Prevalence of thromboembolic events among 8,860 patients with thalassaemia major and intermedia in the Mediterranean area and Iran

Ali Taher1, Hussain Isma’eeel1, Ghassan Mehio1, Daniela Bignamini2, Antonis Kattamis3, Eliezer A. Rachmilewitz4, Maria Domenica Cappellini2

1Internal Medicine, American University of Beirut, Beirut, Lebanon; 2Centro Anemie Congenite, Ospedale Maggiore Policlinico, IRCCS, Milano, Italy; 3First Department of Paediatrics, University of Athens School of Medicine, Athens, Greece; 4Haematology Department, The E. Wolfson Medical Center, Holon, Israel

Summary
Beta-thalassaemia is a congenital haemolytic anaemia characterized by partial (intermedia, TI) or complete (major, TM) deficiency in the production of β-globin chains. The primary aim of this study was to determine the prevalence of thromboembolic events in patients with β-thalassaemia. To achieve this, a multiple-choice questionnaire was sent to 56 tertiary referral centres in eight countries (Lebanon, Italy, Israel, Greece, Egypt, Jordan, Saudi Arabia and Iran), requesting specific information on patients who had experienced a thromboembolic event. The study demonstrated that thromboembolic events occurred in a clinically relevant proportion (1.65%) of 8,860 thalassaemia patients (TI – 24.7% or TM – 75.3%) from the Mediterranean and Iran. Thromboembolism occurred 4.38 times more frequently in TI than TM (p<0.001), with more venous events occurring in TI and more arterial events occurring in TM. Thrombosis in thalassaemia was also more common in females, splenectomized patients and those with profound anaemia (haemoglobin <9 g/dl). Due to the increased risk of thromboembolic events, the rationale for splenectomy should perhaps be reassessed and the role of transfusion therapy for the prophylaxis of thrombosis, among other complications, be evaluated prospectively.

Keywords
Thrombosis, thalassaemia intermedia, thalassaemia major

Introduction
Beta-thalassaemia is a congenital haemolytic anaemia characterized by either partial (intermedia [TI]) or complete (β-thalassaemia major [TM]) deficiency in the production of β-globin chains (1, 2), which determines the clinical outcome (1, 3, 4). TI encompasses a much broader clinical spectrum than TM. At one end of the spectrum of TI, patients are diagnosed between the age of two and six years and may survive without regular blood transfusions but with major impairment in growth and development (3). At the other end of the spectrum, mildly affected TI patients remain asymptomatic until adult life and may require only sporadic transfusions.

Standards of care for thalassaemic patients have improved in recent years, resulting in a concomitant increase in life expectancy (5). As a result, new complications are becoming increasingly evident, such as profound haemostatic abnormalities (5). In 1972, while reviewing 138 cases of TM in Greece, Logothetis et al. described a 'stroke syndrome' in two patients and neurological deficits compatible with transient ischaemic attacks in around 20% of cases (6). Subsequently, similar findings were reported in patients who were not regularly transfused (7–9). Today, thalassaemia is known to be associated with a hypercoagulable state and an increased risk of developing clinical and/or subclinical thromboembolic events, particularly in TI patients who have undergone splenectomy (5).

The aims of this study were to estimate the prevalence of thromboembolic events in a large number of patients with β-thalassaemia, and to determine whether there is a rationale for considering prophylactic treatment with anticoagulants.

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Blood Coagulation, Fibrinolysis and Cellular Haemostasis
Materials and methods

A multiple-choice questionnaire was sent to 56 tertiary referral centres in eight countries: Egypt, Greece, Iran, Israel, Italy, Jordan, Lebanon and Saudi Arabia. The questionnaires requested information on the total number of patients treated at each centre and, specifically, on those who had experienced a thrombotic event. The information included type of thalassaemia, age, sex, history of splenectomy, type, site and diagnostic method of thromboembolic events, frequency of blood transfusions, average haemoglobin level and non-constitutional risk factors. All statistical analyses were performed using SPSS 12.0. Data are summarized as proportions (mean ± SD where applicable). Comparisons between the two types of thalassaemia were performed by odds ratio (OR). P-values < 0.05 are reported as statistically significant, for descriptive purposes only. As the study was a retrospective chart review that precluded the identification of the patient or allowed any breach of confidentiality, the Institutional Review Board of Research Ethics granted exemption from patient consent.

Results

Data were obtained from 8,860 patients with β-thalassaemia with a mean age of 30 ± 13 years, 2,190 with TI (24.7%) and 6,670 with TM (75.3%). Overall, 146 patients (1.65%) had experienced a thromboembolic event, 85 with TI (3.9%) and 61 with TM (0.9%) (p < 0.001 between thalassaemia types; Table 1). There was a slight female preponderance (53.8%) in the occurrence of thrombotic events and patients with TI were significantly older than those with TM at the time of the event (p = 0.02). The OR for a thromboembolic event to occur in TI versus TM was 4.38 (95% confidence intervals [CI]; 3.14 to 6.10; p < 0.001). Diagnosis was made by both clinical and radiological parameters in 63% of cases, clinical parameters only in 27% of cases, and radiological parameters only in 10%. The overall mortality rate from thromboembolic events was 14% (Table 1).

In the overall population, thromboembolic events were predominantly venous (57%), with arterial thrombosis accounting for 40% of events and simultaneous arterial and venous thromboses accounting for 3%. Most patients with TI had venous thrombosis (66%), while, in contrast, arterial events were significantly more common than venous events in patients with TM (p = 0.009). In the overall population, the most common thrombotic events were deep vein thrombosis (DVT; 32%), stroke (18%), portal vein thrombosis (PVT; 16%), pulmonary embolism (PE; 13%) and superficial thrombophlebitis (STP; 4.7%). Patients with TM had a significantly greater risk for stroke (OR = 3.72 [1.48, 9.32]; p = 0.005) and for ‘other’ events (OR = 3.14 [1.33, 7.14]; p = 0.009), while those with TI had a significantly greater risk for DVT (OR = 2.13 [1.02, 4.46]; p = 0.045) (Fig. 1). Superficial thrombophlebitis occurred only among patients with TI.

Most TI patients who had a thromboembolic event were splenectomized (94%) and had average haemoglobin levels < 9 g/dl (68%), and only one-third were receiving regular blood transfusions. The most common thrombotic events in splenectomized patients were DVT (33.3%), stroke (17.4%), PVT (16.0%) and PE (11.6%). Recurrent thrombosis was reported in 25 and 36% of TM and TI patients, respectively, with no difference between thalassaemia groups (OR = 1.6 [0.7–3.6]). In patients with TI, a recurrence of thrombosis occurred in 42.2% of those with an average haemoglobin level < 9 g/dl versus 22.7% of those with an average haemoglobin > 9 g/dl (OR = 0.4 [0.1, 1.2]). The 52% of TI patients who were receiving aspirin after splenectomy had a lower recurrence of stroke, DVT, PE, STP and PVT compared with those who were not, although these differences were not statistically significant. Eleven female patients were receiving oral contraceptive pills (OCP).

Thrombophilia workup for factor V Leiden, prothrombin mutation, antithrombin III deficiency, protein C and S deficiency and hyperhomocystinaemia was carried out in 66 of the 146 thalassaemia patients with thromboembolic events. Of these patients, 27 (41%) were found to have thrombosis predisposing genetic factors (16 factor V Leiden, 5 prothrombin mutation, and 6 hyperhomocystinaemia).

<table>
<thead>
<tr>
<th>Table 1: Patient characteristics*.</th>
<th>TI (n=85)</th>
<th>TM (n=61)</th>
<th>All patients (n=146)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males, %</td>
<td>42.4 (36/85)</td>
<td>51.7 (31/60)</td>
<td>46.2 (67/145)</td>
</tr>
<tr>
<td>Females, %</td>
<td>57.6 (49/85)</td>
<td>48.3 (29/60)</td>
<td>53.8 (78/145)</td>
</tr>
<tr>
<td>Mean age at time of event, years</td>
<td>33.4 ± 14.9</td>
<td>25.1 ± 8.8</td>
<td>30.0 ± 13</td>
</tr>
<tr>
<td>Splenectomized, %</td>
<td>94.0 (78/83)</td>
<td>91.8 (56/61)</td>
<td>93.0 (134/144)</td>
</tr>
<tr>
<td>Haemoglobin &lt; 9 g/dl, %</td>
<td>67.5 (52/77)</td>
<td>43.3 (26/60)</td>
<td>56.9 (78/137)</td>
</tr>
<tr>
<td>Regularly transfused, %</td>
<td>33.3 (28/84)</td>
<td>93.3 (56/60)</td>
<td>58.3 (84/144)</td>
</tr>
<tr>
<td>Recurrent thrombosis, %</td>
<td>35.6 (26/73)</td>
<td>25.0 (13/52)</td>
<td>31.2 (39/125)</td>
</tr>
<tr>
<td>Pulmonary hypertension, %</td>
<td>27.3 (15/55)</td>
<td>19.5 (8/41)</td>
<td>24.0 (23/96)</td>
</tr>
<tr>
<td>Aspirin, %</td>
<td>51.0 (26/51)</td>
<td>52.5 (21/40)</td>
<td>51.6 (47/91)</td>
</tr>
<tr>
<td>Hydroxyurea, %</td>
<td>35.3 (18/51)</td>
<td>4.7 (2/43)</td>
<td>21.3 (20/94)</td>
</tr>
</tbody>
</table>

*Not all patients responded to every question, so presented frequencies are based on responder numbers only. TI, thalassaemia intermedia, TM, thalassaemia major.
Discussion

With the advent of new modalities of treatment such as hypertransfusion and iron chelation, patients with thalassaemia are living longer than ever before. Accordingly, the medical community is facing new challenges in the diagnosis and treatment of complications that were not frequently observed in the past, including thrombotic and ischaemic manifestations (5, 10). In this analysis we observed a clinically relevant prevalence of thromboembolic events (1.65%), with a significantly greater risk observed in TI than TM (3.9 vs. 0.9%, p<0.001). These results correspond with previously published results by other groups, which demonstrated an overall prevalence ranging from 1.1–5.2% depending on the proportion of TM to TI patients involved (11–13).

Risk factors for developing thrombosis in patients with TI are age (>20 years), previous thromboembolic events, family history and splenectomy. Laboratory parameters that indicate an increased risk of thrombosis are a high platelet count (>600 x 10^9/µl) and a low plasminogen level (<60%) (13). In this study, thrombosis was slightly more common among females than males; similar findings were reported in an earlier study (14). Most TI patients who experienced a thromboembolic event were splenectomized and had anaemia (haemoglobin levels <9 g/dl). However, due to the design of the study it was not possible to fully evaluate the role of anaemia and splenectomy on the incidence of thrombosis, or to assess the elapsed time between splenectomy and thromboembolic event. Such knowledge could have helped to explain why thrombosis occurred at a later age in TI than TM patients. However, we do share with others the belief that TI is associated with a higher incidence of thrombosis compared with TM, particularly in patients who are splenectomized. The increased risk of thrombosis in thalassaemia is mainly associated with the co-existence of a chronic hypercoagulable state, which results from oxidative changes to the red blood cell (RBC) membrane by reactive oxygen species (ROS) and from the exposure of phosphatidylserine residues that promote formation of the prothrombinase complex, which in turn converts prothrombin to thrombin (5, 7). In addition, antithrombotic factor such as proteins C and S are depleted. Damage to endothelial cells due to an ongoing inflammatory state, which can be improved by iron chelation therapy (15), may also be a contributing factor (16, 17). PVT and DVT, which were observed in both thalassaemia groups in this study, are well-known complications of splenectomy (7, 18). It is particularly important to be aware of these complications since they may lead to PE and/or pulmonary hypertension (14, 18).

We have previously shown that the prevalence of genetic factors predisposing to thrombosis is similar in thalassaemic and non-thalassaemic populations (9). In the present study, 41% of patients had genetic factors predisposing them to a thromboembolic event. This proportion, which is similar to that found in the general population, supports studies indicating that thalassaemia is a hypercoagulable condition independent of predisposing genetic factors.

The use of prophylactic antiplatelet agents and hydroxyurea to reduce the risk of thrombosis is not an established practice in TI and has not been widely evaluated. Moreover, further doubt exists regarding any benefit of aspirin in the primary prophylaxis of venous thrombosis. In this study, 21.3% of TI patients who experienced a thromboembolic event were receiving hydroxyurea, although we do not know how long patients had been receiving treatment for. It is worth noting that just 52% of patients who had experienced a thromboembolic event were currently receiving aspirin that was initiated after the thrombotic event, suggesting that there is a need to increase the use of, and compliance with, this relatively safe and potentially useful medication.

Clearly, the majority of patients suffering from thrombotic events were splenectomized. However, in the absence of a comparison arm (i.e. patients who did not suffer a thrombotic event) we are conservative in recommending a change in the rationale of splenectomy in thalassaemia based on these results alone. Nevertheless, we underscore the importance of the need to identify whether there is a role for specific treatment to minimize this risk of thromboembolic phenomena. One approach that has been suggested, but that is still in need of a validation trial, would be to decrease the number of circulating pathological RBC in splenectomized patients by initiating regular transfusions. If this is not possible, medications such as hydroxyurea, aspirin and anticoagulants (e.g. coumadin) could be considered, particularly in patients with conditions that are well established, independent predictors of thrombosis such as pregnancy or sepsis, or who are undergoing surgery. Another approach would be to correct the ROS-induced RBC membrane damage using antioxidants, although this approach has not yet been verified in clinical trials. It may also be possible to design a thalassaemia-tailored thrombosis risk-assessment model to estimate thrombotic risk as a function of intrinsic (e.g. thalassaemia type, number of circulating RBC) and extrinsic (e.g. infection, surgery, splenectomy) factors. Moreover, tests for predisposing factors could also be performed, particularly in high-risk patients. If clinically verified, this type of model could serve as a guideline for possible preventative treatment to decrease the incidence of thromboembolic events, which can cause significant morbidity and mortality (as evidenced in 14% of patients in this study). It is well recognized that due to the study design, several limitations may arise including: recall bias, time-from-event bias, changes in staff over time, tertiary centres do not provide a complete denominator as an un-
certain number of patients are treated in secondary centres, failure to ascertain causes of mortality in patients dying outside the centre, and non-standardized diagnostic criteria among different centres. We believe, however, that proper documentation of the events in standardized tertiary care centres might have limited these biases. In addition, as tertiary care centres offer more centralized treatment and are in line with guideline therapy, the population described here is relatively homogeneous despite being from different countries. We acknowledge that our survey serves as a pilot trial for a more detailed future survey. This should include the characteristics of the patients who did not suffer a thromboembolic event in order to further support the conclusions regarding predisposing factors for thrombosis. Moreover, a more detailed questionnaire including information such as complete blood count at the time of the event (because of the importance this might have in analogy to other haemoglobinopathies) and the presence or attribution of thrombosis to central lines and catheters, could offer a more comprehensive picture of thrombosis in thalassaemia.

Limitations
The epidemiology of thrombosis in thalassaemia has assisted with the identification of several risk factors for the development of thrombosis. In line with this, definitive confirmation of these factors would have been more precise in our study had the characteristics of the thalassaemia patients with no thrombotic events been collected in detail and a statistical comparison performed. Such specific characteristics should have included: 1) age at splenectomy; 2) mean annual haemoglobin level; 3) target haemoglobin level for transfusion in those receiving regular transfusions; 4) documentation of the use and dose of hydroxyurea; 5) time to development of thrombotic event and time lapse from splenectomy, if performed; 6) time of initiation of antiplatelet or anticoagulation medications, including type and dose; and finally 7) data about other standard potential risk factors for thrombosis such as smoking, use of OCP (and type), date and type of surgical procedures if performed, travel, family history of thrombosis and personal history of cancer.

In conclusion, thromboembolism occurs in 1.65% of patients with thalassaemia, being 4.38 times more likely to occur in TI than TM. A validated thrombosis risk-assessment model might prove useful for identifying patients at risk for thromboembolism and those who might benefit from prophylactic therapeutic interventions.

Acknowledgements
We would like to acknowledge Dr. P. M. Mannucci for reviewing the questionnaire used in this study, and the following centres for their contribution in providing us with data regarding the patients they follow and completing the questionnaire: Shafa Hospital, Ahwaz-Iran; Kashan University of Medical Sciences; Shiraz University, Dastgeib Hospital; Umia Imam Hospital/Pediatric Hematology-Oncology; Esfahan University, Omid Hospital; Saiedalshahada Hospital, Thalassemia centre; Governmental Zareqa Hospital; Princez Rahmeh centre; Bashir Hospital; Hereditary Blood Diseases centre-Hassa-KSA; King Abdul Aziz University Hospital; Pediatric Hospital-Cairo; Faculty of Medicine, Alexandria University; Alexandria Faculty of Medicine; Zagazeg University; Tanta University; Mansoura University; SIMT; Ambulatorio Emostasi E Terapia Trasfusionale; Azienda Ospedaliera di Busto Arsizio P Ospedaliero di Tradate-U.O. di Pediatria; Ambulatorio Emotopie Congenite (A.E.C.); Dipartimento di Medicina Trasfusionale ed Ematologia Azienda Ospedaliera di Lecco; Oncoematologia Pediatrica-IRCCS Policlinico San Matteo-Pavia; U.O. Ematologia – Ospedali Riuniti; Ambulatorio Trasfusionale del Centro Trasfusionale Dell ‘Ospedale San Gerardo Monza; Clinica Pediatrica di Marchi-IKP; Ospedale di Bollate-pediatria; Servizio Trasfusionale e di Ematologia Azienda Ospedaliera Osp. di Treviglio-Caravaggio; Dip Scienze Ped Med Chir. U.O. di Genetica e Imm Ped – A.O. Policlinico "G. Martino"; Servizio di Immunemometria e Medicina Trasfusionale dell’Ospedale Carlo Poma; Centro della Microcitemia e delle Anemie Congenite; Ospedale V. Cervello Servizio Talassemia; Servizio Immunemometria E Medicina Trasfusionale; Centro Microcitemie – Dipartimento di Scienze Pediatriche e dell’Adolescenza – Università di Torino; Microcitemia A.U.S.L. BR.01; Clinica Pediatrica Ospedale San Gerardo; Azienda Osp. Legnano-u.o. Pediatria; Unita’ Operativa di Pediatria-Ospedale S’Anna; Azienda Ospedaliera di Melegnano-Presidio Ospedaliero di Vizzolo Predabissi; Centro Anemie Congenite-Ospedale Maggiore di Milano IRCCS; University Hospital of Ioannina; University Hospital of Patras; Beer Sheva; Barzilai; Afula; Rabin Medical centre; Hadassah.

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