A prospective study on hyperhomocysteinemia as an aggravating factor in chronic venous insufficiency

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Abstract. The role of hyperhomocysteinemia (HH) in the etiopathogenetics of systemic thrombotic events has been confirmed by numerous studies. However, it has been insufficiently studied as an etiopathogenic factor in chronic venous insufficiency (CVI). The present prospective study included 166 patients with CVI at stages C3-C6. Homocysteine levels and the inflammatory, metabolic and procoagulant profiles of the patients were determined. High-performance liquid chromatography was used to determine the homocysteine level. Within the patients with HH, the thromboembolic risk was analyzed. Smoking was determined to represent the most common procoagulant factor (21.67%), whereas in the subgroup of women, abortions represented a procoagulant factor for 31.93%. The metabolic profile was altered in approximately half of all cases (42.77%), whereas proinflammatory status was a contributing factor in 23.50% of the cases. HH was present in 54.22% of the CVI patients, mainly in the moderate HH category (53.01%), mostly linked to venous ulcers, thrombophlebitis and pulmonary thromboembolisms. The highest average values of homocysteine were recorded in patients >75 years old and when the venous disease age was >20 years (15.03 μmol/l). In summary, in the present study, HH was a contributing factor of CVI alongside the chronic inflammation that is well known in CVI, which increased thrombogenic risk, especially in elderly patients with an advanced age of venous disease.

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Abbreviations: CVI, chronic venous insufficiency; HH, hyperhomocysteinemia; VTE, venous thromboembolism; CEAP, Clinical, Etiological, Anatomy and Pathophysiology criteria for CVI classification; ELISA, enzyme-linked immunosorbent assay; PT, prothrombin time; APTT, activated partial thromboplastin time; IL-1, interleukin-1; MMP, matrix metalloproteinase; DVT, deep vein thrombosis

Key words: hyperhomocysteinemia, chronic venous insufficiency, thrombosis, aggravating factor, procoagulant state

Introduction

Clinical manifestation of chronic venous insufficiency (CVI) in the lower limbs is complex and varies from edema, venectasia and varicosities to ulcerations. CVI has a progressive evolution with a well-known etiopathogeny, prognosis and therapy (1). CVI prevalence increases with age, is more common in elderly females and has certain contributing factors (pregnancy, prolonged standing, menopause and hormone therapy, among others) (2). The reported prevalence of CVI in Romania has a variable value (68.4% in 2015 and 25% in 2020) (3,4), which is similar to the incidence rate in the rest of Europe (20-60%) (2). Etiopathogenic CVI can be congenital (Klippel-Trenaunay-Weber-Parks syndrome), primitive (hydrostatic varicose veins) and secondary (post-thrombotic, as a result of venous compression or external trauma) (5).

Vascular stenosis and valvular incompetence, as well as the procoagulant state represent essential factors for the initiation and evolution of the thrombotic process. The etiology of venous thrombosis is multifactorial (6). The process usually starts with an injury of the vascular endothelium, which is subsequently followed by activation and aggregation of thrombocytes at the lesion, initiating a procoagulant effect and the constitution of the thrombi (7).

Although at present antithrombotic prophylaxis is performed when necessary, an increase in the incidence of thromboses has been observed. This may be due to numerous associated thrombotic factors, including advanced age, invasive investigations, obesity, prolonged immobilization, birth control, hormonal therapy and neoplasia, which increase pressure on the vein wall (8). Furthermore, certain hematological diseases may induce a procoagulant status, such as leukemia and polycythemia verra, and certain medications (such as hydroxyurea) may favor the appearance of lower leg ulcers (9).

Over the last 25 years, hyperhomocysteinemia (HH) has been included as a risk factor for atherosclerosis and thromboembolisms, as well as for pregnancy and cardiovascular and cerebrovascular diseases (10-12). Homocysteine is an amino acid derived from methionine metabolism and is usually excreted renally. Plasmatic levels of homocysteine are

normally <12 μmol/l. Increased levels can occur in congenital HH as a result of genetic deficiencies in cystathionine β synthase or methylenetetrahydrofolate reductase, enzymes which are responsible for the metabolism of homocysteine. In the acquired form, higher homocysteine levels can be a result of folate, vitamin B12 and vitamin B6 deficiencies, senescence, hypothyroidism, connective tissue diseases, nephropathies, neoplasia and drug intake (13,14).

Taking into account that venous thromboembolism (VTE) can appear during CVI and that the etiopathogenic implication of HH in the thrombotic process is recognized, the present prospective study investigated 166 patients with CVI and determined their homocysteine levels, observing their association with factors involved in CVI and VTE aggravation. The main aim of the present study was to determine the association between homocysteine plasmatic levels and the risk of VTE, in the presence or absence of anticoagulant treatments and venous ulcers in patients with CVI. The thrombogenic factors, which are more often linked to HH, including pregnancy, family and personal history of thrombosis, smoking and certain drug treatments, were also investigated. Furthermore, the link between the metabolic, inflammatory and procoagulant status of patients with HH and CVI was explored.

Materials and methods

Patients. Over a period of one year (June 2011 to May 2012), 166 patients with a mean age of 61.59 years (range, 29-89 years) with CVI who were admitted to the Clinic of Dermatology, County Emergency Hospital of Sibiu (Sibiu, Romania), were enrolled in the present study. Patients under the age of 18 were excluded, as well as patients with chronic venous disease stages, Clinical-Etiological-Anatomical-Pathophysiological classifications (CEAP) C0-C2 (C0, no clinical signs but with symptoms; C1, with telangiectasias; and C2, with varicose veins) (15). The experimental protocol was approved by the Ethics Committee of the County Emergency Hospital of Sibiu. All patients provided signed informed consent before participating in the present study.

The following information was collected for all patients: Demographic data, general associated pathology, personal and family history of thrombosis, procoagulant factors and any previous or current anticoagulant therapies. For every patient, the inflammatory, metabolic and procoagulant statuses [activated partial thromboplastin time (APTT) and prothrombin time (PT)] were analyzed, as well as the levels of homocysteine at the time of admission.

High-performance liquid chromatography (HPLC). Homocysteine levels were determined using HPLC and a Homocysteine in Serum/Plasma HPLC kit (cat. no. 45000; Chromsystems Diagnostics). HPLC was carried out with a Homocysteine in Serum/Plasma kit (cat. no. 45000; Chromsystems Diagnostics) and column (cat. no. 39100, Chromsystems Diagnostics). Patient blood samples were drawn following fasting and the plasma obtained was centrifuged at ambient temperature for 30 min at 10,000 x g 4,000 rpm and then stored at 2-4˚C. A 100-μl sample volume was analyzed, and the flow rate was 1.5 ml/min. Levels over 12 μmol/l were considered to represent increased levels of homocysteine.

Statistical analysis. Data were analyzed using SPSS software (version 23.0; IBM Corp.). The nominal variables are expressed as numbers and percentages and the quantitative data as mean ± SD. A Shapiro-Wilk test was applied to evaluate the normal distribution. An ANOVA was used to compare the means of quantitative data with Gaussian distribution. For non-Gaussian distributed data, the Kruskal-Wallis test was used. As appropriate, associations between two categorical variables were analyzed with Pearson's χ^2 test or Fisher's exact test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Demographic profile. The predominant demographic profile of the CVI patients taking part in the study was women (58.43%) of urban origin (55.42%), from the age group 46-75 years (73.49%; P=0.001).

Clinical forms of CVI. Approximately one-half of the patients were stage CEAP C6 of CVI with active ulcers (42.17%; P=0.118). Almost one-quarter of the cases (22.29%) presented with thrombophlebitis at the time of diagnosis (associated with venous ulcer, 16.07%; without ulcer, 6.22%). In less than one-half of the cases (42.77%) the disease was older than 3 years.

Post-thrombotic syndrome was present in 24.09% of patients (superficial thrombophlebitis, 12.04%; profound thrombophlebitis, 10.25%; and pulmonary thromboembolis, 1.80%) and family antecedent of thrombosis in 8.43% of cases.

Procoagulant factors. Procoagulant factors identified in the study group were smoking (21.67%), surgical interventions (16.27%) and prolonged immobilization (3.61%). For the female subgroup, abortions (31.93%) and hormonal treatments (5.42%) were also identified as procoagulant factors.

Blood coagulation, metabolic and inflammatory profiles. The blood coagulation profile demonstrated low values of PT in 1.20% of cases and APTT in 5.42% of the cases. In approximately one-half of the cases the metabolic profile was altered, with hyperglycemia (22.89%; P=0.176), hypercholesterolemia (42.77%; P=0.876), hypertriglyceridemia (28.92%; P=0.752) and hyperlipidemia (14.46%; P=0.05) (Table I) being present. Less than one-quarter of the patients with CVI presented a proinflammatory profile, with increased inflammation marker levels (erythrocyte sedimentation rate, 23.50%; C-reactive protein, 16.27%; and fibrinogen, 13.86%).

Homocysteine levels. In 54.22% of cases (90 patients), increased plasmatic levels of homocysteine were identified. In patients with HH, the average homocysteine level was 17.33±5.75 μmol/l. Moderate forms of HH, with values of 12-20 μmol/l (42.77%) and 20-30 μmol/l (10.24%) and intermediary forms, with values over 30 μmol/l but lower than 100 μmol/l (1.20%) (Fig. 1) were mainly identified. Comparative analysis between average homocysteine levels in patients with CVI and anticoagulant therapy (17.31±4.89 μmol/l) compared with patients without anticoagulant therapy displayed no significant differences.

Table I. Associations between homocysteine levels and the different parameters assessed in the study.

Parameter	Homocysteine level		Total, patients n (%)	P-value
	Elevated, n (%)	Normal, n (%)		
Frequency	90 (54.22)	76 (45.78)	166 (100)	-
Sex				0.412
Male	40 (44.44)	29 (38.16)	69 (41.57)	
Female	50 (55.56)	47 (61.84)	97 (58.43)	
Origin				0.088
Urban	45 (50.00)	48 (63.16)	92 (55.42)	
Rural	45 (50.00)	28 (36.84)	74 (44.58)	
Age, years				0.001
≤45	8 (8.88)	12 (15.79)	20 (12.05)	
46-60	24 (26.67)	39 (51.32)	63 (37.95)	
61-75	42 (46.67)	19 (25.00)	61 (36.75)	
>75	16 (17.78)	6 (7.89)	22 (13.25)	
CVI clinical stages				0.118
CEAP C3-5	47 (52.22)	49 (64.47)	96 (57.83)	
CEAP C6	43 (47.78)	27 (35.53)	70 (42.17)	
CVI age, years				0.217
<1	26 (28.89)	15 (19.74)	41 (24.70)	
1-3	31 (34.44)	23 (30.26)	54 (32.53)	
3-10	22 (24.44)	28 (36.84)	50 (30.12)	
10-20	9 (10.00)	10 (13.16)	19 (11.45)	
>20	2 (2.23)	0 (0.00)	2 (1.20)	
Personal thrombosis history				0.260
Superficial thrombophlebitis	10 (11.11)	10 (13.16)	20 (12.04)	
Profound thrombophlebitis	10 (11.11)	7 (9.21)	17 (10.25)	
Pulmonary thromboembolism	3 (3.33)	0 (0.00)	3 (1.80)	
Family antecedent of thrombosis	4 (2.41)	10 (6.02)	14 (8.43)	
Procoagulant factors				
Study group				
Smoking	15 (16.67)	21 (27.63)	36 (21.67)	0.088
Surgical interventions	14 (15.56)	13 (17.11)	27 (16.27)	0.788
Prolonged immobilization	3 (3.33)	3 (3.95)	6 (3.61)	0.833
Female study group				
Abortions, n				0.218
1	16 (17.77)	14 (18.42)	30 (18.07)	
2	1 (1.11)	7 (9.21)	8 (4.82)	
3	3 (3.33)	4 (5.26)	7 (4.22)	
4	3 (3.33)	5 (6.58)	8 (4.82)	
Hormone treatment	6 (6.66)	3 (3.95)	9 (5.42)	0.697
Blood coagulation profile				
Low PT	0 (0.00)	2 (2.63)	2 (1.20)	0.189
Low APTT	4 (4.44)	5 (6.58)	9 (5.42)	0.752
Inflammatory profile				
Elevated CRP	12 (13.33)	15 (19.74)	27 (16.27)	0.790
Elevated ESR	16 (17.77)	23 (30.26)	39 (23.50)	0.820
Elevated fibrinogen	10 (11.11)	13 (17.11)	23 (13.86)	0.898
Metabolic profile				
High cholesterol	38 (42.22)	33 (43.42)	71 (42.77)	0.876

Table I. Continued.

Parameter	Homocysteine level: Elevated, n (%)	Homocysteine level: Normal, n (%)	Total, patients n (%)	P-value
High triglycerides	24 (26.67)	24 (31.58)	48 (28.92)	0.752
High lipids	15 (16.67)	9 (11.84)	24 (14.46)	0.050

CVI, chronic venous insufficiency; CEAP, Clinical, Etiological, Anatomy and Pathophysiology criteria for CVI classification; PT, prothrombin time; APTT, activated partial thromboplastin time; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.

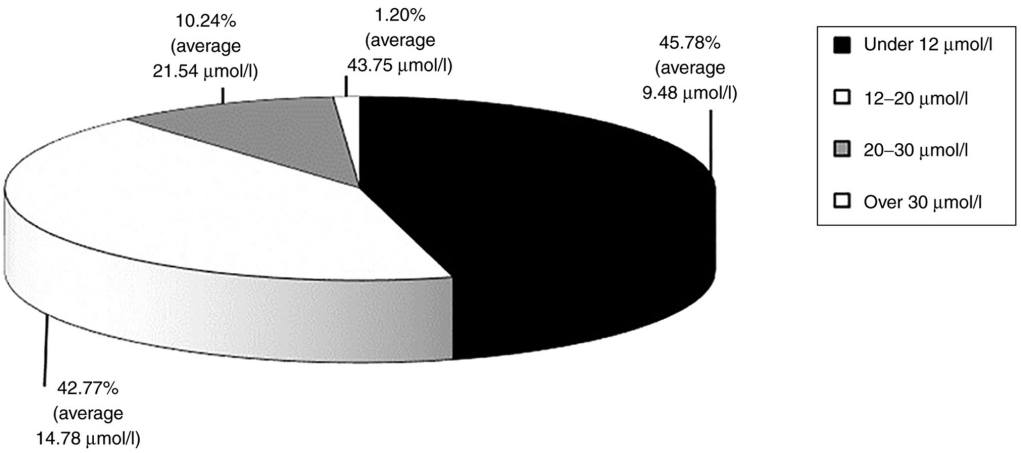

Figure 1. Plasmatic homocysteine levels (using HPLC) in the study group showing that 54.21% of the cases had increased values. HPLC, high-performance liquid chromatography.

Homocysteine levels were linked to a CVI diagnosis with ulcers (15.24±7.02 μmol/l) and a CVI diagnosis with ulcers and thrombophlebitis (14.13±4.08 μmol/l), compared with 13.12±5.81 μmol/l for patients with CVI CEAP C3-5 (P=0.138) (Table II). Homocysteine levels were also linked to pulmonary thromboembolism (15.33±4.07 μmol/l) and deep vein thrombosis (DVT; 14.08±5.97 μmol/l) (P=0.819) (Table II). The average homocysteine levels presented the highest levels for patients >75 years of age (15.03 μmol/l) and the age group 61-75 years (14.53 μmol/l).

The age of CVI was linked to HH (P=0.217). Average homocysteine levels varied between 11.44±3.23 μmol/l in patients with a CVI disease age of <1 year and 15.03±6.49 μmol/l in patients with CVI >20 years (P=0.148) (Table II). The differential analysis of homocysteine on subgroups of patients presenting with CVI without anticoagulant therapies, compared with patients with anticoagulant therapies, demonstrated that for a disease age ≤10 years, 56.40% compared with 35.70% of the cases presented with normal homocysteine levels, and for a disease age of >30 years, 87.50% of the cases compared with 71.40% of cases presented with increased homocysteine levels (P=0.050). The average homocysteine levels in patients with obesity compared with patients of normal weight was similar (13.77±4.07 μmol/l vs. 13.33±7.19 μmol/l; P=0.931) (Table II).

For patients with HH, no statistically significant associations were demonstrated between homocysteine levels and the inflammatory, metabolic and blood coagulation profiles, as well as certain thrombogenic factors, including prolonged immobilization, recent surgical interventions and hormone therapies (Table I). Among the procoagulant factors, smoking was statistically linked to HH in patients with CVI without anticoagulant therapy (P=0.088) compared with patients with CVI with chronic anticoagulant therapy.

Discussion

In CVI of the lower limbs, an increase in the intraluminal pressure determines alterations in endothelial structures with secondary cutaneous lesions. The dysfunction of the endothelium results in endothelial adhesion molecule expression, producing a chemotactic effect for leukocytes. The activated leukocytes release cytokines, including transforming growth factor-β, vascular endothelial growth factor, interleukin-1 (IL-1), tumor necrosis factor α and proteinases, which consequently initiate and maintain the inflammatory signaling cascade resulting in a procoagulant and proatherosclerotic state (16). The procoagulant state is a key etio-pathogenic factor of VTE and indirectly of venous and post-thrombotic ulcers. The interaction between chronic inflammation and thrombosis serves an essential role in post-thrombotic chronic ulceration (16). Matrix metalloproteinases (MMPs), whose activity is stimulated by chronic inflammation, serve an important role in the occurrence of ulcers (17,18). Excess MMP results in changes in fibroblast function alongside dermal

Table II. Mean ± SD homocysteine levels in the study group, associated with the diagnosis of CVI, disease age, personal thrombosis history and obesity.

	Homocysteine			
Parameter	Total patients, n	Mean	Standard deviation	P-value
Diagnosis				
CVI with ulcer and thrombophlebitis	26	14.1319	4.0896	0.138
CVI with thrombophlebitis	11	11.4745	4.2995	
CVI with ulcer	44	15.2450	7.0275	
CVI without ulcer	85	13.1275	5.8134	
Total	166	13.7366	5.9071	
Disease age, years				
<1	4	11.4450	3.2300	0.148
1-3	17	12.4618	4.1013	
3-10	50	12.4368	4.7084	
10-20	54	14.5261	6.7933	
>20	41	15.0339	6.4982	
Total	166	13.7366	5.9071	
Obesity				
Without	37	13.3373	7.1972	0.931
With	129	13.7792	4.0765	
Total	166	13.7366	5.9071	
Personal thrombosis history				
Superficial thrombophlebitis	20	12.6835	2.8670	0.819
Profound thrombophlebitis	17	14.0847	5.9785	
Pulmonary thromboembolism	3	15.3333	4.0723	
Without	126	13.8187	6.3014	
Total	166	13.7366	5.9071	

CVI, chronic venous insufficiency.

fibrosis, proliferation of dermal capillaries and deterioration of extracellular matrix and glycocalyx (19).

Deficiencies in anticoagulant factors (such as anti-thrombin 3, S protein, C protein, factor V) or mutations of certain antithrombotic genes (such as prothrombin gene G20210A), activated C protein resistance and HH, can induce a congenital procoagulant state. Numerous diseases have also previously been determined to induce acquired hypercoagulant states, including neoplasia, cardiovascular diseases, cerebrovascular diseases, venous and hepatic insufficiency, diabetes mellitus, dyslipidemia, connective tissue diseases and HH, as well as tobacco smoking, prolonged immobilization and the long use of oral contraceptives.

As a factor with procoagulant value, HH can occur via congenital deficiencies, including cystathionine β synthase deficiency, methylenetetrahydrofolate reductase deficiency or innate deficiencies in the metabolism of cobalamin. However, HH can also be acquired, such as in folate, vitamin B12 and vitamin B6 metabolism disorders, chronic renal insufficiency, hypothyroidism and neoplasia (breast, ovarian and pancreatic neoplasms, and leukemia), as well as a result of certain treatments (for example, methotrexate, phenytoin, theophylline and phosphodiesterase inhibitors), advanced age and smoking (13,14,20-24). In the present study, a hypercoagulant state induced by CVI, mainly via HH (54.22%), and the general associated pathology (86.14% of cases presented with associated cardiovascular pathology) were identified.

Advanced age is a risk factor for hypercoagulability. This is a consequence of endothelial modifications, which occur during young adulthood and middle age as a result of cardiovascular risk factors (25). Increases in the concentrations of certain procoagulant factors, such as fibrinogen, factor VII, VIII and X and the reduction of certain anticoagulant factors, such as plasminogen, antithrombin III and tissular activator of plasminogen, also contribute towards endothelial modifications (16). In the present study, HH occurred more often in individuals >60 years old (64.45%), with an average homocysteine level of 17.93 μmol/l, compared with that in individuals <60 years old (35.55%), with an average homocysteine level of 15.55 μmol/l (P=0.024).

Blood coagulation and fibrinogen profiles have also been demonstrated to be linked to thrombosis risk. Therefore, low APTT and elevated fibrinogen levels are associated with an increased risk of DVT and pulmonary thromboembolism.

Low PT and international normalized ratio also serve a minor role in determining the procoagulant state (16). In the present study, a small number of patients displayed elevated fibrinogen (13.86%) and low APTT (5.42%) levels.

HH is a risk factor in arteriosclerotic cardiovascular and cerebrovascular diseases, as it is a promoter of endothelial oxidative stress and stimulates the production of reactive oxygen species. Furthermore, HH favors leukocyte inflow mediated by endothelial adhesion molecules, with the infiltration of leukocytes in the vascular wall and the release of chemokines inducing a proinflammatory response (26). In the present study group, in patients with CVI, HH was associated with pulmonary thromboembolism (100%), the presence of ulcers (61.50%), ulcers associated with thrombophlebitis (61.40%; P=0.003) and profound thrombophlebitis (58.80%; P=0.260). Alterations of lipid status (with the implication of adipokines and adiponectin) results in a proinflammatory effect over time. Chronic inflammation of the vascular wall associated with HH can influence the hypercoagulant state (27). In the present study, the analysis of the metabolic profile (glucidic and lipid) in patients with HH compared with patients with normal levels of homocysteine, was not significant. HH also contributes to venous and arterial thrombotic diseases by increasing the expression of tissular factors, alleviating anticoagulant processes, improving the thrombocyte activity, increasing thrombin production, intensifying the activity of factor V, altering the fibrinolytic potential and favoring vascular injuries (28). HH is a recognized thrombotic risk factor and also serves a role in certain complex diseases, such as Klippel-Trenaunay-Weber syndrome (29,30).

Homocysteine treatments are useful, as HH is one of the few post-thrombotic risk factors that can be corrected by administering folic acid and vitamins B6 and B12. However, although treatment using folic acid and vitamin B supplements can lower the homocysteine level by 25%, it does not prevent venous thrombosis from reappearing (31). The prevalence of HH in patients with CVI in the present study was 21.18%. In a meta-analysis published by Ray (32), the prevalence of HH in patients with VTE varied between 5.7 and 34.8% compared with 0-7.1% in healthy individuals. This study also reported that an increase of 5 μmol/l homocysteine can lead to 2-3-fold increase in the risk of VTE. Compared with the study by Ray (32), the present study determined that HH prevalence is relatively similar for patients with CVI and VTE.

HH can be moderate (<30 μmol/l), intermediary (30-100 μmol/l) and severe (>100 μmol/l) (33). In the present study, 54.22% of patients with CVI presented with HH. Almost all patients with HH presented with moderate HH (97.79%) and 2.22% presented with intermediary HH, with no cases of severe HH. These results indicated that moderate and intermediary HH may be an aggravating factor in CVI evolution with DVT and venous ulcer risk.

Increases in plasmatic levels of homocysteine were also demonstrated in the present patients with CVI with ulcers, thrombophlebitis and pulmonary thromboembolic episodes, in elderly patients, in patients with advanced disease age and in smokers.

In conclusion, HH is a well-known risk factor in arterial and venous thrombotic diseases and also serves an important role in CVI, which increases the thrombogenic risk in these patients, especially in the elderly and those with an advanced venous disease age. Treatment of moderate HH in CVI by administration of folic acid, vitamin B6 and vitamin B12 can lead to the alleviation of symptoms, reducing the risk of VTE and their complications and implicitly slowing down the progression of CVI.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

MR and GMI created and designed the present study, analyzed and interpreted the patient data, and drafted and revised the manuscript for important intellectual content. IB was responsible for data acquisition, analysis and interpretation of patient data, manuscript drafting and design. All authors read and approved the final manuscript. All authors confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the County Emergency Hospital of Sibiu (Sibiu, Romania; approval no. 356) and all patients provided written inform consent regarding participation in the study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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