Ultrastructure of internal jugular vein defective valves

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Abstract
Objectives: To study the ultrastructure of intraluminal defects found in the internal jugular vein by using a scanning electron microscopy.

Methods: Using a scanning electron microscopy, intraluminal septa and/or defective valves blocking the flow in the distal internal jugular vein of seven patients were studied together with the adjacent wall and compared with control specimen.

Results: The internal jugular veins' wall showed a significant derangement of the endothelial layer as compared to controls. Surprisingly, no endothelial cells were found in the defective cusps, and the surface of the structure is covered by a fibro-reticular lamina.

Conclusions: Although the lack of endothelial cells in the internal jugular vein intraluminal obstacles is a further abnormality found in course of chronic cerebrospinal venous insufficiency, our investigation cannot clarify whether this finding is primary or caused by progressive loss of endothelium in relation to altered haemodynamic forces and/or to a past post-thrombotic/inflammatory remodelling.

Keywords
Venous valves, intraluminal defects, truncular venous malformation, internal jugular vein, CCSVI, endothelial cell, ultrastructure, scanning electron microscopy

Background
Intraluminal defects such as membranes, immobile leaflets of the valves and septa have been described in the internal jugular vein (IJV) and classified by a quite recent Consensus among the truncular venous malformations. Venous endoluminal obstacles can strike practically any segment of the major veins, including the cava and the iliac veins, due to an incomplete process in the later stage of the developmental process of the major venous trunks.¹

Intraluminal defects are considered one of the main mechanisms causing a significant delay of jugular flow in course of chronic cerebrospinal venous insufficiency (CCSVI), when investigated by an objective standardized catheter venography method.²

High-resolution B-mode ultrasound corroborated by M-mode has been recently recommended, in a blinded study, to detect readily differences between IJV valves in control population with respect to immobile valve cusps, more prevalent in patients affected by CCSVI.³

Finally, according to the blinded PREMiSe Trial, invasive intravascular ultrasound seems to be the methodology with the best diagnostic accuracy to detect the presence of intraluminal obstacles in the IJVs.⁴

However, the ultrastructure of immobile leaflets/septa affecting the IJVs lumen in CCSVI cases was never investigated.

Patients and methods
Seven patients affected by CCSVI, associated with multiple sclerosis (five males and two females; mean age 44±10 years) were investigated by the means of

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Color Doppler ultrasonography for the presence of defective valves, causing bidirectional flow and/or flow blockages in the IJVs, eventually associated with muscular compression. They also underwent a magnetic resonance venography for evaluation of the presence of restricted venous outflow and IJV stenosis. When both the investigations were positive, we assessed brain perfusion by the means of single-photon emission computed tomography, giving indication to surgery in case of reduced cerebral perfusion respect to the data base of normality. This study was approved by the institutional review board of the Aziende Sanitarie della Provincia di Ferrara.

The selected patients underwent an open surgical repair by the means of omohyoid muscular transection complemented by endophlebectomy and patch angioplasty with autologous vein.

The control tissues were obtained from the IJV wall of three healthy subjects (one male and two females; mean age 75±11 years), who underwent emergent vein repair for traumatic reasons.

The above venous specimens intended for scanning electron microscopy (SEM) analysis were collected and rapidly washed and placed in 2.5% glutaraldehyde for 24h at 4°C followed by 2h of incubation in 1% osmium tetroxide at room temperature. The samples were then treated with decreasing concentration of ethanol ending with a passage on propylene oxide. Finally, the samples were removed and covered with gold through sputter deposition (S 150 Sputter Coater Edwards, England) and examined under an AG-EVO®40 SEM (Cambridge, England).

Results
At careful morphological analysis, performed by SEM, control veins showed a virtually intact endothelial layer, with regular disposition of the cells (Figure 1, top). This appearance changed completely in the diseased specimens, displaying areas of partially detached endothelial cells and the loss of the integrity of the luminal monolayer as evidenced by craters or cavities (Figure 1, bottom).

Surprisingly, SEM analysis of intraluminal obstacles revealed the complete absence of endothelial cells, or of whatever cell, on the luminal side. The surface of the intraluminal defects appears as a fibrous lamina covered by micro-reticular structure (Figures 2 and 3).

Discussion
The histological investigation of IJVs’ wall in CCSVI conditions revealed an excess of type III collagen respect to controls, characterized by the predominance of type I collagen. Coen et al. also demonstrated the absence of

![Figure 1](image_url)
inflammatory cells infiltration in the venous wall. The latter aspect clearly indicates that CCSVI cannot be considered an epiphenomenon of multiple sclerosis, since the immune reaction characterizing the disease was not found in the IJV wall. CCSVI picture seems to be even more an independent vascular picture.

Figure 2. SEM study of a long intraluminal septum, appearing at pre-operative ultrasound as a long and immobile monocusp with no commissure, blocking completely the flow downward the brachiocephalic trunk. (a) Particular of the long free border extended upward till the controlateral wall at 60×. (b) Particular of the wall attachment at 60×. (c–e) At any magnification, 1500×, 5000×, 15,000× respectively, no endothelial cells are visible. The surface of the intraluminal obstacle appears as a fibrous lamina, with a microreticulate entrapping micro-lipidic spheres.

Figure 3. Particular of an immobile leaflet of a defective left jugular valve. At any magnification, lack of endothelium layer is evident, with a very irregular and fibrous lamina intima. Red blood cells and lipidic micro-particles are attached to the surface. The latter, well visible at 5400x, come from the close outlet of the thoracic duct.
The present SEM analysis of the jugular lumen (Figure 1) reveals further significant differences between CCSVI and controls, with detachment of the endothelial cells and an irregular intimal layer as compared to controls. The morphology at SEM of control veins is similar to control tissue of the great saphenous vein characterized by laminar flow, whereas CCSVI IJVs resemble the remodelling seen in the lumen of diseased veins exhibiting turbulences and reflux.9

The more surprising finding at SEM analysis is the lack of endothelial cells in all the examined septa/immobile valve leaflets, and open, of course, new questions and clinical implications, as well. It would be expected that a congenital truncular venous malformation should be lined by something like a single-flattened layer of endothelial cells surrounded by sparse, irregularly distributed smooth muscle cells. To the contrary, the findings shown in Figure 2 cannot exclude that intraluminal fibrosis could be a result from a past, resolved inflammatory or thrombotic process that involved the wall of the IJV.

Furthermore, reports of post-procedural thrombosis following endovascular procedures are less surprising and warrant practical guidelines on dosage, intensity and length of anti-thrombotic prophylaxis.10

Despite the novelty of the findings, our study is inconclusive and cannot explain whether the loss of endothelial cells is primary or caused by altered haemodynamic forces or by post-thrombotic/inflammatory remodelling. Further studies are warranted to determine the pathogenesis of these venous abnormalities.

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Conflict of interest

None declared.

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