β-Thalassemia intermedia: morbidity uncovered

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Chapter 4

Vascular Disease

Splenectomy And Thrombosis: The Case Of Thalassemia Intermedia

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Splenectomy and thrombosis: the case of thalassemia intermedia

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Summary. *Background:* Hypercoagulability in splenectomized patients with thalassemia intermedia (TI) has been extensively evaluated. However, clinical and laboratory characteristics of patients who eventually develop overt thromboembolic events (TEE) are poorly studied. *Patients/Methods:* Three Groups of TI patients ($n = 73$ each) were retrospectively identified from a registry involving six centers across the Middle East and Italy: Group I, all splenectomized patients with a documented TEE; Group II, age- and sex-matched splenectomized patients without TEE; and Group III, age- and sex-matched non-splenectomized patients without TEE. Retrieved data included demographics, laboratory parameters, clinical complications, and received treatments that may influence TEE development, and reflected the period prior to TEE occurrence in Group I. *Results:* The mean age of Group I patients at development of TEE was 33.1 ± 11.7 years, with a male to female ratio of 33:40. TEE were predominantly venous (95%) while four patients (5%) had documented stroke. Among studied parameters, Group I patients were more likely to have a nucleated red blood cell (NRBC) count $\geq 300 \times 10^6$ L^{-1}, a platelet count $\geq 500 \times 10^9$ L^{-1} and evidence of pulmonary hypertension (PHT), or be transfusion naïve. The median time to thrombosis following splenectomy was 8 years. Patients with an NRBC count $\geq 300 \times 10^6$ L^{-1}, a platelet count $\geq 500 \times 10^9$ L^{-1}, or who were transfusion naive also had a shorter time to thrombosis following splenectomy. *Conclusion:* Splenectomized TI patients who will develop TEE may be identified early on by high NRBC and platelet counts, evidence of PHT, and transfusion naivety.

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Introduction

The thalassemias, a group of inherited disorders of hemoglobin synthesis, are the most common monogenetic disease worldwide [1]. Extremely diverse phenotypes exist within the thalassemia syndromes. At one end of the spectrum is thalassemia minor, a clinically silent, mildly hypochromic and microcytic anemia. At the other end is thalassemia major (TM), which refers to those patients whose clinical course is characterized by profound anemia, who are presented for medical attention in the first year of life, and who subsequently require regular blood transfusions for survival [2]. The term thalassemia intermedia (TI) was first suggested to describe patients who had clinical manifestations that were too severe to be termed minor yet too mild to be termed major, although there remains substantial overlap between the three conditions [3]. Our understanding of the molecular and pathophysiological mechanisms underlying the disease process in patients with TI has substantially increased over the past decade [4]. Three main factors highlight the pathophysiology of TI: ineffective erythropoiesis, chronic anemia/hemolysis, and iron overload secondary to increased intestinal absorption [4]. However, the extreme diversity in phenotypic expression in TI patients led to a wide variation in observed clinical complications and management practises, which remain solely based on physician preferences rather than evidence-based guidelines [5].

Among the clinical complications of TI that were found to occur at a higher rate than in patients with TM are

thromboembolic events (TEE) [6,7]. The largest epidemiological study to date analyzed data from 8860 thalassemia patients (6670 TM and 2190 TI) and demonstrated that TEE occurred 4.38 times more frequently in TI than TM patients [7]. The hypercoagulability in TI has been attributed to several factors, including a procoagulant activity of hemolyzed circulating red blood cells (RBCs), increased platelet activation, coagulation factor defects, depletion of antithrombotic factors and endothelial inflammation, among others [8]. These factors have been observed at a higher rate in splenectomized patients [8]. Clinical studies also confirmed that splenectomized TI patients have a higher incidence of TEE than non-splenectomized controls [5,7,9,10]. In the OPTIMAL CARE study, 73/325 (22.5%) splenectomized patients developed TEE compared with 9/259 (3.5%) non-splenectomized patients ($P < 0.001$) [5]. However, characteristics of those splenectomized TI patients that eventually develop TEE have never been evaluated.

In this study, we aim to demonstrate clinical and laboratory identifiers that characterize splenectomized TI patients who develop TEE, in an effort to highlight those patients that should be considered for preventive strategies through optimal intervention.

Patients/Methods

This was a retrospective review of data from TI patients currently registered at six comprehensive care centers in Lebanon, Italy, Iran, Egypt, the United Arab Emirates and Oman. Institutional review boards (IRBs) at each center approved the study protocol. All patients were diagnosed with TI based on criteria previously described [11]. All patients had pure β globin gene mutations [IVS-I-6 (T → C), IVS-I-5 (G → C), IVS-II-1 (G → A), or Codon 39 (C → T)]. Three groups of patients were assigned: Group I, splenectomized patients with a documented TEE; Group II, age- and sex-matched splenectomized patients without TEE; and Group III, age- and sex-matched non-splenectomized patients without TEE. For matching, we used the age at development of TEE in Group I, and matched patients from Group II and III accordingly. That age was considered the last patient follow-up and all retrieved data reflected the preceding period. In Group I and II patients, retrieved data also reflected the period after splenectomy. Data included: demographics; type of TEE; duration since splenectomy; evidence of pulmonary hypertension (PHT) (defined as a systolic pulmonary artery pressure > 35 mmHg, which corresponds to a tricuspid regurgitant velocity on Doppler echocardiography of > 2.8 m sec^{-1} + exertional dyspnea without evidence of left heart disease [12]), heart failure (HF) (modified Framingham criteria [13]), diabetes mellitus (DM) (American Diabetes Association criteria [14]), abnormal liver function (alanine aminotransferase level > 50 IU L^{-1}), family history of TEE, thrombophilia (factor V Leiden, factor II [prothrombin] *G20210A*, or methylenetetrahydrofolate reductase *C677T* mutations; antithrombin III, protein C, or protein S deficiency), or malignancy; use of blood transfusions, hydroxyurea (none of the patients received any other fetal hemoglobin [HbF] inducers or erythropoietin), antiplatelets or anticoagulants; and mean total hemoglobin (Hb) level, HbF level, nucleated RBC (NRBC) count, and platelet count of all available laboratory records for each patient.

Statistical analysis

Descriptive statistics are expressed as medians, means ± standard deviation (SD), or percentages. Univariate analysis was performed to determine differences in study parameters between the three groups using the ANOVA test for continuous variables and the chi-square and Fisher's exact tests for categorical variables. Multivariate logistic regression analysis was carried out to determine the independent effect of study parameters, where all significant variables on univariate analysis were entered into the model. In the multivariate model, NRBC counts were categorized as < or ≥ 300×10^6 L^{-1} and platelet counts were categorized as < or ≥ 500×10^9 L^{-1}, as these represented rounded median values. Comparisons of median time to thrombosis (TTT) following splenectomy were carried out by Kaplan–Meier analysis, and *P*-values from the log rank test were reported. All *P*-values are two sided with the level of significance set at < 0.05.

Results

Thromboembolic events

A total of 73 splenectomized TI patients with documented TEE (Group I) were identified. The mean age of patients at development of TEE was 33.1 ± 11.7 years (range, 6–76 years), with a male to female ratio of 33:40. Thromboembolic events were predominantly venous (95%) while four patients (5%) had evidence of stroke (Table 1). None of the patients had recurrent TEE. The diagnosis of deep vein thrombosis (DVT), superficial thrombophlebitis and portal vein thrombosis was based on ultrasonography or venography in all patients. All patients with pulmonary embolism had evidence of DVT, and diagnosis was based on lung ventilation/perfusion scan (23%) or computed tomography pulmonary angiography (77%). The diagnosis of stroke was based on both clinical and radiological grounds in all cases.

Table 1 Type of thromboembolic event in splenectomized TI patients (Group I)

Type of thromboembolic event	*n* (%)
DVT, *n* (%)	46 (63.0)
PE*, *n* (%)	13 (17.8)
STP, *n* (%)	12 (16.4)
PVT, *n* (%)	11 (15.1)
Stroke, *n* (%)	4 (5.5)

DVT, deep vein thrombosis; PE, pulmonary embolism; STP, superficial thrombophlebitis; PVT, portal vein thrombosis. *All patients who had PE had confirmed DVT.

Characteristics of splenectomized TI patients who developed TEE

Group I patients were compared with 73 age- and sex-matched patients from each of Groups II and III (Table 2). There were no statistically significant differences in mean Hb or HbF levels between the three groups. However, mean NRBC counts were significantly higher in Group I, followed by Group II, then Group III ($P < 0.001$). Similarly, mean platelet counts were highest among Group I patients, followed by Group II, then Group III ($P < 0.001$). There was no statistically significant difference in the proportion of patients with HF, DM, abnormal liver function, family history of TEE, thrombophilia or malignancy between the three groups. However, a higher proportion of patients had pulmonary hypertension in Group I as compared with Groups II and III ($P < 0.001$). The highest proportion of patients receiving transfusion therapy was in Group III, followed by Group II, then Group I ($P = 0.001$). There was no statistically significant difference in the proportion of patients receiving antiplatelets, anticoagulants or hydroxyurea between the three groups. Moreover, there was no statistically significant difference between groups in the proportion of patients with co-inheritance of α thalassemia [α^+ ($-\alpha^{3.7}$ and $-\alpha^{4.2}$) or α^0 ($^{-Med}$ and $^{-SEA}$)] or determinants associated with increased γ-chain production (*Xmn*-I +/+ genotype at position −158 of *HBG2*) (data not shown in Table 2).

On multivariate logistic regression analysis, an NRBC count $\geq 300 \times 10^6$ L^{-1}, a platelet count $\geq 500 \times 10^9$ L^{-1}, PHT and transfusion naivety were all independent and significant factors that differentiated the three groups. Group I patients were 11.11 times and 76.92 times more likely to have an NRBC count $\geq 300 \times 10^6$ L^{-1} and a platelet count $\geq 500 \times 10^9$ L^{-1}, respectively. Moreover, Group I patients were 7.30 times and

Table 2 Comparison of study parameters between Group I, II and III patients

Parameter	Group I Splenectomized with TEE $n = 73$	Group II Splenectomized without TEE $n = 73$	Group III Non-splenectomized $n = 73$	*P*-value
Mean age ± SD, years	33.1 ± 11.7	33.3 ± 11.9	33.4 ± 13.1	0.991
Male: female	33:40	35:38	34:39	0.946
Mean Hb ± SD, g dL^{-1}	9.0 ± 1.3	8.8 ± 1.2	8.7 ± 1.3	0.174
Mean HbF ± SD, %	45.9 ± 28.0	54.4 ± 32.8	44.2 ± 27.2	0.429
Mean NRBC count ± SD, × 10^6 L^{-1}	436.5 ± 205.5	279.0 ± 105.2	239.5 ± 128.7	< 0.001
Mean platelet count ± SD, × 10^9 L^{-1}	712.6 ± 192.5	506.3 ± 142.1	319.2 ± 122.0	< 0.001
PHT, *n* (%)	25 (34.2)	17 (23.3)	3 (4.1)	< 0.001
HF, *n* (%)	7 (9.6)	5 (6.8)	1 (1.4)	0.101
DM, *n* (%)	4 (5.5)	5 (6.8)	1 (1.4)	0.256
Abnormal liver function, *n* (%)	2 (2.7)	2 (2.7)	3 (4.1)	0.863
Family history of TEE	3 (4.7)	1 (1.4)	3 (4.7)	0.554
Thrombophilia, *n* (%)	3 (4.7)	2 (2.7)	2 (2.7)	0.863
Malignancy, *n* (%)	1 (1.4)	2 (2.7)	0 (0)	0.363
Transfused, *n* (%)	32 (43.8)	48 (65.8)	54 (74.0)	0.001
Antiplatelet or anticoagulant use, *n* (%)	1 (1.4)	3 (4.1)	2 (2.7)	0.598
Hydroxyurea use, *n* (%)	13 (17.8)	17 (23.3)	29 (27.4)	0.383

TEE, thromboembolic events; Hb, total hemoglobin; NRBC, nucleated red blood cell; HbF, fetal hemoglobin; PHT, pulmonary hypertension; HF, heart failure; DM, diabetes mellitus.

Table 3 Multivariate analysis of parameters that differentiate Group I, II and III patients

Parameter	Group	OR	95% CI	*P*-value
NRBC count $\geq 300 \times 10^6$ L^{-1}	Group III	1.00	Referent	< 0.001
	Group II	5.35	2.31–12.35	
	Group I	11.11	3.85–32.26	
Platelet count $\geq 500 \times 10^9$ L^{-1}	Group III	1.00	Referent	< 0.001
	Group II	8.70	3.14–23.81	
	Group I	76.92	22.22–250.00	
PHT	Group III	1.00	Referent	0.020
	Group II	4.00	0.99–16.13	
	Group I	7.30	1.60–33.33	
Transfusion naivety	Group III	1.00	Referent	0.001
	Group II	1.67	0.82–3.38	
	Group I	3.64	1.82–7.30	

NRBC, nucleated red blood cell; PHT, pulmonary hypertension; OR, adjusted odds ratio; CI, confidence interval.

3.64 times more likely to have PHT or be transfusion naïve, respectively (Table 3).

Duration since splenectomy and development of TEE

In Group I patients, the median TTT following splenectomy was 8 years (range, 1–33 years) (Fig. 1). The median TTT was significantly shorter in patients with an NRBC count $\geq 300 \times 10^6$ L^{-1} compared with those with $< 300 \times 10^6$ L^{-1} (8 vs. 15 years, $P = 0.002$; Fig. 2A). Similarly, the median TTT was significantly shorter in patients with a platelet count $\geq 500 \times 10^9$ L^{-1} compared with $< 500 \times 10^9$ L^{-1} (8 vs. 22 years, $P = 0.008$; Fig. 2B). The median TTT was also significantly shorter in transfusion naïve compared with transfused patients (7 vs. 13 years, $P = 0.009$; Fig. 2C). There was no statistically significant difference in the median TTT between patients with and without PHT (9 vs. 8 years, $P = 0.703$; Fig. 2D). For patients who had an NRBC count $\geq 300 \times 10^6$ L^{-1}, a platelet count $\geq 500 \times 10^9$ L^{-1} and who were transfusion naïve the median TTT was 6 years (range, 2–15 years).

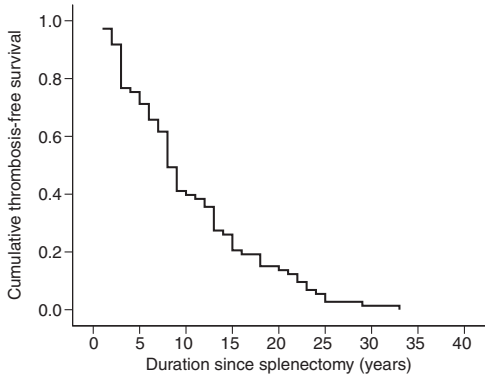

Fig. 1. Thrombosis-free survival in Group I patients.

Discussion

Our study indicates that splenectomized TI patients who develop TEE are characterized by high NRBC and platelet counts, and are more likely to have evidence of PHT and be transfusion naive. Moreover, high NRBC and platelet counts as well as transfusion naivety are associated with earlier development of TEE following splenectomy. The main indications for splenectomy in patients with TI include growth retardation or poor health, leucopenia, thrombocytopenia,

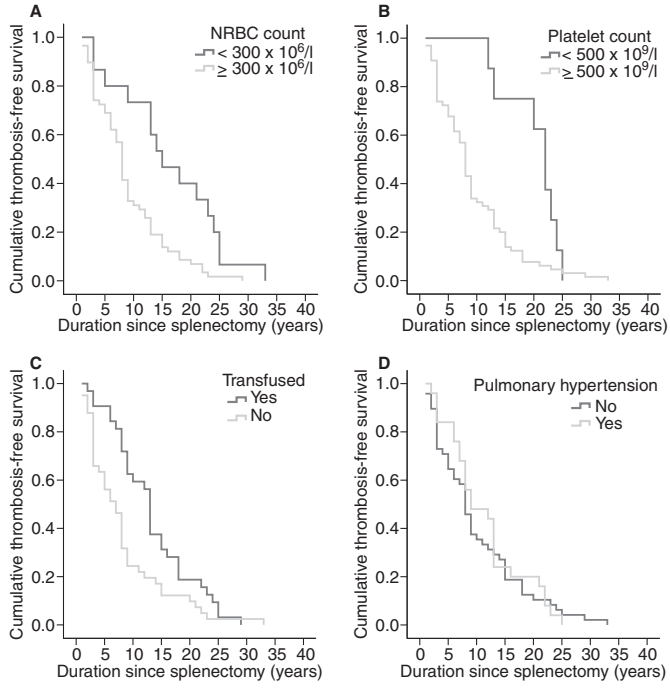

Fig. 2. Thrombosis-free survival in Group I patients according to (A) nucleated red blood cell (NRBC) count ($< 300 \times 10^6$ L^{-1}, $n = 15$; $\geq 300 \times 10^6$ L^{-1}, $n = 58$), (B) platelet count ($< 500 \times 10^9$ L^{-1}, $n = 8$; $\geq 500 \times 10^9$ L^{-1}, $n = 65$), (C) transfusion status (transfused, $n = 32$; non-transfused, $n = 41$), and (D) pulmonary hypertension (yes, $n = 25$; no, $n = 48$).

increased transfusion demand, or symptomatic splenomegaly [4]. A substantial amount of evidence, however, continues to support harmful effects of splenectomy in both normal individuals and patients with hematological disorders [5,9,15]. Complications such as an increased susceptibility to infections and TEE remain the most commonly reported and worrisome [5,7,9,10,15]. However, until a replacement for splenectomy is recommended through evidence-based guidelines, a large number of TI patients will continue to be splenectomized. These, alongside patients who had already undergone splenectomy, constitute a large proportion of TI patients at risk of TEE. In our study, we aimed to identify the characteristics of those splenectomized TI patients who will eventually develop TEE, which should help in undertaking appropriate measures and aid in the design of prospective trials that evaluate the efficacy and safety of intervention. We decided to investigate those characteristics that should be easily identified through careful patient monitoring with history taking and physical examination alongside routine and simple laboratory tests, which are part of regular patient follow-up. Despite the fact that more specific markers and tests of hypercoagulability merit evaluation for such an aim [8], these may not be available, practical or affordable in developing countries where TI is prevalent [16].

The hemolytic anemia implicated in patients with TI causes iron-dependent oxidation of membrane proteins and formation of red-cell senescence antigens such as phosphatidylserine that cause thalassemic RBCs to be rigid and deformed and to aggregate, resulting in premature cell removal [17–20]. Studies have shown that thalassemic RBCs may be a source of negatively charged phospholipids, which can eventually increase thrombin generation [21,22]. An even higher number of circulating RBCs with negatively charged phospholipids was found in splenectomized patients [23]. Moreover, a study evaluating circulating RBC microparticles (submicrometric membrane fragments with procoagulant potential) found significantly higher levels in patients with TI compared with controls, especially in splenectomized patients [24]. These abnormalities have been reduced to normal range after the patients received a blood transfusion that decreases the number of circulating damaged RBCs [25]. These findings may partly explain why patients who had high NRBC counts or were transfusion naïve had a higher occurrence of TEE in our cohort. In TI, the prevailing approach has been avoidance of early blood transfusions and the concomitant requirement for chelation therapy, reserving the introduction of transfusion until later in the disease course when complications arise. Consequently, unlike TM, evaluation of the role of transfusions in the management of TI has been limited. Similar to our findings, few observational studies have also confirmed that transfused TI patients suffer fewer TEE, PHT and silent brain infarcts as compared with transfusion naïve patients [5,7,26,27]. This may be attributed to correction of the underlying ineffective erythropoiesis and the resulting damaged RBCs with thrombogenic potential. As such, earlier introduction of transfusion therapy aiming to prevent the consequences of chronic hemolytic anemia may benefit TI patients by prevention, rather than palliation of late and irreversible hemolysis-related complications. Rather than enforcing the regular transfusion regimens implemented in TM, blood transfusion, if initiated in patients with TI, will require closer monitoring and should be individually tailored to meet patient needs. Although earlier introduction of blood transfusions will increase the rate of iron accumulation, effective methods of iron chelation are available for patients with TI [5,28–31], and the benefits of transfusion therapy may greatly outweigh the cost and inconvenience of iron chelation therapy.

The medical literature is rich in evidence suggesting that patients with thalassemia have activated platelets. Moreover, flow cytometric studies have also confirmed the chronic platelet activation status. In thalassemia, there is evidence of increased platelet aggregation [32], and an increased proportion of platelets expressing CD62P (P-selectin) and CD63 [33,34]. Further evidence of chronic platelet activation in patients with thalassemia was provided by the measurement of urinary metabolites of prostacyclin (PGI2) and thromboxane A2 (TXA2), where a significant increase (4–10-fold) in the urinary excretion of the stable hydrolysis products of TXA2 and PGI2 was found in thalassemia patients compared with controls [35]. The thrombocytosis observed after splenectomy may thus imply increased hypercoagulability and TEE risk in these patients [36]. In our study, higher platelet counts were found in those splenectomized patients who developed TEE. As such, consideration of antiplatelet aggregants (e.g. aspirin) for the prevention of TEE in these patients remains logical. The use of anticoagulant or antiplatelet agents in thalassemia patients has never been prospectively evaluated in large well-designed trials, although patients who were placed on aspirin were found to have lower recurrence rates of TEE than those who were not [7].

The higher occurrence of PHT in splenectomized patients who develop TEE may suggest a common underlying etiology between the two conditions. Unlike patients with sickle cell disease [37], the pathophysiology of PHT in patients with thalassemia has not been extensively studied and is mainly attributed to a state of chronic anemia [38]. PHT has been linked to the intensity of hemolysis, nitric oxide metabolic dysregulation, and hypercoagulability in patients with sickle cell disease; whether the same mechanisms contribute to PHT in TI is not yet known and needs to be investigated. Of note, autopsies of a large series of patients with TI revealed thrombotic lesions in the pulmonary arteries, which may have been due to circulating platelet aggregates [39]. Similar findings of multiple microthrombi, which were composed mainly of platelets, were seen in the pulmonary arterioles and microcirculation in autopsies of two splenectomized patients with thalassemia [40]. Whether these microthrombi contribute to PHT in TI is not well understood. However, the effect of intervention (initiated to prevent TEE) on PHT in TI patients merits evaluation.

The delayed type of TEE (median 8 years) observed in splenectomized TI patients has an important clinical implica-

tion. First, it highlights that TEE in this population is not an acute postoperative complication but rather a late manifestation of a progressive underlying pathology. In fact, a negative impact of time (aging) on physiological adaptation of thalassemia patients to their underlying disease has been documented [41]. Second, it requires that any modality considered for prevention of TEE has to be evaluated for long-term efficacy and safety, as patients may be placed on such interventions for long durations to ensure effective control of hypercoagulability and TEE prevention. The aforementioned suggestions in this report, namely tailored transfusion programs and antiplatelet/anticoagulant therapy, have documented long-term experience in several patient populations and are worthwhile examining for TI.

One limitation in our study is the use of echocardiography instead of cardiac catheterization for the diagnosis of PHT, which may increase the rate of false positive findings. However, our patients were mainly screened for PHT after presenting with exertional dyspnea with no evidence of left heart disease. Moreover, echocardiography is still the modality of choice used in many studies on thalassemia and sickle cell anemia due to financial/practical reasons and reliance on reports of good relationship between Doppler estimates and invasive measurements of pulmonary arterial pressure at baseline and after treatment, despite the variable echocardiographic cut-off values used to label patients with PHT [42–44].

In conclusion, our study demonstrated that splenectomized TI patients who will develop TEE may be identified early on by high NRBC and platelet counts, evidence of PHT, and transfusion naivety. It also calls for prospective clinical trials that evaluate the efficacy, safety and cost effectiveness of transfusion and antiplatelet/anticoagulant therapy in preventing TEE in this patient population. Such studies are expected to evaluate the optimal timing, dose and duration of intervention, and the added advantage of multimodal therapy.

Addendum

A. T. Taher, K. M. Musallam and M. D. Cappellini were responsible for conception and design, data analysis and interpretation, and manuscript writing; K. M. Musallam performed statistical analysis; M. Karimi, A. El-Beshlawy, K. Belhoul, S. Daar and M. Saned gave administrative support and helped in provision of study material or patients; C. Cesaretti helped in collection and assembly of data. All authors gave final approval of the manuscript for submission.

Disclosure of Conflict of Interests

The authors state that they have no conflict of interest.

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