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Rafail Tzanninis [1,*], Efthymia Vlachaki [2], Eleftheria Lefkou [3], Stavroula Tsiara [4], Stamatia Theodoridou [5], Athanasios Vyzantiadis [6] and Miltiadis Matsagkas [7]

[1] Postgraduate studies in Thrombosis and Antithrombotic Education, Department of Medicine, University of Thessaly University, Greece, Larisa, Greece

[2] Aristotle University, Thessaloniki, Greece

[3] Transfusion Center, University of Thessaly, Larisa, Greece

[4] University of Ioannina, Ioannina, Greece

[5] Hippokration Hospital, Thessaloniki, Greece

[6] Hippokration Hospital, Thessaloniki, Greece

[7] University of Thessaly, Larisa, Greece.

[*] Correspondence: raphail2121@gmail.com

Abstract

Sickle Cell Disease (SCD) is one of the most widespread inherited hemoglobinopathies that has been associated with numerous coagulation disorders, hence characterized as a severe hypercoagulable state. We conducted a single-center retrospective observational study of patients with SCD who were monitored at Hippokration Hospital of Thessaloniki between 1999 and 2024. In this study group, demographic data, hemoglobin (Hb) genotype, medical history, use of anticoagulant and antiplatelet treatment, dosage of anticoagulant treatment, recurrence of the first venous thromboembolism (VTE) and laboratory values were examined as possible provoking factors. Twelve patients (12/46-26,1%) developed thrombosis (75% with genotype HbS/β-thal). The prevalence of VTE in this group was 17,4%. Age of thrombosis, age of the patients, anticoagulant treatment, anticoagualants' dosage, antiplatelet treatment and type of transfusion were significantly associated with increased risk of thrombosis (p<0.05). On multivariate analysis, the anticoagulant treatment and its dosage showed statistical significance (p<0.05).Sickle cell disease and thrombosis are strongly associated. Despite current arsenal of drugs and the broad knowledge related to each one, reducing thrombosis' occurrence remains a challenge.

Keywords: sickle cell disease; thrombosis; venous thromboembolism

Introduction

Sickle Cell Disease (SCD) is a monogenic disorder of hemoglobin that leads to production of the abnormal hemoglobin S [1]. As a term, SCD encompasses sickle cell anaemia (SCA) and the various heterozygous states of hemoglobin S with other hemoglobinopathies [2]. According to literature, the prevalence of VTE in this disease is as high as 25%, with various risk factors as key contributors [3]. Despite inherent risk of thrombosis, there are no clear guidelines for thromboprophylaxis, while use rates are concerningly low [4].

SCD is characterized by several pathogenetic mechanisms. The trigger for the pathophysiology of the disease is polymerization of HbS, which converts red blood cells into rigid, fragile sickle cells that adhere more easily to the vascular endothelium [5–7]. This attachment contributes to a second mechanism, the vascular occlusion which is observed in both small and large vessels [8–13]. Simultaneously, the fragility of red blood cells triggers chronic hemolysis that occurs both

intravascularly and extravascularly, resulting in activation of various cell populations and the release of signaling molecules, most importantly hemoglobin [1,6,7,14,15]. The fourth component of pathophysiology is oxidative stress, which, destabilizes the cell membrane, contributing to the acceleration of hemolysis and the activation of coagulation [7,16]. Finally, many disruptions of the coagulation cascade have been observed. Reduction of coagulation factors and anticoagulant molecules and resistance of fibrin to fibrinolysis have been proven. These processes in combination with the mechanism of immunothrombosis, secretion of microvesicles and the activation of platelets and neutrophils promote thrombosis [6,17].

Methods

Delving deeper into the interaction of SCD and thrombosis, we conducted a retrospective observational study at the Adults Thalassaemia and Sickle Cell Disease Unit of the Hippokration Hospital of Thessaloniki using data from 01/01/1999 to 31/11/2024. Objectives of the study were to measure the prevalence of thrombosis and VTE in patients with SCD and the evaluation and comparison of known risk factors.

Inclusion criteria included a genotype compatible with SCD. Three patients were excluded from the study. The first patient had insufficient data regarding the various study parameters as she had only recently started monitoring at the Thalassaemia and Sickle Cell Disease Unit. In the second patient, the molecular genetic testing showed that she did not have SCD genotype. As for the third patient, she has a complex genotype of compound heterozygous sickle cell and β-thalassaemia and heterozygous mild α-thalassaemia being the only individual with that genotype. After conducting both univariate and multivariate studies, it was found that her participation in the study altered the remaining parameters and conclusions to a statistically significant degree, so it was prudent not to be included. (Figure 1)

Patients with SCD monitored between
1/1999 and 11/2024 (n=49)

Patients excluded due to lack of data on
various study parameters (n=1)

Patients excluded due to the presence of
genotype that is not associated with SCD
(n=1)

Patients excluded due to the presence of a
SCD genotype with unique representation
that distorts and prevents drawing safe
conclusions(n=1)

Collection of demographics, medical
history and laboratory values

Division into two groups based on the
presence of thrombosis

Figure 1. Flowchart indicating participation in study after inclusion and exclusion criteria.

After obtaining relevant permission from the hospital's Ethics Committee for each patient, we reviewed all medical files from the Thalassaemia and Sickle Cell Disease Unit. Information relevant to the duration and etiology of anticoagulant treatment, disease genotype, history of splenectomy or functional asplenia, the demographic characteristics and the association of these recordings and other concomitant diseases with thrombosis was analyzed. (Table 1)

Baseline values of hemoglobin, white blood cells, platelets, HbS, HbF were also recorded before the first thrombotic episode. (Table 2)

Table 1. Demographic data, clinical data of study population.

PATIENTS' CHARACTERISTICS

Table 1: Demographic data, medical history		All (n=46)	Thrombosis (n=12)	No thrombosis (n=34)	P-value
Age of patients (Years)		42.9	47	41	0.009
Age of thrombosis (Years)			38,1	-	0.000
Sex	Male	24(52,2%)	6 (50%)	18 (52,9%)	0.861
	Female	22 (47,8%)	6 (50%)	16 (47,1%)	0.861
Genotype	SCA	14 (30,4%)	3 (25%)	11 (32,4%)	0.63
	Double heterozygocity S/βMA	32 (69,6%)	9 (75%)	23 (67,6%)	0.64
Site of thrombosis		12 (26,1%)	-	0,00%	0.000
	PE	4 (8,7%)	4 (33,3%)	-	-
	DVT	4 (8,7%)	4 (33,3%)	-	-
	Other	4 (8,7%)	4 (33,3%)	-	-
Recurrent episode of DVT (1)		1 (2,2%)	1 (8,3%)	-	-
Recurrent episodes of DVT (>1)		2 (4,3%)	2 (16,7%)	-	-
2 ≥ thrombotic episodes		5 (10,9%)	5 (41,7%)	-	-
Anticoagulant treatment		17 (37%)	12 (100%)	5 (14,7%)	0.000
	Before 1st VOE	7 (17,4%)	2 (16,7%)	-	-
	After 1st VOE	10 (21,7%)	10 (83,3%)	-	-
	No anticoagulant treatment	29 (63%)	0 (0%)	29 (85,3%)	-
Antiplatelet treatment		25 (54,3%)	8 (66,7%)	17 (50%)	0.000
	Before 1st VOE	20 (43,5%)	3 (25%)	-	-
	After 1st VOE	5 (10,9%)	5 (41,7%)	-	-
	No antiplatelet treatment	21 (47,5)	4 (33,3%)	17 (50%)	0.000
Anticoagualants' dosage		17 (37%)	12 (100%)	5 (14,7%)	0.000
	Prophylactic	3 (6,5%)	0 (0%)	3 (8,8%)	-
	Therapeutic	14 (30,4%)	12 (100%)	2 (5,9%)	-
Medical history could be the cause thrombosis	Yes	28 (60,9%)	8 (66,7%)	20 (58,8%)	0.63
	No	18 (39,1%)	4 (33,3%)	14 (41,2%)	0.63
Surgical splenectomy		20 (43,5%)	4 (33,3%)	16 (47,1%)	0.405
Fuctional asplenia		15 (32,6%)	4 (33,3%)	11 (32,4%)	0.95
Transfusion		23 (50%)	7 (58,3%)	16 (47,1%)	0.501
Type of transfusion		23 (50%)	7 (58,3%)	16 (47,1%)	0.018
	Exchange transfusion	15 (32,6%)	7 (58,3%)	8 (23,5%)	-
	Packed RBC transfusion	8 (17,4%)	0 (0%)	8 (23,5%)	-
	No transfusion	23 (50%)	5 (41,7%)	18 (52,9%)	-
Use of hydroxycarbamide		46 (100%)	12 (100%)	34 (100%)	-

Table 2. Laboratory values of study group.

		values			
Laboratory tests		All (n=46)	Thrombosis (n=12)	No thrombosis (n=34)	P-value

Hb (g/dl)	<11	39 (84,8%)	10 (83,3%)	29 (85,3%)	0.872
	>11	7 (15,2%)	2 (16,7%)	5 (14,7%)	0.872
WBC (10^3/µL)		9,1 (5,3)	9,1 (3,4)	9,1 (5,9)	0.910
Plt (10^3/µL)		384,6 (159,2)	397,1 (168,6)	349,2 (128,7)	0.474
HbS (%)		60,4 (15)	55,9 (16,4)	62 (14,5)	0.706
HbF (%)		13,7 (8,9)	13,8 (6,9)	13,6 (9,7)	0.239

WBC: White blood cells, Plt: Platelets, HbS: Hemoglobin S, HbF: Hemoglobin F.

Data analysis

The study included quantitative and qualitative variables. Quantitative are described through mean value as well as measures of dispersion (standard deviation, minimum and maximum value), while qualitative variables are expressed in numbers and percentages of participants pertaining to all variables.

Subsequently, the normality hypothesis for quantitative variables was tested using the Shapiro-Wilk and Kolmogorov-Smirnov tests. The former test is designed and widely recommended for samples smaller than 50 experimental units, while the Kolmogorov-Smirnov test is more applicable for samples over 50 experimental units. Given the fact that sample size was near 50, both normality tests were applied and indicated that for most variables, the p-values are less than 0.05, pointing to the rejection of the null hypothesis. Therefore, the variables do not follow a normal distribution and focus was shifted towards a non-parametric statistical analysis.

As a way to evaluate the differences between two or more groups, the Mann-Whitney U Test and Kruskal-Wallis Test were used, suited for groups that do not follow a normal distribution or for small statistical samples. The Crosstabulations test with Chi-Square Test were used to examine the possibility of whether two categorical (qualitative) variables are associated with each other or whether the differences observed are random or statistically significant. Finally, the linear regression test was performed, so as to investigate the relationship between a scalar variable and one or more explanatory variables (or independent variables). Throughout the study, percentages were rounded up to the first decimal place.

Results

1. Patient characteristics

Forty six patients with SCD who are monitored at the Adults Thalassaemia and Sickle Cell Disease Unit were reviewed. Thirty two (69.6%) have the S/βMA double heterozygosity while 14 (30.4%) have the SCA genotype. The sample is almost uniform across men and women, providing balance in the gender analysis, while the average age of the individuals at the time of the analysis is 42.9 years. (Table 1)

It should be clarified that percentages and statistical tests refer to the first episode of thrombosis. The small sample size, in conjunction with an even smaller subpopulation with recurrent episodes, made it impossible to use statistical models in the population with more than one VOE as conclusions would not be objective. Moreover, in the context of anticoagulant and antiplatelet treatment, the "before thrombosis" section included patients who did not experience any thrombotic incidents

2. Frequency of Thrombotic and Venous Thromboembolic events

The prevalence of thrombosis based on the 1st episode was 26.1% (12/46) while the prevalence of VTE was 17.4% (8/46). The median age of the 1st thrombotic event was 38.1 years. (Table 1)

3. Clinical factors associated with thrombosis

The univariate analysis showed that thrombosis is statistically associated with patient's age (p=0.009), age of thrombosis (p=0.000), antiplatelet treatment (p=0.000), anticoagulant treatment

(p=0.000) and its dose (p=0.000). Finally, a significant correlation emerged between the type of transfusion and thrombosis (p=0.018)   (Table 1).

4. Laboratory tests associated with thrombosis

On the contrary, none of the laboratory values studied, were found to be statistically significant in any analysis. (Table 2)

From the linear regression analysis, 12 models emerged. In all models, the administration of anticoagulants and their dose were found to be statistically significant. Blood transfusion and its type were found to be less significant, with two models indicating this correlation. (Table 3)

Table 3. Linear regression analysis and the 12 models arisen.

SS/Model	Unstandardized Coefficients B	Std. Error	Standardized Coefficients Beta	t	Sig.	Correlations Zero-order	Partial	Part	Collinearity Statistics Tolerance	VIF
1. (Constant)	-.293	.229		-1.283	.210					
Sex	.047	.086	.053	.547	.589	-.026	.101	.035	.422	2.370
Patient's age	-.007	.027	-.024	-.271	.788	.196	-.050	-.017	.509	1.965
Genotype	.033	.074	.035	.447	.658	-.070	.083	.028	.677	1.477
Recurrence	.181	.135	.116	1.341	.190	.519	.242	.085	.536	1.865
Anticoagulant treatment	.630	.069	1.145	9.115	**.000**	.882	.861	.579	.256	3.907
Antiplatelet treatment	.019	.055	.029	.348	.730	.384	.065	.022	.585	1.710
Anticoagulant's dose	-.286	.086	-.400	-3.335	**.002**	.547	-.527	-.212	.281	3.562
Splenectomy	.031	.093	.035	.329	.744	-.122	.061	.021	.367	2.722
Surgical asplenia	-.020	.089	-.022	-.228	.821	.009	-.042	-.014	.451	2.218
Baseline Hb	.100	.113	.081	.882	S.385	.024	.162	.056	.473	2.112
WBC	2.695E-6	.000	.032	.376	.710	-.002	.070	.024	.543	1.841
PLT	.000	.000	.057	.567	.575	-.134	.105	.036	.400	2.500
Hb before thrombosis	.003	.004	.067	.874	.389	.011	.160	.056	.692	1.445
H&S	.001	.003	.024	.280	.781	-.179	.052	.018	.545	1.836
Transfusion	-.243	.209	-.277	-1.160	.255	.099	-.211	-.074	.071	14.076
Type of transfusion	.152	.125	.310	1.221	.232	.227	.221	.078	.063	15.942
2. (Constant)	-.296	.225		-1.320	.197					
Sex	.046	.084	.052	.543	.591	-.026	.099	.034	.424	2.361
Patient's age	-.008	.026	-.028	-.324	.748	.196	-.059	-.020	.527	1.899
Genotype	.029	.071	.031	.414	.682	-.070	.075	.026	.711	1.407
Recurrence	.185	.132	.118	1.395	.173	.519	.247	.087	.542	1.844
Anticoagulant treatment	.628	.067	1.141	9.304	**.000**	.882	.862	.582	.260	3.848
Antiplatelet treatment	.021	.053	.033	.406	.688	.384	.074	.025	.607	1.647
Anticoagulant's dose	-.282	.083	-.394	-3.415	**.002**	.547	-.529	-.214	.293	3.410
Splenectomy	.043	.075	.048	.567	.575	-.122	.103	.035	.543	1.842
Baseline Hb	.095	.110	.078	.871	.391	.024	.157	.054	.486	2.057
WBC	2.579e-6	.000	.031	.367	.717	-.002	.067	.023	.546	1.831
PLT	.000	.000	.045	.534	.597	-.134	.097	.033	.551	1.815
Hb before thrombosis	.003	.004	.070	.944	.353	.011	.170	.059	.715	1.399
H&S	.001	.002	.030	.367	.716	-.179	.067	.023	.594	1.685
Transfusion	-.232	.200	-.264	-1.157	.256	.099	-.207	-.072	.075	13.276
Type of transfusion	.147	.120	.298	1.219	.232	.227	.217	.076	.065	15.296

Model	Variable	B	Std. Error	Beta	t	Sig.	Zero-order	Partial	Part	Tolerance	VIF
3.	(Constant)	-.318	.211		-1.506	.142					
	Sex	.040	.081	.045	.489	.629	-.026	.087	.030	.447	2.239
	Genotype	.026	.069	.027	.378	.708	-.070	.068	.023	.725	1.380
	Recurrence	.170	.122	.109	1.389	.175	.519	.242	.086	.619	1.614
	Anticoagulant treatment	.627	.066	1.139	9.440	.000	.882	.861	.582	.261	3.832
	Antiplatelet treatment	.021	.052	.032	.403	.690	.384	.072	.025	.608	1.646
	Anticoagulant's dose	-.282	.081	-.395	-3.467	.002	.547	-.529	-.214	.293	3.410
	Splenectomy	.046	.073	.052	.628	.535	-.122	.112	.039	.554	1.805
	Baseline Hb	.097	.108	.080	.903	.374	.024	.160	.056	.487	2.051
	WBC	3.075e-6	.000	.037	.454	.653	-.002	.081	.028	.573	1.744
	PLT	.000	.000	.054	.695	.492	-.134	.124	.043	.624	1.604
	Hb before thrombosis	.003	.003	.064	.904	.373	.011	.160	.056	.768	1.302
	HbA	.001	.002	.031	.386	.702	-.179	.069	.024	.595	1.682
	Transfusion	-.251	.188	-.286	-1.332	.192	.099	-.233	-.082	.083	12.098
	Type of transfusion	.159	.113	.322	1.405	.170	.227	.245	.087	.072	13.863
4.	(Constant)	-.301	.204		-1.479	.149					
	Sex	.045	.079	.051	.566	.575	-.026	.100	.034	.459	2.177
	Recurrence	.164	.119	.105	1.371	.180	.519	.236	.083	.629	1.589
	Anticoagulant treatment	.626	.065	1.137	9.561	.000	.882	.861	.581	.261	3.826
	Antiplatelet treatment	.021	.051	.032	.406	.687	.384	.072	.025	.608	1.646
	Anticoagulant's dose	-.277	.079	-.387	-3.504	.001	.547	-.527	-.213	.304	3.292
	Splenectomy	.040	.071	.045	.568	.574	-.122	.100	.035	.580	1.724
	Baseline Hb	.089	.104	.073	.853	.400	.024	.149	.052	.510	1.959
	WBC	2.662e-6	.000	.032	.404	.689	-.002	.071	.025	.589	1.699
	PLT	.000	.000	.059	.780	.441	-.134	.137	.047	.641	1.559
	Hb before thrombosis	.003	.003	.069	1.023	.314	.011	.178	.062	.806	1.241
	HbA	.001	.002	.034	.438	.664	-.179	.077	.027	.602	1.660
	Transfusion	-.234	.180	-.266	-1.297	.204	.099	-.223	-.079	.088	11.412
	Type of transfusion	.149	.108	.303	1.373	.179	.227	.236	.083	.076	13.146
8.	(Constant)	-.247	.122		-2.025	.050					
	Sex	.084	.060	.096	1.402	.169	-.026	.228	.082	.725	1.374
	Recurrence	.149	.106	.095	1.406	.168	.519	.228	.082	.736	1.359
	Anticoagulant treatment	.617	.061	1.122	10.041	.000	.882	.858	.585	.271	3.685
	Antiplatelet treatment	.043	.044	.065	.974	.337	.384	.160	.057	.771	1.296
	Anticoagulant's dose	-.265	.071	-.370	-3.725	.001	.547	-.527	-.217	.343	2.914
	PLT	.000	.000	.076	1.193	.241	-.134	.195	.069	.824	1.214
	Hb before thrombosis	.003	.003	.053	.875	.388	.011	.144	.051	.940	1.064
	Transfusion	-.215	.153	-.245	-1.409	.167	.099	-.229	-.082	.112	8.900
	Type of transfusion	.115	.090	.234	1.274	.211	.227	.208	.074	.100	9.953
9.	(Constant)	-.202	.110		-1.833	.075					
	Sex	.078	.059	.088	1.308	.199	-.026	.210	.076	.739	1.353
	Recurrence	.140	.105	.090	1.336	.190	.519	.214	.078	.742	1.348
	Anticoagulant treatment	.615	.061	1.118	10.043	.000	.882	.855	.583	.272	3.677
	Antiplatelet treatment	.046	.043	.070	1.056	.298	.384	.171	.061	.777	1.287
	Anticoagulant's dose	-.260	.071	-.364	-3.681	.001	.547	-.518	-.214	.345	2.897
	PLT	.000	.000	.078	1.218	.231	-.134	.196	.071	.824	1.213
	Transfusion	-.218	.152	-.249	-1.438	.159	.099	-.230	-.083	.112	8.894
	Type of transfusion	.114	.090	.232	1.269	.212	.227	.204	.074	.100	9.952
10.	(Constant)	-.181	.109		-1.664	.104					
	Sex	.065	.058	.074	1.120	.270	-.026	.179	.065	.769	1.300
	Recurrence	.134	.105	.086	1.274	.210	.519	.202	.074	.744	1.343
	Anticoagulant treatment	.626	.060	1.139	10.386	.000	.882	.860	.604	.281	3.558
	Anticoagulant's dose	-.260	.071	-.363	-3.667	.001	.547	-.511	-.213	.345	2.897
	PLT	.000	.000	.092	1.477	.148	-.134	.233	.086	.864	1.157
	Transfusion	-.246	.150	-.280	-1.643	.109	.099	-.258	-.096	.116	8.626
	Type of transfusion	.138	.087	.280	1.575	.124	.227	.248	.092	.107	9.350
11.	(Constant)	-.097	.079		-1.228	.227					
	Recurrence	.133	.105	.085	1.262	.214	.519	.198	.074	.744	1.343
	Anticoagulant treatment	.608	.058	1.106	10.433	.000	.882	.858	.608	.303	3.305
	Anticoagulant's dose	-.243	.069	-.339	-3.498	.001	.547	-.489	-.204	.362	2.766
	PLT	.000	.000	.106	1.720	.093	-.134	.266	.100	.895	1.114
	Transfusion	-.306	.141	-.349	-2.179	.035	.099	-.329	-.127	.133	7.530
	Type of transfusion	.178	.080	.362	2.232	.031	.227	.337	.130	.129	7.749
12.	(Constant)	-.105	.079		-1.324	.193					
	Anticoagulant treatment	.634	.055	1.152	11.506	.000	.882	.876	.676	.344	2.907
	Anticoagulant's dose	-.251	.070	-.351	-3.612	.001	.547	-.496	-.212	.365	2.740
	PLT	.000	.000	.110	1.783	.082	-.134	.271	.105	.901	1.110
	Transfusion	-.323	.141	-.368	-2.295	.027	.099	-.341	-.135	.134	7.459
	Type of transfusion	.194	.080	.393	2.433	.020	.227	.359	.143	.132	7.572

a. Dependent Variable: Thrombosis

5.	(Constant)	-.301	.201		-1.497	.144				
	Sex	.060	.069	.068	.872	.389	-.026	.150	.052	.593	1.687
	Recurrence	.162	.118	.104	1.378	.177	.519	.233	.083	.630	1.587
	Anticoagulant treatment	.627	.064	1.140	9.739	.000	.882	.861	.585	.263	3.807
	Antiplatelet treatment	.027	.049	.040	.544	.590	.384	.094	.033	.657	1.522
	Anticoagulant's dose	-.276	.078	-.385	-3.539	.001	.547	-.524	-.212	.304	3.289
	Splenectomy	.044	.069	.050	.634	.531	-.122	.110	.038	.590	1.695
	Baseline Hb	.073	.096	.050	.767	.448	.024	.132	.046	.590	1.695
	PLT	.000	.000	.062	.825	.415	-.134	.142	.050	.646	1.549
	Hb before thrombosis	.003	.003	.062	.962	.343	.011	.165	.058	.868	1.152
	Hb	.001	.002	.035	.447	.657	-.179	.078	.027	.602	1.660
	Transfusion	-.205	.164	-.234	-1.254	.219	.099	-.213	-.075	.104	9.632
	Type of transfusion	.130	.096	.264	1.347	.187	.227	.228	.081	.094	10.632
6.	(Constant)	-.231	.125		-1.840	.075				
	Sex	.063	.067	.072	.937	.355	-.026	.159	.056	.600	1.668
	Recurrence	.145	.110	.093	1.319	.196	.519	.221	.078	.702	1.424
	Anticoagulant treatment	.624	.063	1.135	9.866	.000	.882	.861	.585	.266	3.761
	Antiplatelet treatment	.032	.047	.048	.680	.501	.384	.116	.040	.697	1.434
	Anticoagulant's dose	-.272	.076	-.380	-3.552	.001	.547	-.520	-.211	.308	3.245
	Splenectomy	.041	.068	.047	.607	.548	-.122	.104	.036	.594	1.684
	Baseline Hb	.064	.092	.052	.696	.491	.024	.118	.041	.619	1.617
	PLT	.000	.000	.062	.846	.404	-.134	.144	.050	.646	1.548
	Hb before thrombosis	.003	.003	.054	.886	.382	.011	.150	.053	.931	1.074
	Transfusion	-.219	.159	-.250	-1.382	.176	.099	-.231	-.082	.108	9.271
	Type of transfusion	.128	.095	.261	1.350	.186	.227	.226	.080	.094	10.622
7.	(Constant)	-.237	.124		-1.903	.065				
	Sex	.069	.066	.079	1.049	.301	-.026	.175	.062	.613	1.631
	Recurrence	.157	.108	.101	1.456	.154	.519	.239	.086	.723	1.384
	Anticoagulant treatment	.622	.063	1.130	9.937	.000	.882	.859	.584	.267	3.747
	Antiplatelet treatment	.035	.046	.053	.758	.455	.384	.127	.044	.705	1.418
	Anticoagulant's dose	-.278	.075	-.388	-3.694	.001	.547	-.530	-.217	.313	3.192
	Baseline Hb	.050	.089	.041	.570	.573	.024	.096	.033	.658	1.520
	PLT	.000	.000	.082	1.255	.218	-.134	.208	.074	.804	1.244
	Hb before thrombosis	.003	.003	.056	.915	.366	.011	.153	.054	.932	1.073
	Transfusion	-.230	.156	-.262	-1.472	.150	.099	-.241	-.087	.109	9.158
	Type of transfusion	.129	.094	.261	1.365	.181	.227	.225	.080	.094	10.622

5. Subpopulation with thrombotic events

Among the 12 patients with thrombosis equal representation of both sexes (6 men and 6 women) is found while the mean age of the first episode of thrombosis was 38.1 years for the first VOE. Eight of the first events were VTE.   (Table 1)

Nine patients (75%) had S/βMA heterozygosity and 3 had SCA (25%). All treated with anticoagulant treatment at a therapeutic dose. Ten (83.3%) started anticoagulants after the first episode of thrombosis while 2 (16.7%) prior (due to a history of AF and tibial artery stenosis). Among the 10 patients, 4 were on LMWH, 2 on DOACs, 2 on VKA, 2 on fondaparinux. The 2 patients with underlying diseases that received anticoagulant treatment, were on VKAs. Regarding antiplatelet treatment, 5 (41.7%) had initiated it after the thrombotic event ,3 (25%) prior, while 4 (33.3%) never received any. The antiplatelet treatment consisted of COX1 inhibitor in 4 patients and P2Y12 inhibitor in 1 patient for the 5patient subgroup. In the subgroup that initiated antiplatelet treatment after the first VOE, 2 received COX1 inhibitor and 1 P2Y12 inhibitor. In eight (66.7%), existing medical history could associate with thrombogenesis. Four (33.3%) had a history of splenectomy, 4 (33.3%) had functional asplenia confirmed by abdominal ultrasound or abdominal CT scan. In addition, 7 (58.3%) were regularly transfused with exchange transfusion. All 12 patients were receiving hydroxyurea throughout the observational period. (Table 1)

6. Subpopulation with more than one thrombotic events

Five of 12 patients (41.7%) experienced more than one thrombotic episode. It is worth mentioning that all had one VOE in the same location as the initial one, while 4 (80%) had at least one episode elsewhere. One patient (20%) had a 2nd PE, 2 (40%) had a recurrence of DVT, 1 (20%) had two superficial thrombosis, and 2 (40%) had a second ischemic stroke.

Patient who had two episodes of PE also had two ischemic strokes. Two patients had the largest number of thrombotic events. The first one experienced 4 incidents, two episodes of PE and two

ischemic strokes. The former experienced 3 episodes of DVT (therefore two recurrences) and 3 episodes of superficial thrombosis.

The subgroup with multiple thrombosis appears to have many common characteristics: a) all patients have the S/βMA double heterozygosity genotype b) they received a therapeutic dose of anticoagulant treatment after the first thrombosis episode c) the level of Hb was<11 and d) all received hydroxyurea.

This subgroup consisted of 3 women (60%) and 2 men (40%). Three (60%) received antiplatelet treatment after the first thrombotic episode while 2 (40%) did not. Two patients (40%) had undergone surgical splenectomy, 1 (20%) had functional asplenia and 2 (40%) had none of the above. Four (80%) were under exchange tranfusions programme. Four (80%) had normal platelet count while 1 (20%) had platelet number above normal. Three (60%) had an increased WBC count and 2 (20%) had normal. In this subpopulation, 4 (80%) had underlying diseases that could also explain the thrombosis. The mean baseline count for their laboratory values were: a) PLT: 308*103, b) WBC: 9,760, HbS: 47.4%, and HbF: 12%.

Regarding the timeline of the succeeding thrombotic episode, 4 had one within 5 years, while 1 had it within 20 years.

Discussion

Prevalence of VTE in the study population

The prevalence of thrombosis in our study was 26.1%, while the prevalence of VTE was 17.4%, consistent with the one described in literature (8-25%). At the same time, however, fairly high percentage compared to the 0,1-0,2% (1 in 1000) the annual incidence of VTE in the general population [18,19].

Keeping in mind only the first thrombotic events, there were identical rates of PE and DVT [33% (4/12) vs 33% (4/12)]. However, taking into account the 26 thrombotic episodes that the group of 12 patients experienced during the 25 years, it appears that DVT is more frequent than PE [30.8% (8/26) >23.1% (6/26)].

Characteristics and risk factors of the study population

Our study underlines that patients with SCD have a strong thrombophilic substrate. The first thrombotic episodes occur at a mean age of 38.1 years, a deviation of approximately 10 years compared to literature (mean age 29.9 years) [20]. No difference was observed between the two sexes, but the first thrombotic episode was found predominantly in S/βMA heterozygosity (75%>25%). The corresponding deduction to 26 thrombotic episodes, reinforces the previous observation (23/26 - 88.5%> 3/26-11.5%).

Compared with large retrospective studies, it is deduced that administration of anticoagulant treatment and specifically its dose, show statistical significance both in univariate (both with p= 0.000) and in the various multivariate models (p= 0.000 and p= 0.001 respectively). This correlation however may not completely be objective as those who had thrombosis certainly received anticoagulant treatment and the majority received a therapeutic dose. What should be noted is that 7 (58.3%) received an anticoagulant dose after the first episode and did not relapse, and of the 5 who had more than one episode, 4 have concomitant diseases (2 have atrial fibrillation, 1 has arterial stenosis, 1 has incompetence of great saphenous vein, polpeteal and its perforating branches, poststenotic lesions in the entire lower limb) that could justify thrombosis (even in the first episode). Adding to this fact, the 5 patients receiving anticoagulant treatment systematically due to comorbidities did not experience any VOE.

There was a recurrence of VTE happened within a 5year window for 1 patient while 2 experienced recurrences within 10 years. This observation digresses from the literature where recurrences appear to be more frequent within a 5 year period. [21,22].

Although the age of the patient is deemed statistically significant, suggesting an increased rate of thrombosis as age increases, this observation is spurious. Examining only the subpopulation that experienced thrombosis (n=12) it is evident that thrombosis occurs more frequently in younger ages

(20-39 years, 58.3%) and decreases progressively with age. This finding is confirmed by previous studies where the combination of thrombosis and SCD seems to have a tropism for younger ages [23]. Also, the age-thrombosis analysis is not valid as it assumes the existence as a prerequisite.

A point of contradiction is that while the association of transfusion with thrombosis was not statistically significant, the relationship between the type of transfusion and thrombosis was (p= 0.018 (<0.05). As seen in the test group, 7/23 (30.4%) who were systematically transfused had thrombosis while 5/23 (21.7%) who were not transfused also had thrombosis. These percentages are not statistically significant as both their size and their deviation are not sufficient to reproduce statistical significance. On the contrary, when thrombosis was analyzed by type of transfusion, which includes the 23 patients who were transfused, the simultaneous presence of the following facts emerged as statistically significant: a) that exchange transfusion (15/23) has the same effect on thrombosis (7/15 who undergone exchange transfusion had thrombosis compared to 8/15 who did not), b) all patients who systematically received regular transfusions did not have thrombosis. These findings could be explained by the fact that exchange transfusion in our unit is performed manually, an issue that needs improvement in our country.

Finally, factors such as surgical splenectomy and homozygous sickle cell anemia did not show a statistically significant difference in contrast to current literature. This result may be a consequence of the insufficient sample size [23–27].

Characteristics of the subpopulation that experienced ≥ 1 thrombosis

Five (41.7%) experienced more than one episode in as early as a few months to as late as 17 years. The 1st VTE recurrence occurred within 5 years in 1 patient and within 10 years in 2, a result that is not consistent with the literature. While this sample is small enough to draw conclusions using statistical methods, it is worth noting the predominance of the S/βMA double heterozygosity genotype and the occurrence of multiple episodes at age ≤ 45 (10 out of 14 heterochronous thrombotic episodes).

Limitations

The present study is a retrospective observational study with data from the patients' medical records. The number of patients which might be the reason for the lack of statistically significant correlations in many of the variables that have been found as such in the literature. Secondly, the absence of other genotypes of SCD seemed critical. In some cases, information collected regarding the patients' concomitant diseases was the result of an interpretation of the therapeutic treatment they were receiving. At the same time, a few patients' reason for administration of anticoagulant treatment was not known.

In variables such as functional asplenia, few patients had imaging tests around the occurrence of an the episode of thrombosis, so interpretations were based on the recorded clinical profile, the platelet count and the last ultrasound (regardless of dating). For the coexistence of PE and DVT that has been examined by various studies, there was no imaging test to document the positive or negative result. In 10 of 12 patients with thrombosis, there was no report on whether testing for hereditary thrombophilia was performed.

Another limitation of this study was if the date of splenectomy had preceded or followed the thrombotic episode was not known. Finally, it is not confirmed whether patients showed complete or relative compliance to their treatment.

Conclusions

There is a significant correlation between the pathophysiology of sickle cell disease and thrombosis, with VTE as the main representative. This interaction is complicated by the increased likelihood of recurrence of VTE. Recurrent VTE events as well as the first thrombotic event tend to manifest at a young age in various anatomical sites. Although the administration of anticoagulant treatment reduces the risk of recurrence of VTE and the occurrence of new thrombotic episodes, it

does not eliminate it, nor does it remove the procoagulant mechanisms. Despite better understanding of the pathogenesis of the disease and the effectiveness of anticoagulant treatment, the innate complexity of the disease makes it difficult to lessen the likelihood of thrombosis which is associated with a broad spectrum of complications ranging from functional deficits to death.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

ACS Acute Chest Syndrome

AF Arial Fibrillation

DOAC Direct Oral Anticoagulant

DVT Deep Venous Thrombosis

Hb Hemoglobin

LMWH Low Molecular Weight Heparin

PE Pulmonary Embolism

RBC Red Blood Cell

SCA Sickle Cell Anaemia

SCD Sickle Cell Disease

VKA Vitamin K Antagonist

VOE Vasoocclusive Event

VTE Venous Thromboembolism

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