Endovascular treatment of chronic cerebro spinal venous insufficiency in patients with multiple sclerosis modifies circulating markers of endothelial dysfunction and coagulation activation: a prospective study

Mariasanta Napolitano[a], Aldo Bruno[b], Diego Mastrangelo[b], Marcella De Vizia[b], Benedetto Bernardo[b], RosaBuonagura [c] and Domenico De Lucia[d]

Objectives **We have performed a monocentric observational prospective study to evaluate coagulation activation and endothelial dysfunction parameters in patients with multiple sclerosis undergoing endovascular treatment for cerebro-spinal-venous insufficiency.**

Materials and methods **Between February 2011 and July 2012, 144 endovascular procedures in 110 patients with multiple sclerosis and chronical cerebro spinal venous insufficiency were performed and they were prospectively analyzed. Each patient was included in the study according to previously published criteria, assessed by the investigators before enrollment. Endothelial dysfunction and coagulation activation parameters were determined before the procedure and during follow-up at 1, 3, 6, 9, 12, 15 and 18 months after treatment, respectively. After the endovascular procedure, patients were treated with standard therapies, with the addition of mesoglycan.**

Results **Fifty-five per cent patients experienced a favorable outcome of multiple sclerosis within 1 month after treatment, 25% regressed in the following 3 months, 24.9% did not experience any benefit. In only 0.1% patients, acute recurrence was observed and it was treated with high-dose immunosuppressive therapy. No major complications were observed. Coagulation activation and endothelial dysfunction parameters were shown to be reduced at 1 month and stable up to12-month follow-up, and they were furthermore associated with a good clinical outcome.**

Conclusions **Endovascular procedures performed by a qualified staff are well tolerated; they can be associated with other currently adopted treatments. Correlations between inflammation, coagulation activation and neurodegenerative disorders are here supported by the observed variations in plasma levels of markers of coagulation activation and endothelial dysfunction. *Blood Coagul Fibrinolysis* 25:000–000 © 2014 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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[a]Hematology Unit, Thrombosis and Hemostasis Reference Regional Center, University of Palermo, Palermo, [b]Division of Endovascular and Vascular Surgery, Clinic GEPOS Telese Terme, Benevento, Italy, [c]Faculty of Medicine, University of Campobasso, Campobasso and [d]Division of Neurology, Second University of Naples (SUN), Napoli, Italy

Correspondence to Napolitano Mariasanta, MD, Hematology Unit, Thrombosis and Hemostasis Reference Regional Center, University of Palermo, Palermo 90127, Italy
Tel: +39 0916554405; fax: +39 0916554402; e-mail: marysanta@libero.it

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Introduction

Multiple sclerosis is a demyelinating disease, which affects approximately 1/1000 of the general population; it has an incidence of approximately 60 000 patients in Italy [1–4]. Multiple sclerosis is a chronic disease characterized by inflammatory lesions with multifocal modification of the coating honey-like state of neurons and subsequent axonal degeneration [2,4–6]. In 2006, it was observed that patients with multiple sclerosis suffered also cerebral and vertebral veins insufficiency; this phenomenon was associated to a slower blood flow with consequent development of collateral veins [7]. The disease was identified with the acronym CCSVI (chronic cerebro spinal venous insufficiency).

Vascular abnormalities, slowing venous outflow, can affect, especially in the brain, the expression of adhesion molecules by the cerebrovascular endothelium, leading to an increased permeability of the blood–brain barrier. Increased vascular permeability is associated with inflammation because of the secretion of pro-inflammatory cytokines from activated endothelial cells. During this process, monocytes have been shown to initiate an autoimmune process against myelin-containing cells [7–13].

Several methods have been evaluated for the diagnosis of CCSVI, and among them, the most suitable for the type of vessels and blood flow has been considered echo-Doppler (ECD), with a specific equipment for slow flows and integrated transcranial Doppler (TCCS).

ECD-TCCS allows the assessment of deep cerebral veins and the detection of any venous reflux [3,10,14–18]. A new access in the ultrasonography evaluation of deep venous cerebral vasculature through the meat of Rosenthal has been recently adopted [18,19]. The same

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expert panel also identified specific parameters to define the diagnosis of CCSVI [18,20].

Activation of coagulation with fibrin deposition has been observed in central nervous system (CNS) blood vessels in animal model of multiple sclerosis [21,22]; growing evidences correlate activation of coagulation to inflammatory disease of CNS [21–23].

We have determined coagulation parameters and markers of endothelial dysfunction to monitor their variations after endovascular treatment of CCSVI.

We here report the results of a prospective study in which patients undergoing endovascular treatment for CCSVI have been evaluated with clinical, instrumental and laboratory determinations before and up to 18 months after the procedure.

Materials

A total of 110 patients were enrolled in this study, from February 2011 to July 2012: 75 females, 35 males (age range: 25–72 years). They all had a definite diagnosis of multiple sclerosis: 23 patients were suffering from a progressive primary-type multiple sclerosis, 32 from relapsing/remitting multiple sclerosis and 55 from a secondary progressive form of multiple sclerosis. Enrolled patients came from all regions of Italy.

Patients treated with immunomodulatory drugs (interferon or glatiramer acetate) were 78% of total, 15% patients were receiving immunosuppressive therapy, and the remaining 7% were off-therapy at study entry. A total of 144 endovascular procedures were performed.

Observation period lasted 12 months in 84 patients and 18 months in 60 patients. Clinical follow-up visits aimed to evaluate the incidence of relapse of multiple sclerosis after endovascular treatment. Patients were regularly followed-up after the procedure at the same institution where the procedure was performed (Clinica Gepos). Informed consent was obtained from each patient enrolled in the current study.

Patients preliminarily underwent ECD-TCCS for CCSVI diagnosis; the examination was always repeated before endovascular treatment, in order to confirm that there were prerequisites for an abnormal discharge of venous vessels (vertebral, jugular veins and deep cerebral veins) of the neck.

ECD-TCCS parameters evaluated are as follows:

(1) Bidirectional flow in one or both of the internal jugular vein (IJV) and/or vertebral vein in both positions or two-way flow in a location with no flow in the other
(2) Bidirectional flow in intracranial veins and sinuses
(3) Intraluminal defect (flaps, valves or septa) associated with hemodynamic changes (blocks, reflux or acceleration) and/or decreased in IJV in supine position to 0.3 cm/q
(4) No flow in IJV and/or vertebral vein and/or no bidirectional flow in one flow and position flow in the other
(5) dCSA IJV greater than or unchanged both at 90 and 0 degree.

The procedure was performed only by expert physicians, when at least two of the criteria for a diagnosis of CCSVI were satisfied [18,20].

Expanded disability status scale (EDSS) was adopted in the evaluation of the clinical picture and of any existing disability [6]. Benefits deriving from endovascular treatment were defined as the disappearance and/or reduction of neurological symptoms with a reduction of at least 0.5 points in EDSS score.

Relapses were defined as the appearance of a new neurological symptom or its deterioration with an increase of at least 0.5 point in EDSS score [24]. EDSS score was calculated by a neurologist the day after endovascular treatment and during follow-up.

EDSS evaluation was based on the following parameters: pyramidal disorder (gait); cerebellar disorders (coordination); cerebral disorders (abnormalities in speech and swallowing); sensorial disorders (abnormal sensations and painful symptoms); bladder and bowel disorders; visual modifications; mental disorders; other (symptoms attributable to multiple sclerosis.)

Endovascular procedure and treatment

The procedure was performed with a femoral access, under local anesthesia with Lidocaine at 2%. The femoral vein was identified on anatomical location or eco-led sting. A short sheath (8 -9 F) was used.

Before the endovascular procedure, 2500 IU of Sodium Heparin were administered. A guidewire 260-cm long, stiff type (Acquatack; Cordis Corporation, Warren, New Jersey, USA), was used in association with 100 from Ber 4F Catheters for jugular veins and Cobra 4F Catheters for azygos veins. Lesions of azygos veins were treated with balloons (Cordis) measuring 8–10 mm in diameter and 4 cm in length, with a pressure from 8 to 10 atm for 120 s.

Lesions of the jugular veins were treated with 10–20 balloons of Cordis with low-compliance chart and a compression between 4 and 8 atm for 120 sec. In the case of hypoplasia of the jugular vein, balloons sized 12–10 mm in diameter were adopted. Hemostasis was achieved with compression.

All patients were discharged on the first day after the procedure. Therapy with low-molecular-weight heparin (LMWH) was started in the evening of the procedure and continued for 20 days after endovascular treatment, at a therapeutic dosage. Following LMWH therapy, the use

of Mesoglycan at a daily dose of 100 mg was recommended up to 24 months after endovascular treatment.

Laboratory assays

Laboratory parameters were first determined in each patient before (from 1 week–1 month) endovascular treatment and they were sequentially repeated during follow-up visits at 1, 3, 6, 9, 12 and 18 months.

The following parameters were assessed: C-reactive protein (CRP), fibrinogen (Fg), coagulation Factor X (FX: c), activated coagulation factor X (FXa), prothrombin fragment (F1+2), homocysteine, tissue-plasminogen activator (t-PA), plasminogen-activated inhibitor (PAI-1), adhesion molecules [intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1)] and Von Willebrand factor antigen (VWF: Ag). All tests were assayed following methods recognized by international guidelines. CRP was determined with an immunoturbidimetric method (Thermofisher, Fisher Diagnostic, Illkirch Cedex - France); Fg plasma levels were assayed by the Clauss functional method. The measurement of factor X activity was performed by a chromogenic method using an automated coagulometer (ACL 2000; Instrumentation Laboratory, Milan, Italy). FXa was determined with a chromogenic assay (Abcam FXa kit Abcam plc, Cambridge, UK). F1+2 were assayed using an ELISA method (Enzygnost F1+2; Behring, Marburg, Germany). Homocysteine was assayed with an automated immunologic method (Hemosil, Instrumantation of Laboratory). T-P and PAI-1 plasmatic antigen levels were measured by enzyme-linked immunosorbent assay (ELISA) (Bouty, Milan, Italy). Circulating VCAM-1 and ICAM-1 concentrations were measured by an immunoenzymatic method (R&D Systems, Minneapolis, Minnesota, USA). Plasma concentrations of VWF: Ag were measured with an ELISA technique (Asserachrom vWF, Diagnostica Stago, Parsippany, New Jersey, USA).

Statistical analysis

The data description was based on standard measures of the distribution, such as mean for the position parameter and standard deviation (SD) for the variability. The normality of the parameter distribution was evaluated through the Kolmogorov Smirnov test. To evaluate parameter linear relations, we also calculated Pearson test and performed regression analysis. To compare median values of different groups, we used the Kruskal–Wallis test when the normality was rejected. Differences were tested with Bonferroni correction at the $P < 0.05$ significance level. Laboratory data that could be expressed in means are shown as means $\pm$ SD. All statistical levels quoted are two-tailed. Correlation analysis between sets of data was performed. Where useful, the confidence interval (CI) of the test value and the P value were provided. Analyses were conducted using the medical software version 7.4.1.2 (http: www.medcalc.be).

This study protocol was approved by the institutional review boards (IRB) of GEPOS Clinic and Second University of Naples. The study has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Results

General demographic data of the patient cohort are shown in Table 1.

Response to treatment was observed in 110 patients after the first endovascular treatment, whereas in 34 cases, endovascular treatment procedure was repeated because of recurrence. Major complications, morbidity and/or disability secondary to endovascular treatment, were not observed. In only two cases, a minor complication (self-limiting inguinal hematoma) was observed; it did not require a prolonged hospital staying. Endovascular treatment procedure was successfully performed in 99% of patients. In only two cases, it was not possible to perform the procedure because of a pre-existing central venous catheter thrombosis.

Clinical and instrumental evaluation

Endovascular procedure for CCSVI determined an improvement of the following neurological symptoms: weakness and paresthesia; functional capacity of arms and legs (with cases of functional recovery immediately after endovascular treatment); sphincter control; vision; headache. There was a low incidence of recurrence during the observation period. A very good correlation (>90%) between ECD-TCCS with dedicated equipment and flebography was observed.

Laboratory determinations

Main results related to laboratory assessment before and after endovascular treatment are shown in Table 2. Values are shown as mean $\pm$ 1 SD. The values reported on the column post endovascular treatment refer to 1 and 12-month follow-up. Interestingly, a statistically significant ($P < 0.05$) reduction in Fg, CRP, coagulation factor X, F1+2, homocysteine, VWF: Ag, t-PA, PAI-1, ICAM-1 and VCAM-1 plasma levels was observed at the first follow-up visit (30 days after endovascular treatment).

Table 1 Demographic and clinical data

Variables (range)	Males, $n = 35$	Females, $n = 75$
Age (years)	40 (28–72)	32 (25–63)
BMI (kg/m^2)	22 (19.1–30.8)	21 (18–27)
	% of subjects	
Smokers	27	
Previous pregnancy	22.6	
NSAID intake	26.2	
Alcohol intake	35	

NSAID, non steroidal anti-inflammatory drugs.

Table 2 Endothelial dysfunction and coagulation activation markers before and after endovascular treatment of CCSVI

Marker	Controls ($n=200$)	After endovascular treatment (1 month) ($n=110$)	After endovascular treatment (12 months) ($n=110$)	Before endovascular treatment ($n=110$)	P value
Fg (mg/dL)	264 ± 75.5	350.5 ± 57.5	425 ± 55.5	425 ± 55.5	0.43
Coagulation factor X (%)	125 ± 32.5	145 ± 35	138 ± 37	165 ± 37.5	0.26
FXa (μg/ml)	0.5 ± 0.25	0.7 ± 0.3	0.6 ± 0.28	0.6 ± 0.34	0.38
F1+2 (nmol/l)	0.45 ± 0.35	0.95 ± 0.75	0.9 ± 0.55	1.95 ± 1.5	0.18
VWF: Ag (%)	145 ± 37.5	155 ± 47.3	147 ± 38.9	200 ± 55	<0.001
t-PA (ng/ml)	6.5 ± 3.5	5.5 ± 2.0	4.9 ± 2.3	11.5 ± 5.6	<0.001
PAI-1 (ng/ml)	37 ± 17.5	30.5 ± 30.5	29.4 ± 23.8	70.5 ± 35.2	<0.001
Hcy (μmol/l)	8.5 ± 4.2	12.5 ± 3.5	11.9 ± 3.7	15.0 ± 1.7	0.03
CRP (mg/dl)	0.95 ± 0.60	2.5 ± 2.5	1.9 ± 1.8	5.5 ± 2.7	0.02
s-ICAM-1 (ng/ml)	157 ± 39	265 ± 60	260 ± 55	305 ± 62	0.115
s-VCAM-1 (ng/ml)	637 ± 99	755 ± 50.5	740 ± 63.5	855 ± 64	0.001

CRP, C-reactive protein; F1+2, prothrombin fragment1+2; Fg, fibrinogen; FXa, activated factor X; Hcy, homocysteine; PAI-1, plasminogen activated inhibitor; s-ICAM-1, soluble intercellular adhesion molecule 1; s-VCAM-1, soluble vascular cell adhesion molecule-1; t-PA, tissue-plasminogen Activator; VWF: Ag, von Willebrand factor antigen.

All these parameters, compared with baseline, were shown to be stably reduced during a 12-month follow-up period (in particular, VWF: Ag: 200 ± 55 vs. 147 ± 38.9, $P < 0.001$; t-PA: 11.5 ± 5.6 vs. 4 0.9 ± 2.3, $P < 0.001$; PAI-1: 70.5 ± 35.2 vs. 29.4 ± 23.8, $P < 0.001$), and they were associated with a good clinical outcome. A very good inverse correlation was furthermore observed between F1+2 and PAI-1 plasma levels ($r = 0.69$, $P = .007$) and homocysteine and F1+2 plasma levels. Controls refer to a population of healthy subjects.

Discussion

Endovascular treatment of CCSVI with angioplasty has been suggested to improve cerebral venous drainage, with significant improvement of symptoms evaluated with EDSS scale [6]. The reason why only some patients respond to endovascular treatment is currently not well understood [14,20]; certainly, a high proportion of patients with multiple sclerosis has lesions of the jugular, vertebral and azygos veins, with a braking in venous outflow.

As regards relapses, they are most frequently recorded in the first 6 months after the procedure, whereas their incidence decreases in the following months. Medical treatment with sodium heparin, LMWH and mesoglycan after endovascular treatment seems to play an important role in preventing re-occlusion but further ad-hoc studies are needed to confirm this hypothesis.

CCSVI treatment offers new perspectives in treating young patients with multiple sclerosis and a low quality of life. The best results are usually obtained in patients with relapsing/remitting multiple sclerosis or secondary progressive multiple sclerosis, a recent diagnosis of disease and a light degree of disability [13,24]. Patients with advanced illness, diagnosed from many years, have a reduced or extremely modest benefit with a high frequency of rest[illegible]is. Some recent studies report discordant results re[illegible] to the clinical relevance of CCSVI [25,26].

Important variables limiting our study are the following: subjectivity of the endovascular treatment procedure, compliance to protocol indications and learning ability of the researchers performing the procedure. Our results, furthermore, derive from a monocentric study even if enrolled patients came from different regions of Italy.

To the best of our knowledge, this is the first report to correlate clinical benefits post endovascular treatment to laboratory markers of endothelial dysfunction and coagulation activation in patients with multiple sclerosis.

We evaluated variations in laboratory markers before and after endovascular treatment and correlated them with the clinical improvement of symptoms. In some cases, a significant reduction of cerebral myelin degeneration was observed after the procedure.

Our findings suggest that endovascular treatment is able to restore a normal endothelial function (stably reduced soluble adhesion molecules) and to control activation of coagulation by reducing fibrinogen, F1+2, coagulation coagulation factor X plasma levels and inflammatory markers like CRP. These results have been observed 30 days after endovascular treatment and they are stable during 12-month follow-up, thus supporting the hypothesis that vascular abnormalities with venous stasis could somehow affect neurological performances of patients with multiple sclerosis and CCSVI.

Our findings support the hypothesis that vascular abnormalities with venous stasis can somehow affect neurological performances of patients with multiple sclerosis and CCSVI, they also suggest that coagulation activation and endothelial dysfunction play a significant role in multiple sclerosis and represent an interesting target for future treatments. Endovascular treatment of stenosis is able to restore a normal coagulation profile and represents a valid therapeutic option.

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Authors' contribution M.N. wrote the article and analyzed data; D.D.L. and A.B. ideated the work and critically

revised all the research process. A.B., M.D.V., B.B. and D.M. enrolled patients, performed the endovascular procedure and critically revised the results. R B.. contribute in writing the article and statistically revise data. All t authors have significantly contributed to the research; they have read and approved the submitted version of the article.

Conflicts of interest

Conflicts of Interest and Source of Funding: None.

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