Inhibitor activity; HTC = Hematocrit; WBC = White Blood Cells; NEU= Neutrophils; PLTS = Platelets; FIB=Fibrinogen; BV = Blood Viscosity.

\*Controls vs B-CVD (see text for details).

BV 230 sec<sup>-1</sup>, BV 23 sec<sup>-1</sup>); in these patients, exclusively mGSVP was slightly higher in comparison to controls ( $p < 0.01$ ), but this result was not present at multivariate analysis. Patients with B-CVD showed much higher values of venous pressure ( $p < 0.0001$ ; Bonferroni post-hoc test); moreover, fibrinogen levels were higher ( $p < 0.021$ ; Bonferroni post-hoc test) in comparison to U-CVD and controls. No difference in all other parameters was observed. Logistic regression confirmed that higher levels of venous pressure and fibrinogen were independently associated to the presence of B-CVD (OR = 1.05, 95%CI [1.03-1.08],  $p < 0.0001$  and OR = 1.01, 95%CI [1-1.02],  $p = 0.004$ , respectively).

## Discussion

It is well-known that CVI is associated with several microvascular alterations involving inflammatory, rheological, coagulative and fibrinolytic aspects but conflicting data are available on the role of these biomarkers in the earlier CEAP classes. Our group observed that long standing workers not affected by CVD showed high levels of venous pressure of the lower limbs and ROS hyperproduction in the blood drawn from the antecubital vein after work<sup>25</sup>. The use of compression stockings was able to prevent venous hypertension and oxidative stress related to work requiring prolonged standing<sup>26</sup>. Condezo-Hoios et al<sup>10</sup> have recently observed significant changes in plasma oxidative stress biomarkers in the earlier stages of CVD. On the contrary, Blomgren et al reported normal protein C, fibrinogen, homocysteine and PGI<sub>2</sub> levels in patients with uncomplicated primary varicose veins (CEAP class C<sub>2</sub>)<sup>16,27</sup>. Moreover, researches on vein walls showed an absence of inflammatory cells, lower C-reactive protein and no difference in fibrinogen and enzymes involved in inflammation between varicose and healthy veins<sup>28</sup>. Thus, it's evident that conflicting etiopathogenic aspects of CVD are present in the literature.

In the last two decades, both clinical evaluation of CVD and the quality of all related studies have dramatically improved. Thanks to the progressive worldwide acceptance and spread of the CEAP classification, to the awareness of the importance of uniform venous terminology in reports on varicose veins and, finally, to the standardized use of the venous Duplex ultrasound, we can now better identify homogeneous groups

of patients affected by diseases of the same severity, anatomical distribution, symptoms and signs established as of venous origin<sup>5-7,22,29,30</sup>. Moreover, disease-specific and patient-reported quality of life evaluation tools are popular today in venous disease management, and contribute to standardize clinical reports. Thus we integrated the CEAP classification with VCSS and VDS to evaluate biomarkers of CVD progression in a well-selected cohort of patients with primary uncomplicated varicose veins, who were slightly symptomatic but able to carry out usual activities without compressive therapy.

Our results confirmed most of previously published data on inflammatory patterns in earlier CVD stages. In particular, the presence of unilateral, uncomplicated varices of great saphenous veins is not associated with an impaired redox state, hemorheology, or fibrinolysis, and the early CVD classes do not show a low grade inflammatory state. As expected at univariate analysis, U-CVD patients present only a mild elevation in mGSVP, which is the mean of the venous pressure in the varicose saphenous vein and in the non-refluxing saphenous vein of the opposite limb.

Above all, interesting considerations can be drawn from patients with bilateral venous disease. In these patients ROS, t-PA, PAI-1, HTC, WBC, NEU, PLT, FIB and Blood Viscosity show no statistical difference when compared with controls, while fibrinogen and mGSVP are significantly higher. Higher mGSVP is caused by the presence of bilateral axial reflux of varicose saphenous veins and represents a sign of venous stasis which in turn influences fibrinogen, as we observed many years ago<sup>16</sup>.

It has long been debated whether hyperfibrinogenemia is an independent risk factor for cardiovascular diseases, but until now, no prospective clinical trial could establish this causality<sup>31</sup>.

Fibrinogen has often been associated with venous thrombosis, atherogenesis, and cardiovascular or cerebrovascular events. It has recently been shown that hyperfibrinogenemia causes thrombosis in a murine model, increases the risk of cardiac events after coronary artery stenting and is associated with specific histo-cytological composition and complications of atherosclerotic carotid plaques in patients affected by transient ischemic attacks<sup>32-34</sup>. Therefore, hyperfibrinogenemia could be considered a warning signal in the development of a number of venous and arterial diseases.

Our findings may suggest a more aggressive treatment of patients with non-complicated, bilat-

eral varicose veins of the lower limbs (CEAP class C<sub>2</sub>), by means of intravenous thermal ablation or classic ligation and stripping of one varicose great saphenous vein, in addition to compressive therapy. The more compromised vein should be selected on the basis of the vein caliber in orthostatism, according to published guidelines and consensus conferences, symptoms related to chronic venous disease and operator's experience<sup>22</sup>. The presence of bilateral disease could represent an additional operating criterion in selecting patients as candidates for surgery, considering that a marked disparity between the predicted number of patients with varicose veins requiring surgical treatment and the actual care given in different countries has been recently shown in Europe<sup>37</sup>.

Finally, this study presents some limitations. The population of patients enrolled is small and includes a large proportion of young women of working age, slightly symptomatic except for the cosmetic consequences of their disease. None of the enrolled patients was affected by hypertension, diabetes or dyslipidemia, conditions associated with a low-grade chronic inflammation, so our data could not be extrapolated to the general population. On the other hand, some of these biases could represent the strength of our work because our population is representative of a large part of patients affected by uncomplicated varicose veins, mainly in Italy, where the use of compressive stockings is very unusual. Moreover, other biomarkers of low-grade inflammation could be measured in varicose patients, but we preferred to evaluate fibrinogen, platelets and white blood cells as worldwide easily repeatable markers of low-grade inflammation<sup>35,36</sup>, and ROS, t-PA, PAI and blood viscosity because of our long lasting experience in these fields<sup>11,12-18,24-26,38</sup>.

## Conclusions

The intriguing result of this pilot study is that unilateral and locally uncomplicated varicose veins are not associated with low-grade systemic inflammation while bilateral disease shows increased fibrinogen levels than unilateral disease. Long lasting bilateral CVD could have systemic effects whose first sign could be a higher fibrinogen level. Thus, our data could encourage the planning of large-scale prospective clinical studies in earlier CEAP classes in order to evaluate the effect of surgical treatments on fibrinogen or

other inflammatory markers. This could augment our knowledge of the natural history of CVD and offer a better prognostic evaluation to our patients affected by varicose veins of the lower limbs.

## Conflict of Interest

The Authors declare that there are no conflicts of interest.

## References

- 1) EVANS CJ, FOWKES FGR, RUCKLEY CV, LEE AJ. Prevalence of varicose veins and chronic venous insufficiency in men and women in the general population: Edinburgh Vein Study. *J Epidemiol Community Health* 1999; 53: 149-153.
- 2) BEEBE-DIMMER JL, PFEIFER JR, ENGLE JS, SCHOTTENFELD D. The epidemiology of chronic venous insufficiency and varicose veins. *Ann Epidemiol* 2005; 15: 175-184.
- 3) ROBERTSON L, EVANS C, FOWKES FG. Epidemiology of chronic venous disease. *Phlebology* 2008; 23: 103-111.
- 4) RABE E, GUEX JJ, PUSKAS A, SCUDERI A, FERNANDEZ QUESADA F. Epidemiology of chronic venous disorders in geographically diverse populations: results from the Vein Consult Program. *Int Angiol* 2012; 31: 105-115.
- 5) PORTER JP, MONETA GM. Reporting standards in venous disease: an update. *J Vasc Surg* 1995; 21: 635-645.
- 6) KISTNER RL, ECKLOF B, MASUDA EM. Diagnosis of chronic venous disease of the lower extremities; the "CEAP" classification. *Mayo Clin Proc* 1996; 71: 338-345.
- 7) EKLÖF B, RUTHERFORD RB, BERGAN JJ, CARPENTIER PH, GLOVICZKI P, KOSTNER RL, MEISSNER MH, MONETA GL, MYERS K, PADBERG FT, PERRIN M, RUCKLEY CV, SMITH PC, WAKEFIELD TW; AMERICAN VENOUS FORUM INTERNATIONAL AD HOC COMMITTEE FOR REVISION OF THE CEAP CLASSIFICATION. Revision of the CEAP classification for chronic venous disorders: consensus statement. *J Vasc Surg* 2004; 40: 1248-1252.
- 8) FOWKES FG, EVANS CJ, LEE AJ. Prevalence and risk factors for chronic venous insufficiency. *Angiology* 2001; 52: S5-S15.
- 9) EBERHARDT RT, RAFFETTO JD. Chronic Venous Insufficiency. *Circulation* 2005; 111: 2398-2409.
- 10) CONDEZO-HOYOS L, RUBIO M, ARRIBAS SM, ESPAÑA-CAPARRÓS G, RODRÍGUEZ-RODRÍGUEZ P, MUJICA-PACHECO E, GONZÁLEZ MC. A plasma oxidative stress global index in early stages of chronic venous insufficiency. *J Vasc Surg* 2013; 57: 205-213.
- 11) POLA P, DAL LAGO A, FLORE R, TONDI P, SERRCCHIO M, GERARDINO L, ET AL. Hyperproduction of oxygen-free radicals in non-complicated varicose disease of the lower limbs; preliminary data. *J Vasc Invest* 1998; 4: 25-29.
- 12) FLORE R, SANTOLIUQUIDO A, ANTONIO DL, POLA E, FLEX A, POLA R, MUZI MG, FARINON A, RULLI F, GAETANI E, TONDI P, GERARDINO L, GASBARRINI A. Long saphenous vein

- stripping reduces local level of reactive oxygen metabolites in patients with varicose disease of the lower limbs. *World J Surg* 2003; 27: 473-475.
- 13) GÖRLACH A. Redox regulation of the coagulation cascade. *Antioxid Redox Signal* 2005; 7: 1398-1404.
  - 14) SEOK SJ, KIM SJ, GIL HW, YANG JO, LEE EY, HONG SY. Tissue plasminogen activator and plasminogen activator inhibitor-1 levels in patients with acute paraquat intoxication. *J Korean Med Sci* 2011; 26: 474-481.
  - 15) JAULMES A, SANSILVESTRI-MOREL P, ROLLAND-VALOGNES G, BERNHARDT F, GAERTNER R, LOCKHART BP, CORDI A, WIERZBICKI M, RUPIN A, VERBEUREN TJ. Nox4 mediates the expression of plasminogen activator inhibitor-1 via p38 MAPK pathway in cultured human endothelial cells. *Thromb Res* 2009; 124: 439-446.
  - 16) BLOMGREN L, JOHANSSON G, SIEGBAHN A, BERGOVIST D. Coagulation and fibrinolysis in chronic venous insufficiency. *Vasa* 2001; 30: 184-187.
  - 17) KHODABANDEHLOU T, BOISSEAU MR, LE DÉVÉHAT C. Blood rheology as a marker of venous hypertension in patients with venous disease. *Clin Hemorheol Microcirc* 2004; 30: 307-312.
  - 18) POLA P, TONDI P, DE MARTINI D, FAVUZZI A, SANTOLIQUIDO A, GERARDINO L, ET AL. Influence of stasis in fibrinogen values. *Int J Angiol* 1994; 3: 180-182.
  - 19) RUTHERFORD RB, PADBERG FT, COMEROTA AJ, KISTNER RL, MEISSNER MH, MONETA GL. Venous severity scoring: An adjunct to venous outcome assessment. *J Vasc Surg* 2000; 31: 1307-1312.
  - 20) VASQUEZ M, RABE E, MCLAFFERTY R, SHORTELL CK, MARSTON WA, GILLESPIE D, MEISSNER MH, RUTHERFORD RB; AMERICAN VENOUS FORUM AD HOC OUTCOMES WORKING GROUP. Revision of the venous clinical severity score: venous outcomes consensus statement: special communication of the American Venous Forum Ad Hoc Outcomes Working Group. *J Vasc Surg* 2010; 52: 1387-1396.
  - 21) MEISSNER MH, NATIELLO C, NICHOLLS SC. Performance characteristics of the venous clinical severity score. *J Vasc Surg* 2002; 36: 889-895.
  - 22) GLOVICZKI P, COMEROTA AJ, DALSING MC, EKLOF BG, GILLESPIE DL, GLOVICZKI ML, LOHR JM, MCLAFFERTY RB, MEISSNER MH, MURAD MH, PADBERG FT, PAPPAS PJ, PASSMAN MA, RAFFETTO JD, VASQUEZ MA, WAKEFIELD TW; SOCIETY FOR VASCULAR SURGERY; AMERICAN VENOUS FORUM. The care of patients with varicose veins and associated chronic venous diseases: clinical practice guidelines of the Society for Vascular Surgery and the American Venous Forum. *J Vasc Surg* 2011; 53: 2S-48S.
  - 23) BARTOLO M, ANTIGNANI PL, DI FOLCA A, PITTORINO L, TODINI AR. Mesure de la pression veineuse avec le Doppler: standardisation de la methode. *Phlebologie* 1984; 37: 97-101.
  - 24) POLA P, DE MARTINI D, GERARDINO L, DE ROSSI S, TONDI P. The action of propionyl-L-carnitine on the basal endothelium: increase t-PA synthesis and a decrease in the activity of PAI-1. A preliminary study. *Drugs Exptl clin Res* 1992; 18: 343-348.
  - 25) FLORE R, GERARDINO L, SANTOLIQUIDO A, POLA R, FLEX A, DI CAMPLI C, POLA P, TONDI P. Enhanced oxidative stress in workers with a standing occupation. *Occup Environ Med* 2004; 61: 548-550.
  - 26) FLORE R, GERARDINO L, SANTOLIQUIDO A, CATANANTI C, POLA P, TONDI P. Reduction of oxidative stress by compression stockings in standing workers. *Occup Med* 2007; 57: 2337-2141.
  - 27) YASIM A, KILINC M, ARAL M, OKSUZ H, KABALCI M, EROGLU E, IMREK S. Serum concentration of procoagulant, endothelial and oxidative stress markers in early primary varicose veins. *Phlebology* 2008; 23: 15-20.
  - 28) GOMEZ I, BENYAHIA C, LE DALL J, PAYRÉ C, LOUEDEC L, LESÉCHE G, LAMBEAU G, LONGROIS D, NOREL X. Absence of inflammatory conditions in human varicose saphenous veins. *Inflamm Res* 2013; 62: 299-308.
  - 29) CAGGIATI A, BERGAN JJ, GLOVICZKI P, EKLOF B, ALLEGRA C, PARTSCH H. Nomenclature of the veins of the lower limb: extensions, refinements, and clinical application. *J Vasc Surg* 2005; 41: 719-724.
  - 30) EKLÖF B, PERRIN M, DELIS KT, RUTHERFORD RB, GLOVICZKI P. Updated terminology of chronic venous disorders: the VEIN-TERM transatlantic interdisciplinary consensus document. *J Vasc Surg* 2009; 49: 498-501.
  - 31) ARIËNS RAS. Elevated fibrinogen causes thrombosis. *Blood* 2011; 117: 4687-4688.
  - 32) MACHLUS KR, CARDENAS JC, CHURCH FC, WOLBERG AS. Causal relationship between hyperfibrinogenemia, thrombosis and resistance to thrombolysis in mice. *Blood* 2011; 117: 4953-4963.
  - 33) GERMING A, VON DRYANDER S, MACHRAOUI A, BOJARA W, LAWOW T, GREWE P, BARMAYER J, LEMKE B. Hyperfibrinogenemia increases the risk of cardiac events after coronary artery stenting. *Heart Vessels* 2000; 15: 263-267.
  - 34) MAURIELLO A, SANGIORGI G, PALMIERI G, VIRMANI R, HOLMES DR JR, SCHWARTZ RS, PISTOLESE R, IPPOLITI A, SPAGNOLI LG. Hyperfibrinogenemia is associated with specific histocytological composition and complications of atherosclerotic carotid plaques in patients affected by transient ischemic attacks. *Circulation* 2000; 101: 744-750.
  - 35) FOX P, HUDSON M, BROWN C, LORD S, GEBSKI V, DE SOUZA P, LEE CK. Markers of systemic inflammation predict survival in patients with advanced renal cell cancer. *Br J Cancer* 2013; 109: 147-153.
  - 36) KARBINER MS, SIERRA L, MINAHK C, FONIO MC, BRUNO MP, JEREZ S. The role of oxidative stress in alterations of hematological parameters and inflammatory markers induced by early hypercholesterolemia. *Life Sci* 2013; 93: 503-508.
  - 37) MOORE HM, LANE TR, THAPAR A, FRANKLIN IJ, DAVIES AH. The European burden of primary varicose veins. *Phlebology* 2013; 28: 141-147.
  - 38) POLA P, FLORE R, TONDI P. Blood and plasma viscosity in experimentally induced hyper- and hypo-fibrinogenemia. *Int J Tissue React* 1986; 8: 333-336.