

The role of anti-endothelial cell antibodies in venous insufficiency

Reason or consequence?

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Keywords

Anti-endothelial cell antibodies, chronic venous insufficiency, varicose veins

Summary

It is a hypothesis that autoimmune factors directed against endothelial cells play a role in developing venous insufficiency. We investigated the association between anti-endothelial cell antibodies (AECA) and the development of venous insufficiency and varicose veins. **Patients, methods:** 44 patients were evaluated with clinical examination and duplex ultrasound for diagnosing chronic venous insufficiency and varicose veins and 120 healthy volunteers were assigned as the control group without evidence of chronic venous insufficiency and varicose veins. All sera samples were analysed by using slides, each containing biochips coated with frozen sections of HUVEC (human umbilical vein endothelial cells) and capillary-rich tissue such as skeletal muscle (Euroimmun, FB 1960–1005–2, Germany). If a positive reaction is obtained, specific antibodies of class IgA, IgG, IgM attach to the antigens. In a second step, the bound antibodies are stained with fluorescein labeled antihuman antibodies and visualised by fluorescence microscopy. **Results:** AECA was positive in 24 out of 44 patients (54.54%) and in 30 out of 120 volunteers (25%). We detected that anti HUVEC antibody occurred significantly more frequent in patients with chronic venous insufficiency

or varicose veins: $p = 0.0007$, OR: 3.60 (1.65 < 7.92). **Discussion:** The presence of antibodies directed against the endothelial structure causes inflammatory cells of the immune system to move towards the location by both forming antigen-antibody complex and activating the complement system. Tissue damage may occur due to inflammation. In our study we have found a statistically significant relationship between antiendothelial cell antibodies and chronic venous insufficiency. **Conclusion:** Early diagnosis or prediction of venous insufficiency and/or varicose veins before the occurrence of symptoms may prevent the damage or even help to establish a prophylactic treatment.

Schlüsselwörter

Anti-endotheliale Zellantikörper, venöse Insuffizienz, primäre Varikose

Zusammenfassung

Autoimmune Faktoren, die gegen endotheliale Zellen gerichtet sind, spielen eine Rolle bei der Entwicklung und Progression degenerativer Venenklappen der Klappeninsuffizienz. Wir untersuchten die Beziehung zwischen anti-endothelialen Zellantikörpern (AECA) und die Entwicklung der venösen Insuffizienz und primären Varikose. **Patienten, Methoden:** 44 Patienten wurden klinisch und mit farbkodierter Duplexsonographie untersucht, um die Diagnose chronische venöse Insuffizienz oder pri-

märe Varikose zu verifizieren. 120 gesunde Freiwillige wurden als die Kontrollgruppe begutachtet. Wir inkubierten venöses Vollblut auf Objektträgern, die mit eingefrorenen Bestandteilen von HUVEC (humane Endothelzellen von Nableschnurvenen) und kapillarreichem Gewebe wie Skelettmuskel (Euroimmun FB 1960–1005–2, Deutschland) beschichtet sind (Biochip). Die Chips wurden fluoreszenzmikroskopisch ausgewertet. **Ergebnisse:** AECA war bei 24 von 44 Patienten (54,54%) und bei 30 von 120 Freiwilligen (25%) positiv. Der Vergleich von Patienten- und Kontrollgruppe zeigte, dass Anti-HUVEC Antikörper im Patienten mit venöser Insuffizienz und primärer Varikose signifikant höher waren: $p = 0,0007$, OR: 3,60 (1,65 < 7,92). **Discussion:** Die Schädigung endothelialer Strukturen scheint ein wesentlicher Faktor in der Entwicklung venöser und arterieller Erkrankungen zu sein. In unserer Studie haben wir eine statistisch signifikante Beziehung zwischen dem Auftreten von AECA und chronischer venöser Insuffizienz gefunden. Dies könnte die frühzeitige Diagnose oder die Vorhersage der Erkrankung vor dem Auftreten von Symptomen erlauben bzw. eine Intervention rechtfertigen, bevor ein irreparabler Schaden entstanden ist. **Schlussfolgerung:** AECA sind leicht zu bestimmen, kostengünstig und können als zuverlässige Marker für prognostische Studien eingesetzt werden.

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Die Rolle anti-endothelialer Zellantikörper bei venöser Insuffizienz – Ursache oder Folge?

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Venous disease, including varicose veins and chronic venous insufficiency (CVI), is one of the most commonly reported chronic medical condition and a substantial source of morbidity. Besides, it is an important socio-economic problem because of its high prevalence, leading to altered quality of life, high treatment costs, and disability payments. Genetic and environmental factors play important roles in the development of CVI. Numerous contributing etiological risk factors have been described such as

- pregnancy,
- obesity,
- heredity and
- ethnicity.

However, there is still no clear basic mechanism explaining how this disease process starts and progresses. In addition to histological and immuno-cytochemical analyses of venous valves and the venous wall from limbs with venous insufficiency CEAP suggests that lesions observed in various stages of venous insufficiency may be associated with an inflammatory process (1, 2). This inflammatory process includes early leucocyte attachment and infiltration into the valve tissue. This ultimately leads to fibrosclerotic remodelling of the valves and weakening of the vein wall.

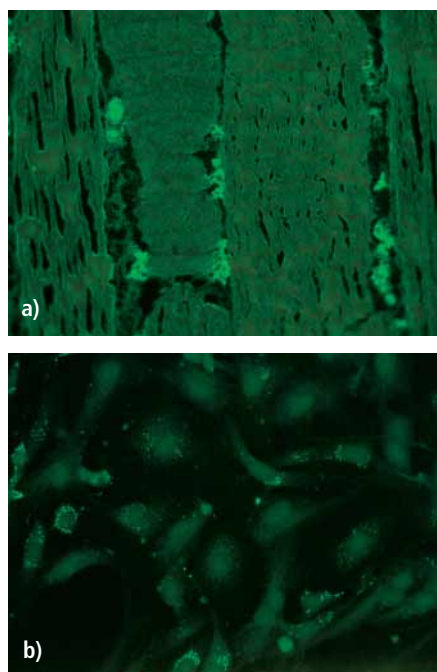


Fig. 1 AECA positivity in **a)** HUVEC; **b)** skeletal muscle (primate)

Haemodynamic forces such as venous hypertension and modified conditions of shear stress in the veins appear to play an important role in triggering this inflammatory reaction. The reaction itself is characterised by early leucocyte activation. This inflammation may be triggered by autoimmune factors.

Antiendothelial cell antibodies (AECA) are a group of antibodies which may have a role in this autoimmune reaction that triggers inflammation. The existence of antibodies reacting with endothelial cells was suggested 20 years ago (3). Furthermore, there are reports concerning the relationship between

- vascular endothelium and AECA and
- AECA and peripheral arterial disease (4).

In this study we tried to investigate the association between AECA and development of varicose veins and venous insufficiency.

Patients, methods

In in this prospective non randomized study 44 patients and 120 healthy volunteers were enrolled. 44 consecutive patients with the diagnosis of varicose veins and/or venous insufficiency according to physical examination and Doppler ultrasound were in the patients' group. The control group included 120 healthy volunteers with no evidence of any venous insufficiency or connective tissue disease.

Diagnosis, therapy

The diagnosis of venous insufficiency was based on medical history, physical examination and venous Doppler ultrasound examination. Demographic data were collected for each group such as age, gender hypertension, hyperlipidaemia, diabetes mellitus, smoking, family history and job history (► Tab. 1). None of the patients had received any treatment for venous insufficiency before admission.

Therapy: The patients received conservative advices, venotropic drugs (diosmin hesperidin, calcium dobesilate or okserutine), compression stockings and they underwent various surgical procedures (sclerotherapy, operation) when needed. The patients were classified in class 0, I or II according to clinical classification of CEAP.

AECA determination

Blood samples were collected both from patients and the volunteers in the control group. All sera samples were stored at -80°C until testing. Each sample was diluted in 1:10 in phosphate buffered saline (PBS)-Tween. We used slides containing BIOCHIPs coated with frozen sections of human umbilical vein endothelial cells (HUVEC) and capillary-rich tissue such as skeletal muscle (Euroimmun, FB 1960–1005–2, Lübeck, Germany). The test specificity for HUVEC (human) is 100%; for skeletal muscle (monkey) it is 100%; the sensitivity for HUVEC (human) is 100%; and for skeletal muscle (monkey), it is 97%. Frozen sections of primate skeletal muscle and cultivated HUVEC covering the reaction areas of the BIOCHIP slides were incubated with a dilute serum sample. A reaction was considered as positive if specific antibodies (classes IgA, IgG, and IgM) attached to the antigens. Levels of AECA were measured according to the fluorescence intensity on microscopy estimated and classified visually as positive (+, ++, +++, +++++). The bound antibodies were then stained with fluorescein-labeled anti-human antibodies and visualized by fluorescence microscopy (► Fig. 1). Results were determined qualitatively and quantitatively.

Statistics

Statistical analysis was performed with SPSS software version 10.0 (SPSS Inc, Chicago, IL). Clinical data were expressed as mean values \pm standard deviation, percents. Chi square and student t test were used when appropriate. Variables of survivors and dead patients were also analyzed with one way Anova. Differences were considered as significant if p was below 0.05.

Results

The mean ages of patients and the volunteers in the control groups were 41.9 ± 2.3 and 40.7 ± 1.6 years, respectively. In the patients' group 24 patients were AECA positive out of 44 patients (54.54%). In the control group 30 volunteers were AECA positive out of 120 (25%); $p = 0.0007$, OR: 3.60 ($1.65 < 7.92$). The demographic data were similar in terms

of age, gender hypertension, hyperlipidemia, diabetes mellitus, smoking, and job history. In patients' group a positive family history was statistically significant when compared with control group. The demographic data are summarized in ► Table 1.

There were 6 patients with CEAP class 0, 16 with class I and 22 with class II. Antiendothelial cell antibodies were positive in 75% of the patients in C I class, positive in 40% of patients in C II class. The distribution of patients with AECA and clinical classification is summarized in ► Figure 2a. In the patients' group in 90% of cases venous reflux according to duplex ultrasound was diagnosed. However, no statistically significant correlation between the reflux incidence and presence of AECA ($p = 0.624$) was found (► Fig. 2b).

No significant differences were found between the two groups with regard to other parameters investigated except for family history. But a correlation between venous insufficiency and varicose veins and AECA positivity was seen: $p = 0.0007$; OR: 3.60 (1.65 < 7.92).

Discussion

The pathophysiology of chronic venous disease is related with several risk factors including older age, female gender, family history, obesity, and standing occupation. However, there are other potential factors that may play a role in disease development and progression which have not been well studied so far and warrant further investigation.

Some studies suggest that lesions observed in various stages of venous insufficiency may be associated with an inflammatory process (1, 2). In this inflammatory process early leucocyte attachment and infiltration into the valve tissue is included. This ultimately leads to fibrosclerotic remodelling of the valves and weakening of the vein wall which is triggered by haemodynamic forces such as venous hypertension and modified conditions of shear stress in the veins. All these events are associated by the release of many inflammatory molecules, and inflammatory regulators (5, 6).

These regulators in addition to leucocyte and endothelial cell activation may be important factors for the remodelling of veins. Autoimmune factors may trigger inflammatory factors. These autoimmune factors may

play a role in endothelial cell activation, leucocyte activity and vein wall injury.

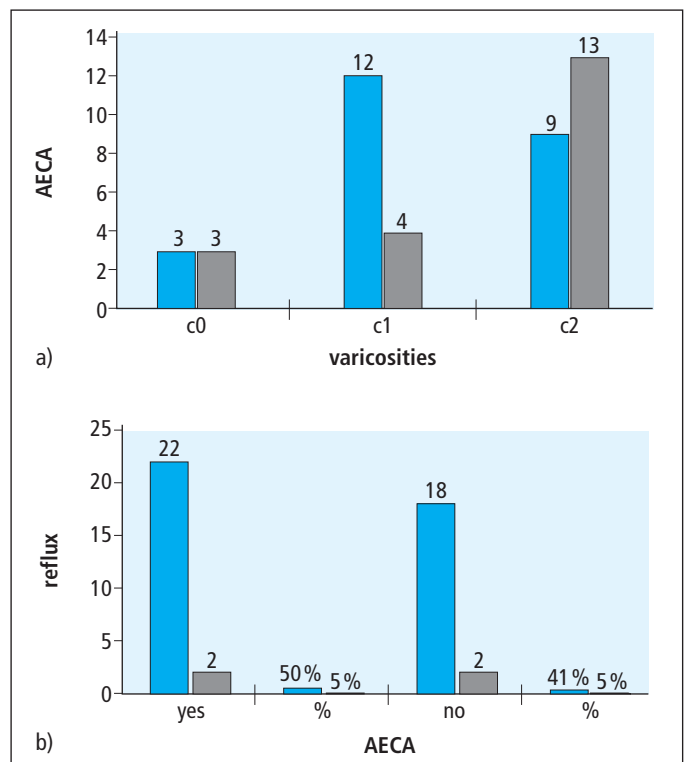
The initiation and progression of vascular wall injury is a complicated process with participation of macrophages, endothelial cells,

lymphocytes, and smooth muscle cells. Endothelial cells, which play a major role in this process, show functional and phenotypic differences with regard to their anatomic locations and/or the vessel type in which they are

Tab. 1
Demographic data

patients			AECA positive	p
age (years) mean (range)	patients	41.9 (30–62)	120/30 (25%)	
	controls	40.7 (25–55)		
gender	women	29	17	> 0.05
	men	15	7	
diabetes mellitus	+	23	5	> 0.05
	–	21	3	
hyper-tension	+	27	11	> 0.05
	–	17	9	
smoking	+	28	12	> 0.05
	–	16	9	
family history	+	32	22	0.013
	–	12	2	
occupation	+	26	12	> 0.05
	–	18	8	
hyper-lipidaemia	+	25	8	> 0.05
	–	19	5	

Fig. 2
Relationship between AECA and
a) clinical classification
c0: no evidence of venous disease;
c1: superficial spider veins (reticular veins) only;
c2: simple varicose veins only
(AECA ■ yes; ■ no)
b) reflux
(■ yes; ■ no)



located (7). Actual studies have reported that the immune system may be responsible for changes in the vascular wall (4,8,9). Therefore, they can be easily recognized by the antibodies in circulation (10). It has been shown that the positive reaction of AECA is effective on a complement system or antibody-related endothelial cell cytotoxicity (11–13).

AECA can be detected in plasma samples from certain diseases (such as Wegener's granulomatosis, Takayasu's arteritis, Kawasaki syndrome) in which various degrees of vascular wall injury are associated. It also has been reported that the incidence of vasculitis is rare without AECA in patients with positive c-ANCA. However, the presence of AECA increases the incidence of vasculitis (14).

We found out a relationship between AECA positivity frequencies and varicose veins or venous insufficiency in the patients' group. In patients with AECA positivity there was also a statistical significance concerning family history, which may support the relationship between autoimmune antibodies and family history. The relation between AECA presence and activation of the disease supports the assumption that AECA may exert important effects on vascular wall injury. The presence of antibodies against the endothelial structure causes inflammatory cells of the immune system to move toward the location by both forming an antigen-antibody complex and by activating the complement system. Tissue damage may occur owing to inflammation. Activated inflammatory mediators and endothelial cell activation may degrade extracellular matrix constituents by release of oxygen free radicals and matrix metalloproteinases (MMPs) activity

(6, 15). Venous hypertension induces morphological changes in valves including fibrosis with strong MMP-2 and MMP-9 activity in valves and during remodelling (16).

We did not study the activity of extracellular matrix constituents and metalloproteinases. Our study only suggests the association of anti-endothelial cell antibodies with venous insufficiency and varicose veins.

Limitations

This study contains a relatively small number of patients. Thus, there is no predictive power. A positive bias in the choice of the control group is another limitation.

Conclusion

The detection of antibodies directed against endothelial cells is

- easy,
- cost effective and
- can be used as a reliable parameter for predictive and prognostic purposes.

Is the presence of such antibodies a result of venous insufficiency? Or are these antibodies one major factor in the development of this disease?

There still is an ongoing debate about the answer to this question. Future studies will help to enlighten this topic. The long term follow up of patients after venotropic drug treatment will also help to enlarge our knowledge concerning the quantity of anti-endothelial cell antibodies.

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Comment

Anti-endothelial cell antibodies in venous insufficiency

Reason or consequence?

This very interesting paper mentions whether up to now unknown autoimmune mechanisms may play a role in the development of venous insufficiency. To investigate anti-endothelial cell antibodies in varicose veins in comparison to competent veins can be seen as an important first step of research that may lead to further autoimmune studies. The

results of the paper suggest that autoimmune mechanism may be relevant for varicose veins, but the number of the patients enrolled in the study is quite small to underline this hypothesis. It could be very interesting to reinvestigate the healthy control persons in some years.

Birgit Kahle, Lübeck
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