ORIGINAL ARTICLE

Impact of coagulation in the development of thromboembolic events in patients with spinal cord injury

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Study design: Although the knowledge described about risk factors and venous thromboembolism (VT) in the general population, the impact of these factors in the development of thromboembolic events in patients with spinal injury (SI) caused by spinal cord injury (SCI) is poorly understood.

Objective: Evaluate the impact of risk factors in the development of thromboembolic events in patients with SCI.

Setting: Brazil, São Paulo.

Methods: Observational, prospective and cross-study. Eligible patients ($n=100$) had SI by SCI, > 18 years. The degree of motor and sensory lesion was evaluated based on American Spinal Injury Association (ASIA) Impairment Scale (AIS). Blood samples were collected for coagulation exams, hemogram, laboratory and biochemical analyses. Ultrasonography analyzes were performed from deep and superficial venous systems of lower limbs. Quantitative real-time PCR experiments were performed in order to investigate mutations in the prothrombin (G20210A) and Leiden factor V (G1691A) genes.

Results: The main finding of this study was the higher occurrence of deep venous thrombosis (DVT) in patients with Leiden factor V and hyperhomocysteinemia. There was no association between SI for DVT, VT and thrombophilia. Also, there was no relation between lupus anticoagulant and anti-cardiolipin.

Conclusion: There is an important difference in the incidence of DVT in patients with SI by acute and chronic SCI. Therefore, the conduct of the investigation for thrombophilia should be based on clinical factors, risk factors for DVT and family history of thrombosis.

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Keywords: thromboembolism; spinal cord injury; Leiden factor V; hyperhomocysteinemia.

The venous thromboembolism (VT), that involves deep venous thrombosis (DVT) and pulmonary embolism, is considered a common disease, with annual incidence of one to three cases per 1000 individuals.¹ There are many factors related to VT, including age, male gender, pelvic and lower limb fractures and spinal injury (SI).²

It is well established in the literature the relation between DVT and SI. This incidence has increased in the last years,³ ranging between 18 and 100%, depending on the diagnostic methods used, lesion time, associated risk factors and prophylactic treatment.⁴ Sequelae from SI can affect the three components from classic Virchow triad, responsible for the genesis of VT: blood flow changes, endothelial cell damage and production of procoagulants, leading to hypercoagulability state. Besides, pulmonary embolism is the serious complication and is the second leading death cause in these patients. The incidence of pulmonary embolism varies between 4.6 and 14% of the cases, being fatal between 1.7 and 4.7%.⁵

In the venous system vascular, many changes can induce DVT, for example, partial or total paralysis of the muscles, affecting the blood flow due changes in the venous competence, causing the reduction in the capacity and distensibility of vascular bed and increase in the

venous flow resistance. These factors promote metabolic changes in blood vessels generating hypoxia associated to endothelial cell damage in the vessel wall, adhesiveness and platelet aggregation, coagulation cascade activation and venous thrombosis. The hypercoagulability in the patients with SI is due to multiple mechanisms, including coagulation cascade activation, modifications in endothelial and other blood cells.⁶

Many genetic components constitute risk factors for VT. The majority affects the natural pathways of coagulation, mainly the C protein system. Resistance to activated C protein is the hypercoagulability state more frequently associated with the pulmonary embolism development, caused by a Leiden factor V gene mutation (Arg 506 for Gly).¹ This mutation is found only in Caucasians, but the prevalence varies among the different countries.⁷ The second most frequent mutation occurs in the not translated position 3 in the prothrombin gene (G 20210 A).⁸ Less frequent genetic changes are the scarcity in the natural anticoagulant proteins (anti-thrombin, C and S protein).¹ Other acquired risk factors are sedentarism and tabagism,⁹ lupus anticoagulant, anti-cardiolipin antibody, hyperhomocysteinemia and increase of coagulation factors, mainly factors I and VIII.¹

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Although the knowledge described about risk factors and VT in the general population, the impact of these factors in the development of thromboembolic events in patients with SI caused by spinal cord injury (SCI) is poorly understood, and is the object of this study.

Design and study population

Observational, prospective and cross-study. Patients ($n=100$) were recruited from the Physical Medicine and Rehabilitation Institute, University of Sao Paulo, Faculty of Medicine Clinics Hospital USP—IMREA/Lucy Montoro Network, from January 2011 to April 2012. Eligible patients had SI by SCI, > 18 years; the exclusion criteria were patients with SI for other reasons (not SCI), as cancer, lupus, multiple sclerosis, spinal compression by disc herniation, motor sequelae from stroke or subjects that presented clinical signs compatible with infection. The fluxogram is represented in Figure 1.

Figure 1 Fluxogram of the study. It was included SCI patients ($n=100$), with and without clinical suspicion for DVT. PT, prothrombin time; aPTT, activated partial thromboplastin time.

Variables from the study

The variables investigated were: age, gender, race, type of SI (paraplegia or tetraplegia), injury time, etiology of SI and degree of motor and sensory lesion, using the American Spinal Injury Association (ASIA) Impairment Scale (AIS), or Scale of Deficiency from ASIA, created in 1992, with review in 2011, and adopted worldwide in the specialized centers in the care subjects with SI. This classification is defined, according to modifications from Frankel,¹⁰ as ASIA A (complete motor and sensory impairments), ASIA B (loss of motor function with sensitive function preserved, below the neurological level, including the sensitive sacral nerve S4–S5), ASIA C (> 50% of the major muscles below of the neurological lesion, presenting strength less than degree 3), ASIA D (> 50% of the major muscles below of the neurological lesion, showing strength greater than or equal degree 3) and ASIA E (normal sensory and motor functions). Summarily, the classifications ASIA A and B are complete motor lesion and ASIA C and D incomplete motor lesion.

Other variables analyzed were VT evaluation by duplex scan ultrasonography, laboratory exams, prothrombin time, clottable fibrinogen, dosage of factor VIII, C protein of coagulation, antigen-free S protein, anti-thrombin, search for lupus anticoagulant, cardiolipin antibodies IgG, IgM, IgA, Leiden factor V, prothrombin mutation (factor II) and D-Dimer dosage.

Blood sampling and exam analyses

Blood samples were collected for coagulation exams, hemogram, real-time PCR and biochemical analyses. Plasma was freed to -70°C for evaluating prothrombin time, activated partial thromboplastin time, fibrinogen, Factor VIII, quantitative D-Dimer, C, S and anti-thrombin proteins, search for lupus anticoagulant. Serum was refrigerated to -4°C for searching of IgG and IgM anti-cardiolipin antibodies and homocysteine. DNA was obtained from samples collected in EDTA tubes refrigerated to -4°C , for analyzing the presence of mutations in Leiden factor V and prothrombin (G20210 A).

Laboratory exams

The analyzes of laboratory exams were performed in the Clinical Pathology Department from Hospital Israelita Albert Einstein for determining the International Normalized Ratio, fibrinogen, factor VIII dosage, C, S and anti-thrombin proteins, quantitative D-Dimer, search for lupus anticoagulant, cardiolipin antibodies IgG, IgM, IgA, homocysteine and genetic tests.

Real-time PCR

Genomic DNA was obtained from 200 μl of peripheral blood using the bioMérieux NucliSens easyMAG. Quantitative real-time PCR experiments were performed in order to investigate mutations in the prothrombin (G20210A) and Leiden factor V (G1691A) genes, using Taqman probes ABI7500 instrument and kits for allelic discrimination ABI Taqman (Applied Biosystem, San Diego, CA, USA). Primer sequences and probes are shown in Tables 1 and 2.

Ultrasonography exams

Ultrasonography analyses were performed from deep and superficial venous systems of lower limbs (proximal and distal veins), using linear transducer of 7.5 MHz and Siemens equipment Sonoline G40 (Siemens, Malvern, PA, USA). Two-dimensional echocardiography, pulsed Doppler and Color Doppler flow mapping were employed. Appropriate probes were selected according to the depth of the vessels examined. Doppler ultrasonography was executed in common

Table 1 Primer sequences and probes for Leiden Factor V

Sequence	Leiden Factor V		Start	Stop	Tm
Type	Name	Sequence	bp	bp	
Forward primer	FVPF	CGCCTCTGGGCTAATAGGACTA	70	91	59
Reverse primer	FVPR	TGTTCTAGCCAGAAGAAATCTCAGAA	194	168	60
Probe1	FVS1	CCTGGACAGGCAAG	115	128	66
Probe2	FVS2	CCTGGACAGGCGAG	115	128	67

femoral vein for analyzing the flow as a measure of evaluation of iliac ptery, as well as b-mode image.

The venous system was evaluated bilaterally. Veins were investigated in longitudinal and transversal views. Diagnostic criteria used for confirming or exclude DVT was based on compression test and the absence or presence of endoluminal material. The test was considered negative if the veins were totally compressible and without thrombus, positive when the veins were incompressible combined with the direct image of an endoluminal thrombus.

Statistical analyses

Categorical variables are presented as *n* (%) and absolute frequencies and numerical variables are expressed as median (interquartile range). The investigation to the factors associated to the thrombosis occurrence was performed in two stages: simple analysis, using Pearson's Chi-square, exact Fisher or Mann-Whitney tests for evaluating the association between the characteristics of interest and the occurrence of thrombosis and multiple analysis, using logistic regression models for verifying the influence of the variables of interest in the thrombosis occurrence. Statistical significance was set at a *P*-value of <0.05. All analyses were made using the SPSS 17.0 for Windows (SPSS Inc, Chicago, IL, USA).

The trial protocol was conducted in accordance with ethical standards of the institute on human experimentation and approval was obtained from the local ethics committee for analysis of research projects (1201/09). Informed consent was obtained from all participants prior to inclusion.

RESULTS

The subjects (*n*=100) were 72% men, 65% white race, 83% did rehabilitation, with a mean age of 39 years and a s.d. of 13.11 (range 20–76) years. Concerning the type of SI, 63% presented paraplegia and the most frequent etiologies were motor vehicle accident (35%), accidental fall (25%) and injury by firearms (17%). Other etiologies were observed in 23% of the patients (Table 3).

Clinical presentations were ASIA A (41%), followed by B (23%), C (19%) and D (17%); 9% of the patients had heterotopic ossification and 83% made rehabilitation. All the patients performed laboratory and image exams. The DVT occurrence was confirmed in 17% of the patients that made Doppler ultrasonography of bilateral lower limb.

The multivariate approach showed that the occurrence of DVT is bigger among individuals with homocysteinemia, with values between 15 and 30 $\mu\text{mol l}^{-1}$ (odds ratio 25.67, *P*-value<0.001) and among individuals with presence of Leiden factor V (odds ratio 51.51, *P*-value=0.013) (Table 4, Figures 2 and 3).

The investigation of the factors associated to the occurrence of thrombosis showed more frequency in individuals with paresis, that made big surgeries, with lower C and S protein dosage, increased

Table 2 Primer sequences and probes for prothrombin

Sequence	Prothrombin		Start bp	Stop bp	Tm
Type	Name	Sequence			
Forward primer	PROTF	GGAACCAATCCCGTGAAAGA	76	95	58
Reverse primer	PROTR	TGAATAGCACTGGGAGCATTGA	173	152	59
Probe1	PRO1	TAAAAGTGACTCTCAGCAAG	130	149	66
Probe2	PRO2	TAAAAGTGACTCTCAGCGAG	130	149	67

homocysteine (between 15 and 30 $\mu\text{mol l}^{-1}$). Other factors associated to DVT occurrence were the presence of genetic polymorphism of Leiden factor V (Table 5). There were changes in the quantitative D-Dimer in 70.6% of the patients that showed DVT and 43.4% in those patients without DVT. Concerning the time of lesion, the median was 1.3 years among patients without DVT and median of 2.4 years among patients with DVT.

It was investigated the association between some characteristics observed and changes in D-Dimer (Table 5). Values of D-Dimer above the reference were found in 46% of the patients with paraplegia and 51.4% of the patients with tetraplegia, showing that there were no differences in the lesions (*P*=0.607). There were no significant differences between D-Dimer greater than reference value and ASIA classification. There was association between changes in D-Dimer and fibrinogen (*P*<0.001), and the changes in D-Dimer in patients with values >400 are more frequent (72.3%) than between patients with normal dosages (26.7%). This evaluation was performed in the subgroup without DVT and the results were similar (not shown).

DISCUSSION

The main finding of this study was the higher occurrence of DVT in patients with Leiden factor V and hyperhomocysteinemia. There was no association between SI for DVT, VT and thrombophilia, contrary that was thought in the study design. Also, there was no relation between lupus anticoagulant and anti-cardiolipin.

DVT is a multigenic and multifactorial disease; the interrelation of the risk factors lead to the thrombosis.¹ Besides, age is an isolated risk for DVT, mainly after 40 years.¹¹ In this study, 51% of the patients had age <40 years (80.4% without DVT and 19.6% with DVT). Although the results not show statistical significance, the population was young, most men that can have association with the results, impacting in the low frequency of thrombosis, 17%. Experimental studies with fibrinogen scanning marked with I¹²⁵ showed presence of DVT in 100% of the patients with SI for acute SCI evaluated.¹²

The majority of the studies in the literature evaluated patients with SI for acute SCI.^{9,13–15} In this study, patients were analyzed with time lesion >1 year (median of 1.3 years among the patients without DVT and 2.4 years among patients with DVT). There are studies that show adaptation of many organic and functional systems in patients with SI.^{15,16} Many factors can contribute to the higher occurrence of thrombosis in these patients. The permanent venous vasodilatation state in the lower limbs promotes lower speed of blood flow and stasis as constant risk factor. Experimental studies of spinal transection in mice showed increase in 1.5 times the diameter in femoral and saphenous veins.¹⁵ Patients with SI present frequently dehydration and secondary increase of blood viscosity that can complicate the stasis and local hypoxia.¹⁷

The higher the degree of muscle paralysis, the greater the tendency of slowing the blood flow, because of the loss of

Table 3 Factors associated to DVT – simple analysis

	DVT				P-value
	No		Yes		
	n	%	n	%	
Gender					0.555
Female	22	78.6	6	21.4	
Male	61	84.7	11	15.3	
Age					0.775
Until 39 years	41	80.4	10	19.6	
Between 40 and 69 years	39	84.8	7	15.2	
70 years or more	3	100	0	0	
Obesity					>0.99
No	81	82.7	17	17.3	
Yes	2	100	0	0	
Paresis					0.182
No	19	95	1	5	
Yes	64	80	16	20	
Trauma					0.742
No	16	80	4	20	
Yes	67	83.8	13	16.2	
Big surgeries					0.02
No	21	100	0	0	
Yes	62	78.5	17	21.5	
Respiratory failure					0.531
No	80	83.3	16	16.7	
Yes	3	75	1	25	
Diaphyseal fracture					0.585
No	78	82.1	17	17.8	
Yes	5	100	0	0	
Severe and/or hospital infection					>0.99
No	24	82.8	5	17.2	
Yes	59	83.1	12	16.9	
Coagulable fibrinogen (mg dl ⁻¹)					0.284
Until 399	46	86.8	7	13.2	
400 or more	37	78.7	10	21.3	
Factor VIII dosage (%)					0.357
Until 149	64	85.3	11	14.7	
150 or more	19	76.0	6	24.0	
Proteina C from coagulation dosage (%)					0.170
Until 59	0	0	1	100	
60 or more	83	83.8	16	16.2	
Antigen-free S protein (%)					0.015
Until 54	1	25	3	75	
55 or more	82	85.4	14	14.6	
Homocystein (μmol l ⁻¹)					<0.001
Until 14	76	90.5	8	9.5	

Table 3 (Continued)

	DVT				P-value
	No		Yes		
	n	%	n	%	
15–30	7	43.8	9	56.2	
31–100	0	0	0	0	
>100	0	0	0	0	
Search of lupus anticoagulant					>0.99
Negative	79	82.3	17	17.7	
Positive	4	100	0	0	
Antibodies anti-cardiolipin IgG GPL					0.320
Until 14.9	75	82.4	16	17.6	
15–20	2	66.7	1	33.3	
20.1 or more	6	100	0	0	
Anticorpos anti-cardiolipin IgM MPL					0.683
Until 12.4	78	83	16	17	
Between 12.5–20	2	66.7	1	33.3	
20.1 or more	3	100	0	0	
Leiden Factor V					0.074
No	82	84.5	15	15.5	
Yes	1	33.3	2	66.7	
Protrombina Factor II mutation					0.432
No	81	83.5	16	16.5	
Yes	2	66.7	1	33.3	
D-Dimer dosage (ng ml ^{−1})					0.050
≤500	47	90.4	5	9.6	
500–1000	19	86.4	3	13.6	
1000–2000	13	65	7	35	
2000–3000	3	75	1	25	
>3000	1	50	1	50	
D-Dimer ng ml ^{−1} dosage					0.041
≤500	47	90.4	5	9.6	
>500 (unsettled)	36	75	12	25	
ASIA					0.869
A	33	80.5	8	19.5	
B	19	82.6	4	17.4	
C	17	89.5	2	10.5	
D	14	82.4	3	17.6	
Lesion level					0.218
Cervical spine injury	43	84.3	8	15.7	
(T1–T12) thoracic spine injury	33	86.8	5	13.2	
Lumbar spine injury	7	63.6	4	36.4	
Hemoglobin dosage–median (IQR)	14 (13–15)		14 (13–14)		
Platelet count × 10 ³ ul ^{−1} –median (IQR)	258 (218–301)		280 (195–335)		0.701
Lesion time (years) – median (IQR)	1.3 (0.8–2.5)		2.4 (1.6–4.0)		0.014

Abbreviations: GPL, IgG phospholipid units; IQR, Interquartile range; MPL, IgM phospholipid units. Bold and italic values are statistically significant.

Table 4 Factors associated to DVT – multiple regression analysis

	Odds ratio	95% CI for odds ratio		P-value
		LL	UL	
Age (reference: until 39 years)				
40 or more	0.486	0.111	2.125	0.337
Homocystein (reference until 14)				
15–30	25.670	5.110	128.948	<0.001
Leiden Factor V (reference: no)				
Yes	51.508	2.333	1137.433	0.013
ASIA (reference: A)				0.731
B	1.435	0.255	8.071	0.682
C	0.407	0.049	3.372	0.405
D	0.622	0.069	5.622	0.672
Lesion time (reference: paraplegia)				
Tetraplegia	0.338	0.049	2.347	0.273
Lesion level (reference: cervical spine injury)				0.575
(T1–T12) thoracic spine injury	0.595	0.104	3.384	0.558
Lumbar spine injury	1.674	0.160	17.471	0.667

Abbreviations: LL, lower limit; UL, upper limit.

Table 5 Association between characteristics and changes of D-Dimer dosage

	D-Dimer dosage (<i>ng ml</i> ⁻¹)				P-value
	≤500		> 500		
	<i>n</i>	%	<i>n</i>	%	
<hr/>					
Type of SI					0.607
Paraplegia	34	54	29	46	
Tetraplegia	18	48.6	19	51.4	
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ASIA					0.921
A	20	48.8	21	51.2	
B	12	52.2	11	47.8	
C	10	52.6	9	47.4	
D	10	58.8	7	41.2	
<hr/>					
Age					0.707
Until 39 years	28	54.9	23	45.1	
40–69	23	50	23	50	
70 or more	1	33.3	2	66.7	
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Coagulable fibrinogen (<i>mg dl</i> ⁻¹)					<0.001
Until 399	39	73.6	14	26.4	
400 or more	13	27.7	34	72.3

Figure 2 Relation between Leiden Factor V and DVT. **Figure 3** Relation between homocystein and DVT.

compressive effect of the muscle pumping, explaining higher occurrence of DVT in flaccid paralysis.¹⁸ Boudaoud *et al.* (1997) simulated venoestase state in upper and lower limbs in 15 patients with paraplegia or tetraplegia, up to 6 months of SCI, compared with 10 normal volunteers. The aim of the study was to evaluate the fibrinolytic system and the risk of DVT after SCI. After venoestase upper limbs, the tissue plasminogen activator increased significantly in patients as in the control group, but no significant changes in the lower limbs. The prolonged stasis not allowed that the fibrinolytic response level in the lower limbs was the same as observed in the upper limbs. The dosage of plasminogen activator inhibitor (PAI-1) did not change significantly after hypoxic state, although there was variation in dosages between individuals. In this study, 75% of patients with thrombosis had increased levels of D-Dimer but all patients had normal levels of thrombin–anti-thrombin complex, prothrombin fragments 1 + 2. The permanence of the thrombotic process characterized by increased levels of D-Dimer without recovery of the fibrinolytic system suggests perennial antithrombotic treatment in therapeutic doses.¹⁹

Iversen published a study in 2002 about hemostatic abnormalities and tetraplegia, concluding that circadian variations of several coagulation factors and fibrinolytic system are increased in patients with SI, due to a possible deregulation of the autonomic nervous system reflex, leading to a dysfunction between the variations central and peripheral circadian.²⁰ This could possibly reflect the deregulated autonomic nervous system, leading to a dysfunctional link between central and peripheral circadian oscillators.

In this study, 75% of patients with altered D-Dimer (>500 ng ml⁻¹), had no DVT (Table 3), confirmed by Doppler ultrasonography, which could suggest that changes in the fibrinolytic system may be a protective mechanism for patients outside the acute phase of SCI (> 1 year of SI).

CONCLUSIONS

There is an important difference in the incidence of DVT in patients with SI by acute and chronic SCI.

Laboratory investigation for thrombophilia, including genetic tests, immunologic, coagulation and biochemistry exams, should not be requested in a systematic manner for SI patients by SCI. The conduct of this decision should be based on clinical factors, risk factors for DVT and family history of thrombosis.

The adaptation of venous vascular system and changes in the fibrinolytic system need to be better understood and studied in SI patients by SCI for >1 year.

Patients in this study are accompanied in an important National Rehabilitation Center, where all clinical support, mainly psychological and physical therapy, with complete guidance for patients and caregivers are provided. This might have impacted on the results and may not reflect the reality of other services.

DATA ARCHIVING

There were no data to deposit.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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