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Malattia trombotica: condizioni cliniche ad aumentato rischio e terapia anticoagulante

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INDICE

INTRODUZIONE

Con il termine di trombosi si intende l'ostruzione di un vaso venoso o arterioso.

Il processo trombotico è alla base dei tre maggiori eventi cardiovascolari: l'ischemia miocardica, l'ictus cerebri e il tromboembolismo venoso[1]. E' stato calcolato dall'Organizzazione Mondiale della Sanità che nel 2010 circa 7 milioni di morti possono essere ricondotte ad una ischemia cardiaca e più di 2 milioni di morti ad una ischemia cerebrale[2]. In Italia l'ictus è la terza causa di morte dopo le malattie cardiovascolari e le neoplasie, causa il 0%- 2% di tutti i decessi per anno e rappresenta la principale causa d'invalidità[3]. Nel 2004 in Europa sono state segnalate più di cinquecentomila morti associate a tromboembolismo venoso[4]. L'incidenza di trombosi venosa si attesta attorno allo 0.75-2.69 per mille persone, e raggiunge la soglia di 2-7 malati ogni 1000 persone, se si considera una popolazione di più di 70 anni[5].

Classicamente si riconoscono alcuni fattori di rischio per lo sviluppo di trombosi. Sin dagli inizi del 1900 è noto che la presenza degli elementi della cosiddetta triade di Virchow (ipercoagulabilità, rallentamento del flusso sanguigno e lesione vascolare) sono alla base dello sviluppo di trombosi venosa. Vengono considerati fattori di rischio trombotico: l'immobilizzazione, la presenza di malattia neoplastica, la chirurgia recente, soprattutto ortopedica, o la presenza di predisposizione genetica (trombofilia)[6]. I due meccanismi associati allo sviluppo di uno stato trombofilico sono rappresentati dalla ridotta attività dei fattori anticoagulanti e dall'aumentata attività dei fattori procoagulanti (per aumentata sintesi, per mancata inattivazione di elementi normali o

[1] ISTH Steering Committee for World Thrombosis Day; ISTH Steering Committee for World Thrombosis Day. Thrombosis: A major contributor to global disease burden. Thromb Res. 2014 Oct 7

[2] Lozano, R., Naghavi, M., Foreman, K.. et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet. 2012; 380: 2095–2128

[3] SPREAD 2012

[4] Cohen, A.T., Agnelli, G., Anderson, F.A.. et al. Venous thromboembolism (VTE) in Europe. The number of VTE events and associated morbidity and mortality. Thromb Haemost. 2007; 98: 756–764

[5] Deitelzweig, S.B., Johnson, B.H., Lin, J., and Schulman, K.L. Prevalence of clinical venous thromboembolism in the USA: current trends and future projections. Am J Hematol. 2011; 86: 217–220

[6] Heit JA , O'Fallon WM , Petterson TM , et al . Relative impact of risk factors for deep vein thrombosis and pulmonary embolism: a population-based study . Arch Intern Med .2002 ; 162 (11): 1245 - 1248

per la produzione di elementi iperfunzionanti)[7]. Le condizioni trombofiliche possono essere ereditarie come la mutazioni del fattore V di Leiden, la mutazione G20210 delle protrombina, il deficit di proteine C ed S oppure possono essere acquisite come in caso dello sviluppo di anticorpi antifosfolipidi; le mutazioni del fattore V o della protrombina rappresentano la forma di trombofilia più frequente nella popolazione (2-5% nell'ambito della razza caucasica) e sono associate ad un aumento del rischio di sviluppare trombosi di 2- 5 volte[8].

La chirurgia ortopedica maggiore è associata ad un rischio di trombosi venosa maggiore rispetto a tutte le altre specialità chirurgiche; tanto che in questi casi è raccomandato l'utilizzo di una profilassi antitrombotica che deve essere portata avanti per almeno 35 giorni[9].

Sul versante arterioso l'ipertensione, il diabete, il fumo di sigaretta, la dislipidemia sono riconosciuti essere fattori che favoriscono la formazione di placche aterosclerotiche e quindi la successiva occlusione vascolare[10].

Recentemente è stata posta l'attenzione sulla necessità di una valutazione del rischio cardiovascolare totale, che tenga conto di tutti i fattori di rischio che,quando presenti contemporaneamente, possono potenziarsi a vicenda, risultando in un maggior rischio rispetto alla somma dei singoli componenti[11]. La prevalenza complessiva dell'ipertensione risulta compresa tra il 30% e 45% nella popolazione generale, con un netto incremento con il crescere dell'età e rappresenta la principale causa di ictus.

[7] Martinelli I, De Stefano V, Mannucci PM. Inherited risk factors for venous thromboembolism. Nat Rev Cardiol. 2014 Mar;11(3):140-56

[8] Kearon C. Influence of hereditary or acquired thrombophilias on the treatment of venous thromboembolism. Curr Opin Hematol. 2012 Sep;19(5):363-70

[9] Falck-Ytter Y, Francis CW, Johanson NA et al. Prevention of VTE in Orthopedic Surgery Patients Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. CHEST 2012; 141(2)(Suppl):e278S–e325S

[10] Wilson PW , D'Agostino RB , Levy D , et al. Prediction of coronary heart disease using risk factor categories . Circulation . 1998; 97(18):1837 - 1847

[11] Mancia G, Fagard R, Narkiewicz K, et al. 2013 ESH/ESC Guidelines for the management of arterial hypertension. The Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). J Hypertens 2013;31:1281-357

Il fumo di sigaretta rappresenta il principale fattore di rischio modificabile per eventi cardiovascolari, esso agisce determinando una disfunzione endoteliale ed un aumento dell'attivazione piastrinica creando un ambiente protrombotico[12].

I pazienti affetti da diabete mellito sono sottoposti ad un rischio 2-3 volte maggiore di sviluppare eventi cardiovascolari e gli eventi cardiovascolari rappresentano la principale causa di morte per i pazienti diabetici[13].

Nel recente passato si è posta l'attenzione sulla ricerca di nuovi condizioni a rischio, per poter giustificare lo sviluppo di trombosi definite "idiopatiche". Ad esempio ci si è chiesti se i classici fattori di rischio per trombosi arteriosa potessero avere un ruolo anche nello sviluppo di trombosi venosa; sono stati pubblicati quindi studi riguardanti l'associazione tra aterosclerosi o sindrome metabolica e la trombosi venosa[14].

Recentemente si è posta l'attenzione sul ruolo dello stress psicologico come fattore di rischio per lo sviluppo di accidenti cerebrovascolari.[15]

Inoltre il sesso maschile che è classicamente associato ad un maggiore rischio di trombosi arteriosa, sembra essere protettivo per lo sviluppo di recidive di trombosi venose.[16]

Nell'ambito delle nostre osservazioni abbiamo voluto porre l'attenzione su alcune condizioni particolari, di frequente riscontro nella popolazione generale, e sulla loro possibile correlazione con lo sviluppo di trombosi. In particolare abbiamo analizzato la correlazione tra trait talassemico e lo sviluppo di eventi cardiovascolari, abbiamo analizzato l'effetto pro coagulante mediato dalla presenza di tireotossicosi e il rischio trombotico correlato; è stata inoltre analizzato la terapia anticoagulante ottimale da

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[12] Messner B, Bernhard D. Smoking and cardiovascular disease: mechanisms of endothelial dysfunction and early atherogenesis. Arterioscler Thromb Vasc Biol.
2014 Mar;34(3):509-15

[13] Seshasai, S.R.; Kaptoge, S.; Thompson, A., et al. Diabetes mellitus, fasting glucose, and risk of cause-specific death. N. Engl. J. Med. 2011, 364, 829–841

[14] Ageno W, Prandoni P, Romualdi E, et al. The metabolic syndrome and the risk of venous thrombosis:a case–control study. J Thromb Haemost 2006; 4: 1914–8.

[15] Everson-Rose SA, Roetker NS, Lutsey PL, et al. Chronic stress, depressive symptoms, anger, hostility, and risk of stroke and transient ischemic attack in the multi-ethnic study of atherosclerosis. Stroke. 2014 Aug;45(8):2318-23

[16] Kyrle PA., Minar E, Bialonczyk C, et al. The Risk of Recurrent Venous Thromboembolism in Men and Women N Engl J Med 2004; 350:2558-2563

attuare in corso di una condizione fisiologica come la gravidanza, che si associa ad un aumentato rischio di sviluppare tromboembolia; infine ci siamo occupati della terapia anticoagulante orale e dell'individuazione di soggetti a maggior rischio di complicanze trombotiche o emorragiche perché impossibilitati a mantenere un'adeguata stabilità della terapia.

OBIETTIVI

Nell'ambito di questo progetto di dottorato ci si è occupati dell'analisi di nuovi ed inusuali fattori di rischio trombotico, della terapia antitrombotica attuata in particolari condizioni a rischio e, nell'ultima sezione, della terapia anticoagulante orale al fine di individuare i pazienti esposti ad un aumentato rischio trombotico perché geneticamente predisposti ad una maggiore instabilità della terapia con i classici anticoagulanti orali.

Talassemia

Le sindromi talassemiche[17] rappresentano un gruppo di disordini ereditari caratterizzati dall'abolizione o dalla riduzione della sintesi di una o più catene globuliniche, con conseguente anemizzazione ed eritropoiesi inefficace.

La talassemia tipo beta è caratterizzata dalla diminuita o assente sintesi di catene beta-globuliniche. Dal punto di vista clinico si distinguono varie forme a seconda della gravità:

- Talassemia maior: forma più grave che esordisce precocemente e determina marcata anemia con ittero, epatosplenomegalia e alterazioni ossee
- Talassemia intermedia: caratterizzata da anemia di grado variabile ad esordio più tardivo, senza evidenti alterazioni dello sviluppo psicomotorio ma con evidente eritropoiesi extramidollare e possibile sviluppo di calcolosi della colecisti
- Talassemia minor o trait talassemico: condizione asintomatica caratterizzata da microcitosi, e lieve anemia

In alcuni studi è stata evidenziato un aumentato rischio trombotico in pazienti affetti da talassemia intermedia[18], mentre risultava meno definito il rischio trombotico in pazienti affetti da trait talassemico.

Abbiamo quindi deciso di condurre una revisione sistematica della letteratura valutando l'associazione tra eventi cardiovascolari arteriosi e trait talassemico.

Utilizzando i motori di ricerca MEDLINE ed EMBASE abbiamo eseguito una ricerca utilizzando le parole chiave: *Thalassemia, beta-Thalassemia, Acute Coronary Syndrome, Myocardial Infarction, Unstable Angina, Stroke, Transient ischemic attack.*

L'identificazione e la selezione degli studi inerenti è stata eseguita indipendentemente da due ricercatori. E' stata analizzata la prevalenza di trait talassemico in paziente

[17] Malattie del sangue e degli organi ematopoietici. G. Castoldi e V.Liso. McGraw Hill 5°edizione

[18]Taher AT, Otrock ZK, Uthman I, Cappellini MD. Thalassemia and hypercoagulability. Blood Reviews 2008; 22: 283-292

affetti da patologia cardio-cerebrovascolare. Inoltre sono state eseguite sottoanalisi riguardanti pazienti di sesso maschile. Nel nostro lavoro sono stati identificati più di 300 articoli, ma solo 8 , per un totale di più di 3000 casi e 6000 controlli, sono risultati eleggibili per l'esecuzione della metanalisi. I nostri risultati hanno evidenziato che il trait talassemico sembra essere un fattore protettivo rispetto allo sviluppo di accidenti cerebro o cardiovascolare solo nei soggetti di sesso maschile.

THALASSEMIA TRAIT AND ARTERIAL THROMBOEMBOLIC EVENTS: A SYSTEMATIC REVIEW AND A META-ANALYSIS OF THE LITERATURE[19]

F. Dentali, E. Romualdi, W. Ageno, MD Cappellini, PM Mannucci

Introduction:

β-thalassemia is a congenital haemolytic anaemia characterized by a deficiency in the production of β-globin chains [1]. An increased incidence of venous thromboembolic events has been reported in thalassemic patients, especially in patients with β-thalassemia intermedia [2]. A number of prothrombotic conditions have been described in this population which support the biological plausibility of this observation. These in particular include increased thrombin generation by structurally abnormal red blood cells [3], increased platelet activation [4], endothelial inflammation, reduction of protein C and S levels, elevated levels of von Willebrand factor, and specific changes in the lipid membrane composition of abnormal red blood cells [2].

On the other hand, the risk of atherothrombotic complications is well established in β-thalassemia trait carriers. β-thalassemia trait carriers usually present with anaemia with low blood viscosity levels, secondary to microcytosis [5], and hypolipidaemia [6]. A number of studies have suggested that because of low serum cholesterol levels, chronic anaemia and reduced blood viscosity, these patients may actually be protected from atherothrombotic cardiovascular disease [7-9]. On the basis of these observations, we performed a systematic review of the literature and a meta-analysis of all studies that compared the prevalence of β-thalassemia trait in patients with arterial thromboembolic events or objectively documented major atherosclerotic lesions and in healthy controls.

Methods:

Studies were identified using the MEDLINE (1950 to July Week 4 2010) and EMBASE (1980 to July Week 4 2010) electronic databases. The search strategy was

developed without any language restriction, and used the following keywords: Thalassemia, beta-Thalassemia, Acute Coronary Syndrome, Myocardial Infarction, Unstable Angina, Stroke, Transient ischemic attack. Research was supplemented by manually reviewing abstract books from the Congress of the International Society on Thrombosis and Haemostasis (ISTH), from the Congress of the American Society of Haematology (ASH) and the reference lists of all retrieved articles. Case control or cohort studies were included if they met the following criteria: i) diagnosis of arterial thromboembolic events was objectively confirmed; ii) patients were compared to a control group of healthy subjects without a history of thromboembolic disease or genetic relationship with the patients or to subjects with similar characteristics of cases; iii). an objective definition of β-thalassemia was provided.

The following data were extracted: study characteristics (year of publication, design, study centre) and patients and controls characteristics (number of subjects studied, mean age,

variation in age, gender and race).

Study identification, study selection and data extraction were performed independently in duplicate by two reviewers, with disagreements resolved through discussion or by the

[19] Journal of Thrombosis and Haemostasis 2012

opinion of a third reviewer, if necessary. The agreement between the reviewers was calculated using the kappa (k) statistic [10].

Review Manager (RevMan; version 4.2 for Windows; Oxford, England; The Cochrane Collaboration, 2003) was used to pool data for each risk factor. Odds ratios (ORs) and
95% confidence intervals (CIs) were calculated using a random-effects model [11]. Statistical heterogeneity was evaluated using the I2 statistic, which assesses the appropriateness of pooling the individual study results [12]. The I2 value provides an estimate of the amount of variance across studies due to heterogeneity rather than chance. $P < 0,05$ was considered to denote statistically significant heterogeneity. To further explore potential sources of heterogeneity, studies with different design were analyzed separately.
The proportion of arterial cardiovascular events in the population that could be attributed to the presence of β-thalassemia trait (population attributable risk [PAR]) was estimated as follows:
PAR = 100 x [Prevalence (OR – 1) / Prevalence (OR – 1)+ 1]
For this calculation we estimated the prevalence of exposure as the genotype frequency among control subjects.
A subgroup analysis on studies that included only patients with clinical events was performed. Furthermore, another subgroup analysis including only patients with cardiovascular disease was performed. Since some studies suggested a different role of β-thalassemia trait in male and female [14,15,17,18,21] we performed a subgroup analysis according to gender.
The presence of publication bias was explored using funnel plots of effect
size against standard error [13].

Results:
A total of 342 (139 Medline and 203 Embase) citations and 12 abstracts from ISTH congresses were identified by our systematic search. A total of 325 studies were excluded after reviewing the study abstracts. We retrieved the full text of 17 articles, 9 were excluded because they did not meet the predefined inclusion and 7 case-control studies and 1 prospective cohort study were eligible for inclusion in our systematic review [14-21]. Two studies were from Iran [17,18], two from Greece [19,20], one from the US [14], one from Taiwan [21] , one from India [16], and one from Italy [15]. All studies were written in English.
The inter-observer agreement for the study selection was total (k=1.0).
Five studies enrolled patients with acute myocardial infarction [14-16, 19,21], one enrolled patients with acute cerebrovascular ischemic events [18], one enrolled patients with coronary atherosclerosis diagnosed angiographically [17], and one enrolled patients with clinical and electrocardiographic findings suggestive for stable angina [20]. It remains unclear if (and how many) patients enrolled in the last two studies had previous concomitant cardiovascular events. Three studies [14,19,21] enrolled only male patients and controls whereas the other five [15-18, 20] enrolled male and female patients and controls.

The prevalence of β-thalassemia trait was evaluated in 3172 patients with cardiovascular or cerebrovascular events and in 6307 controls. Baseline characteristics of included studies are summarized in table 1.

β -thalassemia trait was associated with a reduced risk of developing arterial cardiovascular and cerebrovascular events (OR 0.45; 95% CI 0.45-0.60), Figure 1. Heterogeneity among studies was low (I2 = 13 %).

The funnel plots for arterial cardiovascular and cerebrovascular events appeared symmetric, suggesting the absence of publication bias.

The proportion of arterial cardiovascular and cerebrovascular events that could be avoided due to the presence of β-thalassemia trait in this pooled cohort was 4.4%.

Subgroup analysis that excluded the two studies that only enrolled patients with coronary atherosclerosis diagnosed angiographically and with electrocardiographic findings suggestive for stable angina [17, 20] gave similar results (OR 0.45; 95% CI 0.33-0.63).

Subgroup analysis that included only studies that enrolled patients with cardiovascular complication gave similar results (OR 0.40; 95% CI 0.30-0.54).

Five studies [14,15,17,18,21], with a total of 1883 patients and 3583 controls, considered the role of β-thalassemia trait in male subjects, whereas only 3 studies [15,17,18], with a total of 332 patients and 2236 controls, considered the role of β-thalassemia trait in female subjects. Subgroups analysis in male subjects confirmed the results of the principal analysis (OR 0.39; 95% CI 0.24-0.62), whereas the presence of β-thalassemia trait did not appear to affect the risk of arterial cardiovascular and cerebrovascular events in female subjects (OR 0.89; 95% CI 0.52-1.53). Figure 2.

Discussion:

This is the first systematic review and meta-analysis that assessed the association between β-thalassemia trait and arterial cardiovascular and cerebrovascular events. Our results suggest that the presence of β-thalassemia trait could be protective against arterial thrombotic disorders. This conclusion is strengthened by the large number of included cases that exceeded three thousand patients and by the uniform nature of our results. Subgroups analysis in male subjects confirmed the results of the principal analysis. On the other hand, the presence of β-thalassemia trait did not appear to be protective in female subjects.

Our findings are different from those obtained in previous studies carried out in patients with β-thalassemia major or intermedia, in which both conditions, and in particular thalassemia intermedia, were associated with an increased risk of venous thromboembolic events [2]. Furthermore, recent studies have shown a high risk of symptomatic and asymptomatic brain ischaemia in patients with β-thalassaemia intermedia [23,24].

This increased risk has been supported by the identification of a number of prothrombotic conditions such as the increased thrombin generation due to structurally abnormal red blood cells and increased platelet activation, which are likely absent or at least less pronounced in patients with β-thalassemia trait [25]; unfortunately there are not specifically designed studies to identify the real incidence of venous thrombotic events in β-thalassemia trait carriers. Conversely, the protective role of β-thalassemia trait against arterial cardiovascular or cerebrovascular disease could be explained by a number of different mechanisms including a reduction in serum cholesterol levels [6] and in blood viscosity [5,9]. In addition, some of the selected studies [15,18] also reported a reduced prevalence of arterial hypertension in β-thalassemia trait carriers than in controls suggesting a protective role of this condition. This was recently

confirmed in a study performed by Vyssoulis et al in which β-thalassemia trait carriers had a better 24-h blood pressure profile in comparison with anemic and non-anemic hypertensives patients [26].

Interestingly, the protective effect of β-thalassemia trait was considerably stronger in male patients (61% risk reduction) than in female patients (11% risk reduction), and in this latter group this effect was not statistically significant. This result may be due to truly existing, gender related, protective mechanisms of β-thalassemia trait, or simply to the relatively low number of female patients with cardiovascular events included in this subgroup analysis.

Our meta-analysis has limitations. First, it was restricted to case-control and cohort studies, and the application of formal meta-analytic methods to observational studies is controversial, since bias implicit in the study design may misrepresent the strength of associations within the data [20]. Second the definition of β-thalassemia trait was heterogeneous across studies. However, we only included studies in which an objective definition of β-thalassemia trait was reported. Third, since we have no access to the raw data of the single studies, it was not possible to perform separate analyses evaluating the association between beta-thalassemia trait and cardiovascular events in normotensive and hypertensive patients. Last, despite a careful review of references and research in the abstracts books of ISTH and ASH we failed to identify any published or unpublished study not found in our initial literature search. Because it is recognized that publication bias can affect the results of meta-analyses, we attempted to assess this potential bias using a funnel plot. However, funnel plot appeared symmetric suggesting the absence of publication bias.

In conclusion, the results of this systematic review of the literature and meta-analysis suggest that β-thalassemia trait may act as a protective factor against the developing of major atherothrombotic lesions or acute arterial thromboembolic events. Larger prospective studies are necessary to confirm these preliminary findings and to further investigate the mechanisms underlying this protective effect.

Figure 1 Association between cardiovascular events and β-thalassemia trait

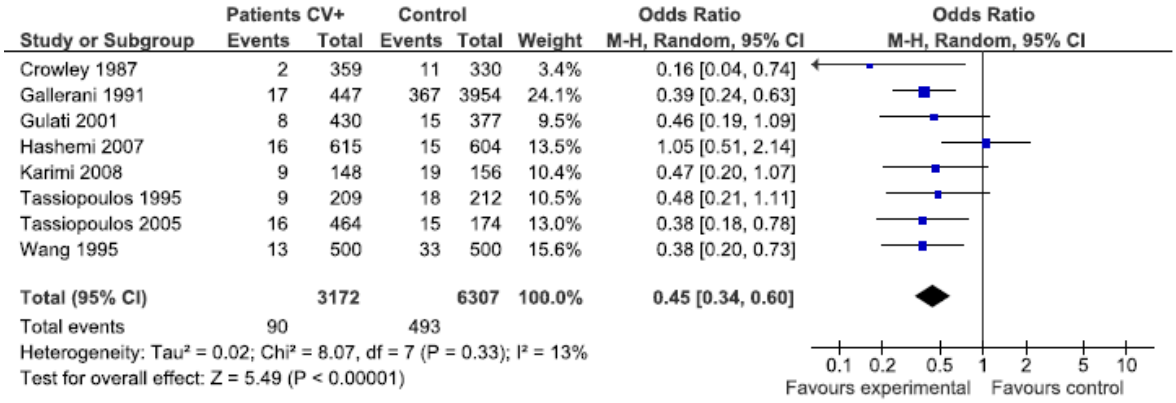


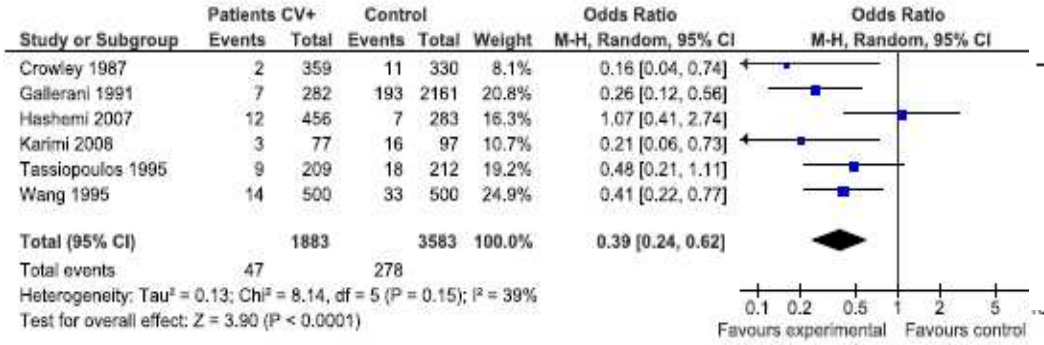
Table 1. Baseline characteristics of included studies

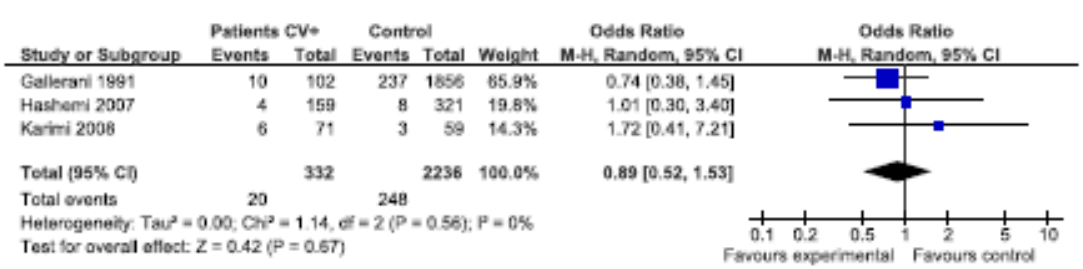
Study	Type	Inclusion	End-point	Other	Results	Other	Diagnosis
Hashemi et al. Cardiovasc J 2007	Case-control	Pts underwent coronary angiography	Association of beta thal trait with CAD	Stepwise multivariate regression to study thal mechanism	CAD+/TT+:16 of 615 CAD-/TT+:15 of 604 P:n.s.	Only hematocrit was different	MCV <78, MCH<27, HbA2>3.5%
Karimi et al. J of Stroke and Cardiovasc Dis 2008	Case control	Pts with acute focal neurologic deficits; Controls: age and sex matched	Presence of beta thal trait	Presence of hypertension, diabetes, hyperlipidemia	TT+: in 9 of 148 case and in 19 of 156 controls P:0.066 In male pt p: 0.008	Negative association hypertention and TT only in male pt	MCV <80, MCH<27, HbA2>3.5%
Tassiopoulos et al. Ann NY Academy of Sc 2005	Case control	Pts with stable angina pectoris with advanced CAD or without CAD	Presence of beta thal Trait	Smoke, hypertension, diabetes, hypercholesterolemia	aCAD+/TT+: 16/464 pt aCAD-/TT+: 15/174 pt (8.62%) p:0.001		Hemoglobin electrophoresis
Tassiopoulos et al. Haematol 1995	Case control	Male pts with AMI (cases) and 212 males orthopedic pts (controls)	Presence of beta thal Trait	-	TT+/AMI+: 9/209; TT+/AMI-: 18/212	-	Red cells morphology, HbA2 and HbF
Crowley et al.Acta Haematol 1987	Case control	Males with AMI (cases), males >24 years admitted for other reasons (controls)	Presence of beta thal Trait	-	TT+/AMI+: 2/359; TT+/AMI-: 11/330 P<0.01	-	MCV <80, England and Frasier index neg

Wang et al Am J Haematol 1995	Case control	Males with AMI (cases); Males without MI (controls)	Presence of beta thal Trait	cholesterol	TT+/AMI+: 14/500; TT+/AMI-:33/500 P<0.01	Cholesterol was significantly lower in TT+	MCV <80, England and Frasier index neg
Gulati er al Journ of Intern Med of India	Case control	Pts with AMI (cases); blood donors (controls)	Presence of beta thal Trait	-	TT+/AMI+: 8/430; TT+/AMI-:15/377 P<0.01		Clinical and laboratory criteria
Gallerani et al. J Intern Med 1991	Prospective study	Pts admitted to hospital; no thal (cases) and beta thal trait (controls)	Prevalence of thromboembolic events	DM, hypertension	AMI+/TT+: 17/447 AMI+/TT-:367/3954 P=n.s. P sign only in males: AMI+/TT+: 7/200 AMI+/TT: 275/2243	DM and hypertension were significantly lower in TT+	Abnormal red cell morphology, decreased osmotic red cells fragility , HbA2>3 % England and Frasier index neg

DM diabetes mellitus; AMI acute myocardial infarction; TT+ trait carriers; coronary artery disease

Figure 2 Association between cardiovascular events and β-thalassemia trait in male and female patients





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Distiroidismo

Si tratta di una condizione caratterizzata da un'alterata concentrazione degli ormoni tiroidei nell'organismo; si parla di tireotossicosi quando sia presente un eccesso di ormoni tiroidei nel torrente ematico (sia per produzione endogena sia per assunzione incongrua di terapia con levotiroxina).

In caso patologia della ghiandola tiroidea si definisce ipotiroidismo conclamato la situazione caratterizzata da una riduzione dei livelli di tiroxina (T4) ed un aumento dei livelli di tireotropina (TSH), al contrario si definisce ipertiroidismo conclamato la condizione caratterizzata da un aumento dei valori di T4 con TSH ridotto.

Il legame tra disfunzione tiroidea e sistema emostatico è stato ipotizzato fin dagli inizi del ventesimo secolo.

In pazienti ipotiroidei è stata osservata una tendenza al sanguinamento, soprattutto interessante il distretto muco-cutaneo[20]. Inoltre pazienti affetti da ipotiroidismo conclamato spesso hanno presentato lo sviluppo della malattia di von Willebrand acquisita[21].

In corso di ipertiroidismo sono state evidenziate alcune alterazioni del sistema emocoagulativo tali da suggerire la presenza di uno stato trombofilico. In particolare è stata evidenziata un'aumentata attività del fattore VIII e del fattore IX ed una riduzione dell'attività fibrinolitica[22]; tali alterazioni sono state evidenziate anche in corso di terapia ormonale.

Data la rilevanza clinica abbiamo deciso di condurre una revisione sistematica della letteratura e una metanalisi al fine di valutare gli effetti della tireotossicosi sul sistema coagulativo e il rischio di tromboembolia venosa.

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Utilizzando i motori di ricerca MEDLINE ed EMBASE sono stati identificati gli studi tesi a valutare l'effetto della tireotossicosi sul sistema emocoagulativo; sono stati esclusi case reports, review e studi in vitro.

Sono stati selezionati più di 1000 articoli, di questi solo 29 sono stati utilizzati nell'analisi.

Con il nostro studio abbiamo osservato che in corso di tireotossicosi si sviluppa un evidente stato trombofilico, determinato da un netto aumento di alcuni fattori della coagulazione a scapito del decremento di elementi determinati nelle fibrinolisi; nonostante ciò resta ancora da definire la vera incidenza di trombosi venosa in tali circostanze.

THE EFFECT OF HYPERTHYROIDISM ON PROCOAGULANT, ANTICOAGULANT AND FIBRINOLYTIC FACTORS

A SYSTEMATIC REVIEW AND META-ANALYSIS[23]

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Introduction

Hyperthyroidism is associated with a hypercoagulable state (1, 2). Several coagulation and fibrinolytic parameters appear to be affected by thyrotoxicosis; elevated plasma levels of factor VIII (FVIII), factor IX (FIX), von Willebrand factor (VWF), and fibrinogen, and a reduced fibrinolytic activity due to increased levels of plasminogen activator inhibitor-1 (PAI-1) have been reported in both hyperthyroid patients and healthy subjects after taking thyroid hormones (1, 3–9). However, in a previous systematic review, we found the majority of these studies to have major methodological flaws (1). This left the net effect of thyroid hormone excess on the haemostatic system unclear. Moreover, the question as to whether thyrotoxicosis enhances the risk of venous thrombosis, and to which extent, still remains controversial. In this review, we aimed to update our previous systematic review and systematically summarise and meta-analyse the data by assessing the effects of thyrotoxicosis on the coagulation and fibrinolytic system in vivo (1). For the interpretation of the results we will also discuss the recent studies on the relationship between (supra) physiological thyroid hormone levels and VTE.

Methods

Study identification

A computer-assisted search of the MEDLINE and EMBASE electronic databases from July 2006 to March 2012 was performed to identify published studies that evaluated the effect of thyroid hormone excess on the coagulation-fibrinolytic system. The following search terms were used for the MEDLINE search: “haemostasis, blood coagulation tests, blood coagulation, blood coagulation fac- tors, blood coagulation disorders, thyroid diseases, thyroid hormones, thyroid dysfunction, thyroid receptors, thyroid hormone, hyperthyroidism”. For the EMBASE database search, the terms “haemostasis, blood clotting, blood clotting test, blood clotting factor, blood clotting disorders, thyroid disease, thyroid hormone, thyroid hormone receptor, and hyperthyroidism” were used. Reference lists of all included studies were manually searched for other potentially eligible studies. All included studies were merged with the studies found in our previous search from January 1980 until June 2006 (1).

Inclusion criteria

Two investigators (D.S. and B.v.Z) performed the study selection independently. Main inclusion criterion was that the study had to evaluate the effect of thyrotoxicosis, overt and/or subclinical, on the coagulation-fibrinolytic system in vivo. The following study designs were allowed: 1) observational cross-sectional studies in which hyperthyroid individuals were compared to euthyroid controls or 2) observational intervention studies in which laboratory tests were performed before and after treatment to

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correct hyperthyroidism, or 3) experimental clinical trials in which euthyroid subjects received a thyroid hormone analogue to induce exogenous thyrotoxicosis. Case reports, case series, reviews, editorials, in vitro, and non-human studies were excluded. Moreover, studies on cancer patients (as cancer itself influences the coagulation system) and studies without statistical analysis were excluded. No language restrictions were initially applied to the search strategy, but only articles written in English, French, Spanish, German, Dutch, and Italian were evaluated. The two investigators independently reviewed titles and/or abstracts from the initial search to determine whether the inclusion criteria were satisfied. The full text of the study was obtained when an article could not be excluded with certainty. Decisions regarding inclusion were made separately, results were compared, and any disagreement was resolved by discussion. When multiple articles for a single study had been published, it was decided to use the latest publication and to supplement it, if necessary, with data from the earlier publications.

Quality assessment

The quality of randomised and non-randomised clinical trials was assessed in an extended version of the Delphi list (the Maastricht- Amsterdam list) (10, 11). The Newcastle-Ottawa Scale (NOS scale) for assessing quality of observational studies was used as a guide to assess study quality of cross-sectional and intervention studies (12). For summarising study validity, we adopted a simple Cochrane Collaboration approach (13). Three categories were therefore identified: high quality (low risk of bias), medium quality (moderate risk of bias), or low quality (high risk of bias). Quality of the included studies was assessed independently by the same two reviewers and any differences were resolved by consensus or the opinion of the third reviewer, if necessary. No attempts to mask for authorship, journal name or institution were made.

Statistical analysis

For each outcome and for each study we extracted the effect sizes comparing the exposed and control situations. This implies that in cross-sectional studies hyperthyroid patients and controls were compared, whereas in intervention studies pre- and post-treatment values were evaluated. In experimental trials, post- versus pre-exposure values, or post-intervention versus values without intervention, were compared. A positive effect size reflected higher values in the exposed situation (i.e. thyroid hormone excess), whereas a negative effect size reflected lower values in the exposed situation. Results were expressed as standardised mean difference, i.e. the difference between the group means divided by the pooled standard deviation (14). If, instead of standard deviations (SD), standard errors of the mean (SEM) were provided, the SD based on the SEM and the number of subjects ($SEM = SD / \sqrt{n}$) was calculated. If, instead of SD, the 95% confidence interval (CI) was given, the SEM was calculated based on the z-score, the upper limit and the mean value (SEM = upper limit-mean/z-score). For those articles that did not report exact statistics, we only documented the authors' statements on the statistical difference per outcome using notations of statistical significant increase, statistical significant decrease or no statistical significant difference. Data were categorised according to 1) study design and 2) subclinical and overt hyperthyroidism (in cross-sectional studies). These data were pooled using a fixed-effects model and comparing these findings with the results obtained using a random-effects model, in particular in case of significant statistical heterogeneity (15, 16). In case of high statistical heterogeneity, results using random- effects model are reported. Statistical heterogeneity was evaluated using the I2 statistic, which assesses the appropriateness of pooling the individual study results. The I2 value provides an estimate

of the amount of variance across studies due to heterogeneity rather than chance. I2< 30% indicates mild heterogeneity, 30–50% moderate, and >50% severe heterogeneity. When heterogeneity was present, we repeated the analysis removing one study at time to assess the source of heterogeneity. All statistical calculations were performed using Review Manager 5.0 computer software (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2008).

Results

Search results and included studies

From our previous search from January 1980 to June 2006, a total of 18 articles had been identified (3–6, 8, 17–29). Our new search identified a further 1,233 citations from the database and reference searches. Of these, 96 publications were considered potentially relevant. Forty casereports and 31 reviews were excluded. Of the remaining articles, the full text was retrieved and assessed for eligibility. Six articles were further excluded based on an in vitro design (30), a study population aged below 18 years (31, 32), or involving cancer patients (33–35). Only 10 publications actually reported on the effect of thyrotoxicosis on haemostatic parameters and were therefore included in the present analysis (7, 9, 36–43). In addition, we included one previously excluded publication from before June 2006 as the previous review did not take exogenous thyrotoxicosis into account (44). Taken together with the articles published before June 2006, a total of 29 articles were included. In several articles, more than one study or study design was described. The 29 articles contained a total of 51 studies: seven experimental studies (thyroid hormones given to healthy subjects) and 44 observational studies. The latter consisted of 15 intervention studies (pre- versus post-treatment in hyperthyroid patients) and 29 cross-sectional studies (hyperthyroid patients versus euthyroid controls). Table 1

Methodological quality of included studies

Five observational cross-sectional studies, and 4 observational in- tervention studies of medium quality were identified. In the experimental studies, two high-quality studies and one mediumquality study were identified. The remaining studies were considered of low quality.

Outcome parameters

Observational cross-sectional studies

Four medium quality studies involved subclinical thyrotoxicosis and only one study compared patients with overt hyperthyroidism to euthyroid controls. Levels of VWF, fibrinogen, and D-dimer were significantly increased in subclinical hyperthyroid individuals, whereas only fibrinogen levels were measured in overt hyperthyroid individuals and appeared to be slightly increased compared to euthyroid controls. Figure 1-2

Observational intervention studies

In the four medium quality studies, increased levels of plasma fibrinonectin, VWF, thrombomodulin and PAI-1, and decreased levels of tissue type-plasminogen activator (t-PA) were found during hyperthyroidism compared to the euthyroid state after normalization of thyroid hormone levels by anti-thyroid agents. Figure 3

Experimental studies

Four studies investigated laboratory parameters in healthy volunteers taking thyroid hormone for a specific period of time. Thyrotoxicosis increased plasma levels of tissue factor, FVIII, FIX, VWF,

fibrinogen, d-dimer, and PAI-1. For the remaining parameters, no univocal statistical differences were observed. Figure 4

Discussion

This meta-analysis shows that thyrotoxicosis shifts the haemostatic balance towards a hypercoagulable and hypofibrinolytic state with a rise in FVIII, FIX, VWF, fibrinogen, and PAI-1. This was observed in both endogenous and exogenous thyrotoxicosis, and in subclinical as well as overt hyperthyroidism. Several pathophysiological mechanisms have been suggested to underlie the relation between thyroid hormone excess and haemostasis. One of these mechanisms is the activation of the immune system in thyroid disease (45, 46). As we found an equal effect on haemostatic parameters in subclinical thyrotoxicosis compared to overt thyrotoxicosis, the role of the immune system on the haemostatic system may be relevant. However, similar alterations were observed in exogenous thyrotoxicosis in which auto immunity does not come into play. This gives way for another possible mechanism involving the direct effect of thyroid hormones on the synthesis of coagulation and fibrinolytic proteins due to thyroid-receptor mediated upregulation of gene transcription in hepatic and endothelial cells (47, 48). As antibody concentrations have been found to decrease with anti-thyroid treatment, these two mechanisms might not be mutually exclusive, but together may result in a prothrombotic state with an increased risk of both venous and arterial thrombosis during thyrotoxicosis (49, 50). Nowadays, thrombosis is known as a "multi-causal" disease in which multiple genetic or environmental risk factors coincide to push over a so-called 'thrombotic threshold'. If hyperthyroidism is a risk factor for venous thromboembolism (VTE), this will be clinically relevant both for the treatment and prevention of venous thrombosis as well as for the treatment of hyperthyroidism. It will be important for the distinction between provoked or unprovoked thrombosis and decisions on the duration of anticoagulant therapy. Moreover, it may lead to more vigilant monitoring for possible signs and symptoms of VTE in patients with recently diagnosed hyperthyroidism, but could also be relevant for prophylactic strategies in hyperthyroid patients undergoing surgery or other interventions associated with a high risk of venous thrombosis. In recent years, a few studies on the relationship between hyperthyroidism or thyroid hormone levels and VTE have been published (51–58). Four population-based follow-up studies explored the risk of VTE in individuals with, amongst others, hyperthyroidism (51, 54, 55–57). The diagnosis of hyperthyroidism was, however, solely based on diagnostic codes. A recently performed cohort study, performed by our group, included a small number of patients with biochemically confirmed overt hyperthyroidism (53). Two case-control studies investigated the risk of VTE associated with varying levels of thyroid hormone (52, 56). A rise in VTE risk was found with higher levels of thyroid hormone, yet only a small number of patients had thyroid levels above the upper end of the normal range. These studies mostly point towards an increased risk of VTE associated with hyperthyroidism. However, limitations due to study design and contradicting results drastically reduce the strength of evidence and do not allow for definitive conclusions to be drawn about the clinical relevance of the findings. Further prospective studies are needed to provide robust evidence on the VTE risk involved in hyperthyroidism, and subsequently the mechanisms behind this presumed relationship. Ideally, a prospective clinical study including consecutive patients with a well-defined degree of hyperthyroidism, with proven VTE as main outcome should be performed. In addition, further insight could be obtained by assessing the relation between thyroid hormone levels and venous thrombosis in

patients already at high risk of venous thrombosis, such as patients undergoing major orthopaedic surgery. Several limitations need to be addressed. Most importantly, the use of quality scoring in meta-analysis for observational studies is controversial. Also, the quality scoring is arbitrary. However, we do feel that it is the only way to gain insight on adequate study design and identify risks of bias. Even when combining the more recent studies (published after June 2006) with the older studies, only a limited number of medium and high-quality studies were found. As a result, most observed alterations have not been confirmed in other studies. However, we were able to confirm main alterations in different study designs, and most low-quality studies provided similar results.

In conclusion, this meta-analysis showed consistent evidence of a hypercoagulable and hypofibrinolytic state in thyrotoxicosis. Well-designed studies with clinical outcomes are needed to provide more definitive data. Only then, the clinical relevance of these findings, especially in terms of prevention and treatment of venous thrombosis in hyperthyroid patients, can be determined.

Table 1: Characteristics of included studies

Author	**Study design**	**Quality**	**Population (n)**	**Treatment or intervention**	**Blood sampling**	**Outcome parameters**
Arnaout, 1992	A. Observational: Cross-sectional B. Observational: Intervention	low	Hyperthyroid patients (12) Euthyroid controls (15) Hyperthyroid patients (10)	Anti-thyroid treatment	A.Before start of treatment B.Before treatment and after euthyroidism wasachieved	A.pFN, FVIII:C, vWF:Ag, AT-III □1antitrypsin:Ag B.pFN, FVIII:C, vWF:Ag, AT-III □1antitrypsin:Ag
Burggraaf, 2001	A. Observational: Cross-sectional B. Observational: Intervention	low medium	A.Hyperthyroid patients (14) Euthyroid controls (14) B.Hyperthyroid patients (14)	B.Propranolol and thiamazol 10 mg 3 times a day and levothyroxine 100 ug starting dose	ABefore start of treatment B At time of diagnosis and after euthyroidism was achieved (at least 1 months after propranol was stopped)	F1+2, cFN, pFN, VWF:Ag, tPA:Ag, tPA:C, PAI-1:Ag, PAP, thrombomodulin, Plasminogen, □-2-antiplasmine, Fibrinogen: Ag,
Coban,	Observational:	mediun	Subclinical hyperthyroid		Before start	VWF:Ag

2006	Cross-sectional		patients (20) Euthyroid controls (20)		of treatment	
Dörr, 2005	A Observational: Cross-sectional B. Observational: Cross-sectional	medium	A General population of unselected patients divided into TSH<0.1 mU/l (11) compared to TSH 0.3–3.0 mU/l (3362) B .General population of unselected patients divided into TSH<0.3 mU/l (388) compared to TSH 0.3–3.0 mU/l (3362)			Fibrinogen:Ag
Erem, 2002	Observational: Cross-sectional	low	Hyperthyroid patients (41) Euthyroid controls (20)		Before start of treatment	PT, APTT, Fibrinogen, FV:C, FVII:C, FVIII:C, FIX:C, FX:C, AT III:Ag, Protein C (%), Protein S, vWF:C, t-PA:Ag, PAI-1:Ag
Erem, 2006	Observational: Cross-sectional	low	Subclinical hyperthyroid patients (20) Euthyroid controls (20)		Before start of treatment	Platelet count, MPV, PT, APTT Fibrinogen:Ag, D-Dimer, FV:C, FVII:C, FVIII:C, FIX:C, FX:C, AT-III:Ag, Protein C, Protein S, VWF:C,t-PA:Ag, PAI-1:Ag
Graninger, 1986	A. Observational: Cross-sectional B. Observational:	A. low B.medium	A. Hyperthyroid women (27) Euthyroid women (30) B.Healthy women (7)	B. 25 ug T3 three times a day for 14 days	A.Before start of treatment B. On day 4,7, 14 and 24 after start of	pFN, FVIII:Ag, AT-III:Ag

	Intervention				treatment	
Li, 1998	A. Observational: Cross-sectional B. Observational: Intervention	A. low B.medium	A. Hyperthyroid patients (14) Euthyroid controls (10) B. Hyperthyroid patients (14)	B. Iodine radiotherapy	A. Before start of treatment B. Before and 30 days after treatment	vWF:Ag, t-PA:ag, PAI-1:Ag
Liu, 1993	A. Observational: Cross-sectional B. Observational: Intervention	A. low B.medium	A. Hyperthyroid patients (35) Euthyroid controls (20) B.Hyperthyroid patients (7)	B. Anti-thyroid treatment	A. Before treatment B. Before treatment and after euthyroidism was achieved	VWF:Ag
Marongiu, 1988	Observational: Cross-sectional	low	Hyperthyroid patients (14) Euthyroid controls (25)		One time point	Fibrinopeptide B□□15–42 Fibrinogen: C
Marongiu, 1991	Observational: Cross-sectional	low	Hyperthyroid patients (65) Euthyroid controls (58)		One time point	Fibrinopeptide a, GLP, MLP
Marongiu, 1991	A. Observational: Cross-sectional B. Observational: Intervention	low	A. Hyperthyroid patients (50) Euthyroid controls (14) B. Hyperthyroid patients (40)	B. Methimazole or 131I treatment	A. Before start of treatment B. Before treatment and after euthyroidism was achieved	fibrinopeptide a, fibrinopeptide B□□15–42, Fibrinogen:C
Morishita, 1998	A. Observational: Cross-sectional B. Observational: Intervention	low	A. Hyperthyroid patients (15) Euthyroid controls (25) B. Hyperthyroid patients (10)	B Antithyroid drugs	A. Before start of treatment B. Before treatment and after euthyroidism was achieved	vWF:Ag, thrombomodulin, Free TFPI, Total TFPI
Myrup, 1995	A. Observational:	A. low B.mediu	A. Hyperthyroid patients (10)		A. Before start of	Platelets, Bleeding time, RIPA, Platelet

	Cross-sectional B. Observational: Intervention	m	Euthyroid controls (15) B. Hyperthyroid patients (10)	B. Carbimazole	treatment B. Before treatment and after euthyroidism was achieved	aggregation (ADP/collagen), pFN, vWF:Ag, Fibrinogen:Ag, □-2 macroglobulin
Ozcan, 2003	A. Observational: Cross-sectional B Observational: Intervention	low	A Hyperthyroid patients (10) Euthyroid controls (16) B Hyperthyroid patients (10)	B Anti-thyroid therapy (not further specified)	A. Before start of treatment B. Before treatment and after euthyroidism was achieved	free TFPI, total TFPI, tPA:Ag, PAI-1:Ag
Rogers, 1982	A. Observational: Cross-sectional B. Observational: Intervention	low	A Hyperthyroid patients (22) Euthyroid controls (24) B Hyperthyroid patients(10)	B. Levothyroxine, radioactive iodine, propylthiouracil	A. Before start of treatment B. Before treatment and after euthyroidism was achieved	FVIII:C, FVIII:Ag: FVIII:RiCo
Rosc, 1998	Observational: Cross-sectional	low	Hyperthyroid patients (33) Euthyroid controls (34)		Before start of treatment	t-PA:Ag, u-PA:Ag, PAI-1;Ag
Wahrenberg, 2002	A. Observational: Cross-sectional B. Observational: Intervention	low	A Hyperthyroid patients (10) Euthyroid controls (16) BHyperthyroid patients (10)	B Anti-thyroid therapy for at least 8 weeks (not further specified)	A. Before start of treatment B. Before treatment and after euthyroidism was achieved	PAI-1:C, PAI-1:Ag
Rogers, 1983	Experimental: Clinical trial	medium	Healthy volunteers (14 and 9)	A. Levothyroxine (LT4) 0.6 mg daily B. Liothyronine 50 ug three times	A. On day 1,7 and day 14 B. On day 1 and 7	FVIII:C, FVIII:Ag: FVIII:RiCo

				daily		
Akinci, 2007	Observational: Cross-sectional	low	Overt hyperthyroid patients (14) Euthyroid controls (26)		Before start of treatment	PAI-1:Ag, TAFI:Ag
Akinci, 2011	A.Observational: Cross-sectional B. Experimental: Clinical trial	A low B medium	A. Subclinical hyperthyroid patients (10) euthyroid controsl (18) B Premenopausal women with benign thyroid nodules (20)	B Levothyroxine (LT4) suppression therapy	A. Before start of treatment B Before and one year after LT4 suppression therapy	PAI-1:Ag, TAFI:Ag
Brona, 2011	Observational: Intervention	low	Overt and subclinical hyperthyroid women (15 and 20)	Radioiodine therapy	Before treatment, and between 24–28 or 12-16 weeks after treatment	Fibrinogen:C, D-Dimer
Coban, 2008	Observational: Cross-sectional	Medium	SC hyperthyroid patients (36) Euthyroid controls (36)		One time point	Fibrinogen:C, D-Dimer
Demir, 2009	Experimental: Clinical trial	High	Premenopausal women with benign thyroid nodules (30)	Levothyroxine (LT4) suppression therapy	Before and one year after start of LT4 suppression therapy	Fibrinogen :C, D-Dimer, vWF:C, Tissue Factor, t-PA:C, PAI-1:Ag, TFPI:Ag
Erem, 2009	Observational: Cross-sectional	low	Hyperthyroid patients (30) healthy controls (25)		Before start of treatment	FV:C, Protein C, Protein S, TFPI:Ag, TAFI:Ag
Homoncik, 2007	A. Observational Crossectional B. Observational Intervention		AOvert hyperthyroid patients (30) Euthyroid controls (30) B Overt hyperthyroid patients (30)	B Thiamazole started with 60 mg/d adjusted depending on T4, T3 and TSH concentrati	A One time point B At baseline and after therapy with thiamazole	Platelet count, PT, APTT, platelet aggregation (epinephrine), vWF:Ag, vWFRiCo, FVIII:C

				ons		
Lippi, 2008	Observational: Cross-sectional	medium	General population of unselected patients devided into TSH<0.2 (54) compared to TSH 0.2–2.5 (943)		Retrospective analysis of routine blood testing	APTT, PT, Fibrinogen:C
Mohamed-Ali, 2008	Observational: Cross-sectional		Overt and subclinical hyperthyroid patients (30) Euthyroid controls (30)		Before start of treatment	Platelet count, PT, APTT, Fibrinogen: Ag
Van Zaane, 2010	Experimental: Randomised clinical trial	High	A Healthy volunteers (16) B Healthy volunteers (12)	A Levothyroxine 0.3 mg/d or placebo for 14 days B Levothyroxine 0.45 mg/d or 0.6 mg/d depending on weight or placebo for 14 days	At baseline and on day 14	PT, APTT, Fibrinogen:Ag, VWF:Ag, VWF:RiCo (%), Factor II:C, FVII:C, FVIII:C,FIX:C, FX:C, protein C, APCsr, total protein S, free protein S, F1+2, ETP, PAI-1:Ag, PAP, D-dimer, Clot-lysis time

PT indicates protrombin time; APTT, activated partial thromboplastin time; ADP, adenosine 5'-diphosphate; RIPA, ristocetin platelet agglutination; F1+2, prothrombin fragment 1+2; pFN, plasma fibronectin; cFN, cellulair fibronectin; F, factor; Ag, antigen; C, activity; RiCo, ristocetin-cofactor activity; VWF, von Willebrand factor; ATIII, antithrombin III C; GPL, anti-cardiolipin antibody IgG; MLP, anti-cardiolipin antibody IgM; TFPI, tissue factor pathway inhibitor; TAFI, thrombin-activatable fibrinolysis inhibitor; PAP plasmin-antiplasmin complexes; t-PA, tissue type-plasminogen activator; u-PA, urokinase-type plasminogen activator; PAI, plasminogen activator inhibitor; ETP, endogenous thrombin potential; APCsr, activated protein C sensitivity ratio

Fig.1 Haemostatic parameters for all medium-/high-quality cross-sectional studies: subclinical hyperthyroidism

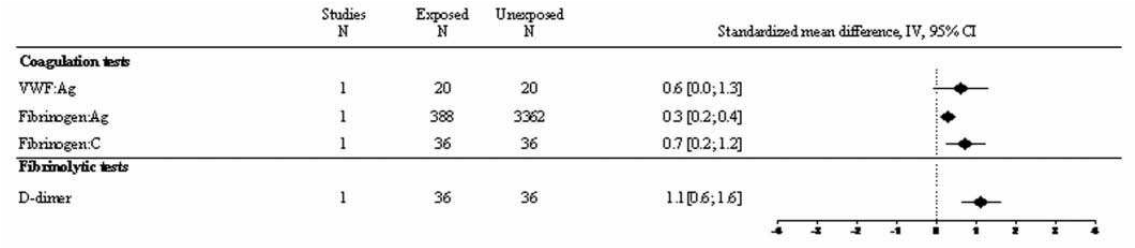


Fig. 2 Haemostatic parameters for all medium-/high-quality cross-sectional studies: overt hyperthyroidism

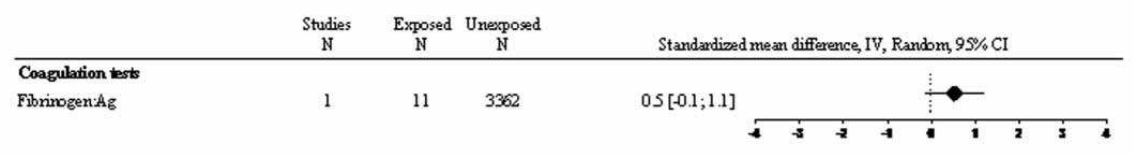


Fig 3 Haemostatic parameters for all medium-/high-quality observational intervention studies

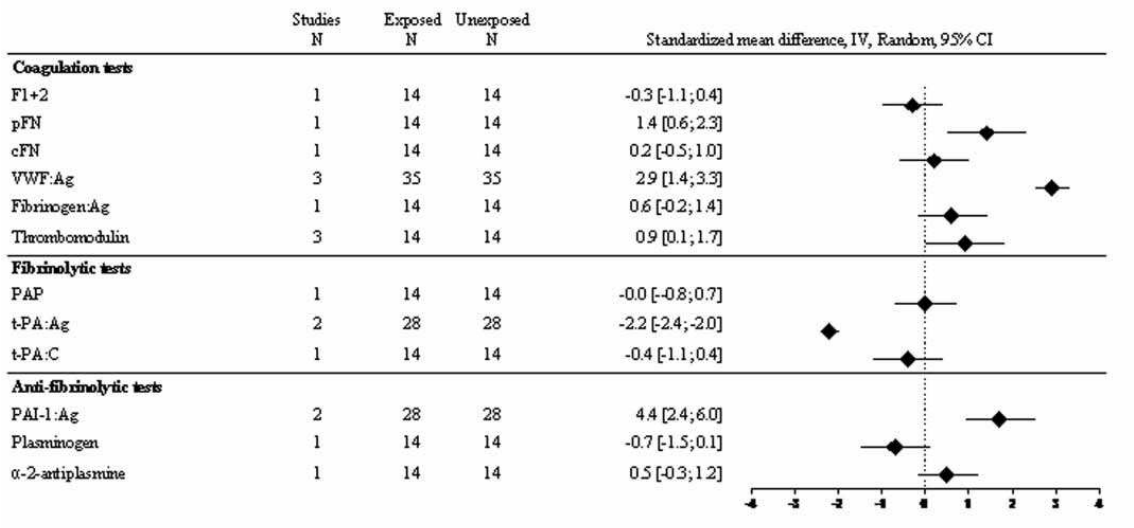
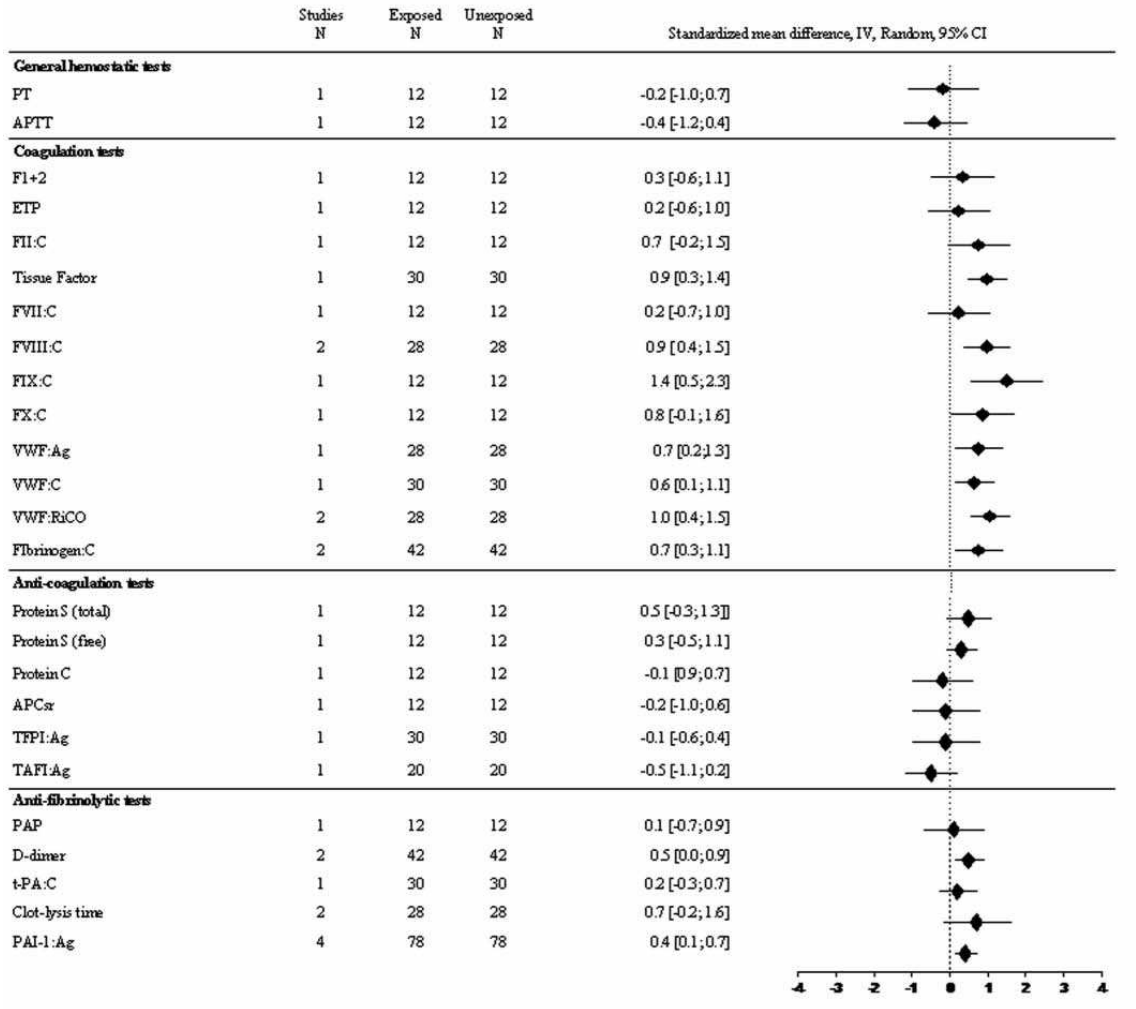


Fig 4 Haemostatic parameters for all medium-/high-quality experimental studies



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Gravidanza

Durante la gravidanza il rischio di sviluppare trombosi venosa aumenta di dieci volte, probabilmente come conseguenza dell'attivazione del sistema emostatico, della riduzione del ritorno venoso e della riduzione del sistema fibrinolitico che si sviluppano durante la gestazione[24]. Inoltre anche gravidanze multiple, età maggiore di 35 anni e l'obesità possono essere considerati fattori di rischio per lo sviluppo di trombosi in gravidanza.[25] La patologia tromboembolica comunque rappresenta la prima causa di morte materna nei paesi occidentali e anche durante il puerperio il rischio trombotico è cinque volte maggiore rispetto a donne di pari età[26].

In letteratura si ritrovano diverse linee guida circa la terapia anticoagulante in gravidanza, ma i dati riguardano poche pazienti che spesso trattate per patologie diverse dalla trombosi venosa.

Quindi in considerazione della rilevanza clinica e della mancanza di dati certi sull'argomento, abbiamo deciso di condurre una revisione sistematica della letteratura riguardo il trattamento della tromboembolia in gravidanza. Utilizzando i motori di ricerca EMBASE e MEDLINE sono stati identificati gli studi riguardanti la terapia anticoagulante attuata per un episodio di tromboembolismo venoso verificatosi in gravidanza, la ricerca è stata poi completata considerando gli abstracts congressuali.

Sono stati identificati più di 5000 articoli, ma sono stati selezionati per le analisi 15 articoli e 3 abstracts. In totale sono stati evidenziati 981 casi di pazienti affette da trombosi venosa profonda o embolia polmonare durante la gravidanza. Per la maggior parte la terapia di scelta è stata rappresentata da eparina a basso peso molecolare che si è dimostrata efficace ed anche sicura in questa particolare popolazione di pazienti;

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infatti il rischio emorragico non si è discostato da quello rilevato in pazienti trattati per patologia trombotica non in corso di gravidanza.

ANTICOAGULANT THERAPY FOR VENOUS THROMBOEMBOLISM DURING PREGNANCY: A SYSTEMATIC REVIEW AND A META-ANALYSIS OF THE LITERATURE[27]

Erica Romualdi, Francesco Dentali, Elena Rancan, Alessandro Squizzato, Luigi Steidl, Saskia Middeldorp , Walter Ageno

Introduction

Venous thromboembolism (VTE), comprising deep vein thrombosis (DVT) and pulmonary embolism (PE), is one of the most relevant causes of maternal death in the developed countries with a reported mortality rate of 1.56 per 100,000 maternities in the United Kingdom [1,2]. Symptomatic VTE is estimated to occur in 5 to 12 women per 10,000 pregnancies ante-partum and in 3 to 7 women per 10,000 deliveries post-partum [3,4]. During pregnancy and puerperium, the risk of developing VTE is five times higher than in the general female population of childbearing age [5].

Treatment of VTE in pregnant women poses some particular challenges, because it requires taking into account the safety of the selected drugs not only for the mother, but also for the fetus. Vitamin K antagonists (VKAs) cross the placenta and have the potential to cause fetal bleeding and teratogenicity[6]. Unfractionated heparin (UFH) does not cross the placenta and does not cause fetal teratogenicity, but its use may be associated with additional maternal safety issues including the risk of heparin-induced thrombocytopenia (HIT) and heparin-associated osteoporosis [6]. Furthermore, the use of therapeutic doses of UFH requires regular laboratory monitoring of the activated partial thromboplastin time (aPTT). As UFH, low-molecular weight heparin (LMWH) does not cross the placenta and there is no evidence of teratogenicity or risk of fetal bleeding [6,7]. In addition, LMWH offers a number of advantages over UFH thanks to its better bioavailability, longer plasma half-life, more predictable dose response and improved safety profile with respect to osteoporosis and HIT [6]. For these reasons, LMWH is currently recommended as the treatment of choice for patients with acute VTE occurring during pregnancy [2-4,6-10]. However, evidence to support this recommendation is largely based on case reports or case series of pregnant patients, or on data derived from studies carried out in non-pregnant patients. Consequently, limited data exist on the incidence rate of recurrent VTE or bleeding during treatment with LMWH or UFH during pregnancy and puerperium, and little is known on their optimal therapeutic dosage. Thus, different therapeutic strategies are proposed and used in clinical practice. [3,6,8,9]. Although a few systematic and narrative reviews have tried to assess the risk to benefit profile of anticoagulant treatment of VTE in pregnancy [3,9,10,11], these studies often mixed together data from patients receiving anticoagulant therapy for different indications such as VTE treatment or prophylaxis, prevention of obstetrical complications, prevention of arterial thrombosis in patients with mechanical cardiac valves or, conversely, only focused on a single therapeutic agent.

We therefore decided to carry out a systematic review of the literature and a meta-analysis of the studies that have reported on recurrent VTE or bleeding events in patients receiving anticoagulant drugs for the treatment of pregnancy related VTE with the aim to provide an estimate of the incidence rates of these complications.

[27] Journal of Thrombosis and Haemostasis 2012

Methods

A protocol was prospectively developed. Specific objectives, criteria for study selection, approach to assess study quality, outcomes, and statistical methods were a priori defined.

Study identification

Studies were identified using the MEDLINE (1966 to April Week 2 2012) and EMBASE (1980 to April Week 2 2012) electronic databases. The search strategy was developed using the following keywords: "Venous Thrombosis", "Pulmonary Embolism", "Pregnancy", "Therapy", "Treatment".

The electronic search was supplemented by manual search of reference lists and recent reviews and by reviewing abstracts books from the Congress abstracts from the International Society on Thrombosis and Haemostasis (ISTH) annual meetings, from the European Society of Human Reproduction and Embryology (ESHRE) annual meetings, and from the American Society of Haematology (ASH) meetings from 2003 to 2011.

Studies were selected if they reported data about recurrent VTE (any PE or DVT) and/or about any haemorrhagic event that occurred during pregnancy or the post-partum periods.

Study selection

Study selection was performed independently by 2 reviewers (ERo, ERa), with disagreements resolved through discussion and by the opinion of a third reviewer (FD), if necessary. Any type of randomised controlled trials or cohort studies were included if they met the following criteria: I) deep venous thrombosis (DVT) or pulmonary embolism (PE) were objectively confirmed (with Doppler ultrasound, computer tomography, angiography, lung scan) and occurred during pregnancy; II) patients were treated with one or more anticoagulant drug.

We excluded studies that considered VTE occurring before pregnancy, studies in which pregnant women were treated for reasons other than the treatment of acute VTE, and when it was impossible to obtain separate data about patients treated for acute VTE only.

To reduce the risk of biased results, we decided to only include studies that enrolled more than 10 patients.

When multiple publications for a single study had been published, we decided to use the latest publication and to supplement it, if necessary, with data from the earlier publications.

To assess the agreement between reviewers for study selection, we used the k statistic, which measures agreement beyond chance [12]. Values higher than 0.6 were considered to represent a substantial agreement and values higher than 0.8 an almost perfect agreement.

Data Extraction

Two reviewers (ERo, ERa) independently completed data extraction using a standardized form. Disagreement was resolved by consensus and by the opinion of a third reviewer (FD), if necessary.

The following data were extracted: study characteristics (year of publication, design, study centre), patients characteristics (number of subjects studied, mean age, mean gestational age at diagnosis of VTE), number of DVT and PE events, drugs and regimens used for the acute treatment, drugs and regimens used for the long-term treatment, haemorrhagic events occurred both ante-partum and during post-partum period, recurrent VTE events occurred both ante-partum and during post-partum period.

The acute treatment phase was defined as the first week of anticoagulant therapy, the long-term treatment period was defined as the remaining treatment period.

To define the severity of bleeding events, we aimed to use the ISTH classification for haemorrhages occurred during the ante-partum period [13] . Post-partum haemorrhage (PPH) was defined as those bleeding events occurring within the first 24 hours after delivery and we aimed to use the classification proposed by the Royal College of Obstetricians and Gynaecologists (RCOG)[14]. Based on this definition, major PPH is adjudicated after the loss of 1000 ml or more of blood from the genital tract; clinically relevant, non- major bleeding after the loss of more than 500 ml of blood; and minor bleeding in case on any other blood loss reported. In the case the quantity of blood loss was not specified, we took into account the definition of bleeding given by the authors and considered wound haematomas as minor bleedings and atonic post-partum bleedings as clinically relevant, non-major bleedings. In addition, we aimed to collect information about bleeding that occurred after the first 24 hours from the delivery and to classify again using the ISTH definitions in the absence of other available classifications.

We considered recurrent VTE as any new thromboembolic event occurred during anticoagulant therapy and after the index VTE, as adjudicated by the authors. In addition, information was collected on the occurrence of heparin induced thrombocytopenia (HIT) during treatment. In case of necessity we contacted the authors for additional information.

Study Validity Assessment

A protocol was prospectively developed. Specific objectives, criteria for study selection, approach to assess study quality, outcomes, and statistical methods were a priori defined and reported according to the proposal for reporting of Meta-analysis of Observational Studies in Epidemiology (MOOSE) group [15]. The same 2 unmasked investigators independently completed the assessment of study validity. We assess the methodological quality of each study with the Newcastle-Ottawa scale that was developed to assess the quality of non-randomised studies with its design, content and ease of use directed to the task of incorporating the quality assessments in the interpretation of meta-analytic results [16] . A 'star system' was developed for this scale in which a study is judged on three broad perspectives: the selection of the study groups; the comparability of the groups; and the ascertainment of either the exposure or outcome of interest for case-control or cohort studies respectively [16]. We awarded studies a maximum of 3 points for selection, 2 points for comparability, and 2 points for outcome assessment, with more points indicating better quality. The maximum possible score was 7: studies that obtained 6 or 7 points were considered of high quality, studies that obtained 3 or less points of low quality and the others studies were considered of medium quality.

The quality scale was supplemented by other quality criteria that were specific for this study: description of the methods used for the diagnosis of VTE (how was the diagnosis made in each study), definition of recurrence, and the use of standardized methods for determining haemorrhage.

Statistical analysis

Weighted mean incidence of bleeding complications and of VTE recurrence were calculated using the random effect model [17] Events occurring during the ante-partum and post-partum period were analyzed separately. Statistical heterogeneity was evaluated using the Cochran's Q and the I^2 statistic [18]. Analysis was performed with StatsDirect software (Version 2.7; StatsDirect Ltd, England).

Results

A total of 5011 (2831 Medline 2180 Embase) citations, 6 abstracts from ISTH and 2 abstracts from ASH meetings were identified by our systematic search. A total of 4988 studies, 3 ISTH abstracts and 2 ASH

abstracts were excluded after reviewing the study titles or abstracts. We subsequently retrieved the full text of 31 potentially eligible articles and 3 ISTH abstracts. Among the studies published as full papers, we further excluded 1 article because no antithrombotic therapy was used, 7 because they included 10 or less patients, 2 because no data about follow-up were provided, and 6 because data on patients treated for acute VTE were undistinguishable from data on women receiving antithrombotic prophylaxis. Thus, a total of 15 full papers [19,20,22-26,28-30,32-36] and 3 abstracts [21,27,31] were eligible for inclusion in our systematic review. (Figure 1)

The inter-observer agreement for the study selection was optimal (k=0.91).

According to the Newcastle-Ottawa scale, no studies were of high quality and four studies were of medium quality [26,29,33,36]. Eight studies specified that diagnosis of VTE was made using imaging techniques [20,22,24,26,28,30,35,36], two studies defined the diagnosis of recurrent VTE [20,35] and only one study used the classical definition of PPH to describe post-partum haemorrhages [33].

Among the selected studies, 17 were written in English [19,21-36] and 1 in German [20].

The corresponding authors of 11 of the selected studies were contacted with the aim to receive missing information and 6 authors kindly provided the requested data.

A total of 981 pregnant women with acute VTE were included, with their age ranging from a minimum of 17 years to a maximum of 43 years. Only 13 of the selected studies, including a total of 632 patients, provided separate data on the number of DVT and PE (in total: 506 DVT and 127 PE) [19,20,22,24-28,31-33,35,36.] (Table 1). The timing of VTE onset during pregnancy was not reported in all studies. Where available, this ranged from the 7^{th} gestational week to the 38^{th} gestational week.

Overall, 822 patients received LMWH and 155 UFH for the acute phase treatment of VTE. None of the studies reporting on patients treated with fondaparinux met our selection criteria. Dosing regimens of LMWH were heterogeneous among studies and are summarized in Table 2. Only in two studies, during the acute phase, the dosages were adjusted according to the measurement of anti-Xa levels [26,35]. UFH was administered both intravenously and subcutaneously, in most cases, but not always, according to aPTT values. (Table 2)

Treatment regimens during the long-term treatment period are summarised in Table 3. The majority of patients initially treated with UFH were switched to LMWH, others were continued on UFH, in most cases administered subcutaneously, and a few (36 from a single study[22]) were switched to warfarin. In one study, 4 patients initially treated with LMWH were switched to UFH during the late phase of pregnancy[36]. During the long-term treatment period, six studies reported on dose changes to LMWH according to anti-Xa levels in some patients [26,31,32,34-36]

Duration of the follow-up period was heterogeneous among studies and, in some cases, it was not reported. In one study, follow-up was limited to the first three months after the diagnosis of VTE[22]. Based on information provided, we could not estimate the total duration of treatment period.

<u>Bleeding complications in the ante-partum period</u>

During the ante-partum period, a total of 28 bleeding events were reported in 16 studies over a total of 944 patients [19,21-24, 26-36]. Regrettably, available data were not sufficient to enable us to apply the ISTH classification of bleeding severity, so we could only use the definitions given by the authors in each study. Overall, 5 events were defined by the authors as major, 1 was defined as clinically relevant non-major, 16 were defined as minor, and 6 events were not classified [29-30]. In one patient major bleeding

developed after severe eclampsia complicated by thrombocytopenia [31]. Four of the 5 major bleedings occurred during the acute phase treatment [19,22], and the only 1 clinically relevant non-major bleeding after 2 weeks of treatment [28]. Of the 4 major bleeding events that occurred during the acute phase treatment, 2 occurred in patients treated with LMWH and 2 in patients treated with UFH.

The use of anticoagulant therapy was therefore associated with an ante-partum incidence of haemorrhagic complications of 3.28% (95% CI:2.10-4.72;I^2: 14.6%). The ante-partum incidence of major bleeding was 1.41 % (95% CI:0.60-2.41;I^2:0%); the incidence of major bleeding during the acute treatment phase only was 1.00 % (95% CI:0.4-2.0%;I^2:0%) (Figure 2). Based on available data, it was not possible to separately estimate the incidence of bleeding complications for UFH and LMWH.

Bleeding complications in the post-partum period

A total of 260 PPH were reported in 13 studies [21,23-29,31-33,35,36], over a total of 725 patients. In most studies, information on blood losses was sufficient to enable us to use the classification of the RCOG. In total, 14 PPH were defined as major, 41 were defined as clinically relevant non-major (4 of these were atonic post-partum bleedings), and 205 were defined as minor. (Table 4)

The incidence of major PPH was 1.90% (95% CI:0.80-3.60%; I^2:36.8%). (Table 5, Figure 2)

Only 7 studies with a total of 350 patients provided information on bleeding events occurring after the first 24 hours after delivery. Information provided on bleeding events was insufficient to apply any classification, and the definition of severity provided by the authors was used. Information on the duration of follow-up was not available. A total of 14 haemorrhagic events were reported: 3 were defined as major (1.2%, 95% CI:0.30-2.50%; I^2:0%)

Thromboembolic complications

Information on recurrent venous thromboembolic events occurred during the ante-partum period was available in 16 studies including a total of 922 patients. There were 16 recurrent VTE events: 11 were PE (one fatal) and 5 were DVT. (Table 4)

The estimated weighted-mean incidence of ante-partum recurrent VTE was 1.97% (95% CI:0.88-3.49% I^2: 39.5%).(Figure 3) The estimated weighted mean incidence of recurrent PE was 1.30 % (95%CI: 0.6 %-2.3%; I^2 13.3%).

Seven VTE recurrences occurred in the first 7 days after the start of treatment, 5 of these occurred on UFH treatment [19,20,23,27].

The incidence rate of recurrent VTE during the acute phase treatment period was 1.41% (95%CI: 0.44-2.90%; I^2 38%); during the long-term treatment period this incidence was 0.95% (95%CI: 0.37-1.82%; I^2:0%). Once again, based on available data we could not separately estimate the incidence of bleeding complications for UFH and LMWH.

Two studies [19,23], including 41 patients, reported on 4 cases of recurrent VTE occurred during the post-partum period.

Finally, we found no reported cases of HIT in the 13 studies that addressed this complication, for a total of 860 patients. [21-24,26-29,31,32,34-36]. The occurrence of HIT was prospectively assessed in only 2 studies.

Discussion

The results of this systematic review of the literature substantially support the efficacy and safety of currently used therapeutic strategies for the treatment of pregnancy-related VTE, but suggest that some work should be done to improve their efficacy and safety profile, in particular during the highest risk periods. In fact, the estimated incidence of recurrent events during pregnancy remains substantial during the first week of treatment, as well as the rates of major bleeding complications, again during the first week of treatment and in the first 24 hours after delivery.

We observed a substantial heterogeneity in the reported treatment regimens: although most patients received LMWH, various dosing regimens for the acute phase treatment and, in particular, for the long-term secondary prevention were prescribed in the studies, and no direct comparisons are currently available. Heterogeneity was also present among definitions of major bleeding events prior and after delivery in individual studies. Because the application of a single, more widely accepted definition was not always feasible given the limited information provided in some studies, the observed rates of bleeding should be interpreted cautiously.

The 2012 version of the guidelines of the American College of Chest Physicians (ACCP) recommends the use of adjusted-dose LMWH for the treatment of VTE during pregnancy [6.] LMWH is considered the best option based on its greater bioavailability and its favourable safety profile. It is also recommended that anticoagulant therapy should be continued with the same dose throughout pregnancy, and until at least 6 weeks after delivery [6]. Clear-cut indications about the need for dose adjustments over the course of pregnancy or the usefulness of routine measurement of anti-Xa activity were not provided [6]. In the RCOG guidelines published in February 2007 [2], the authors suggested to treat VTE with LMWH given twice daily throughout pregnancy and discouraged physicians from the routine measurement of peak anti-Xa activity, with the exception of women at extremes of body weight or with other complicating factors like renal insufficiency. In our search, we found six studies in which, in some patients, the LMWH dosages were modified according to anti-Xa levels. Unfortunately, a separate assessment of clinical outcomes in this subgroup of patients was not feasible. During the acute phase treatment period, most patients have received weight-adjusted, full dose LMWH with an acceptable incidence of major bleeding events; during the long-term treatment phase period, in most patients treatment doses were empirically reduced, while a minority of patients have received dose adjustments based on the measurement of anti-Xa levels.

Of note, the rates of major bleeding events in our study are consistent with the rates of major bleeding events reported in studies carried out in non-pregnant patients treated for acute VTE with standard anticoagulant therapy [4,37]. Conversely, the estimated incidences of bleeding and recurrent VTE events in our study somewhat differ from the results of a previous systematic review published in 2005 by Greer et al. [9]. In this study, the authors assessed the safety and the efficacy of LMWH during pregnancy, and included both patients treated for pregnancy-related DVT or PE (n: 146) and patients receiving antithombotic prophylaxis. The overall rate of what the authors defined significant bleeding was 1.98% (95% CI: 1.50%-2.57%) including both the ante-partum and the post-partum periods, and 0.43% (95% CI: 0.22%-0.75%) in the ante-natal period only. The rate of bleeding in the post-partum period only in patients on LMWH for the treatment of acute VTE was 1.72%. These rates are lower than the rates reported in our study, probably because we included only patients receiving therapeutic doses of

anticoagulants and patients on UFH, and possibly because of a different definition of bleeding events. Finally, also the rates of recurrent VTE in our study were higher than those reported in the review by Greer et al (1.15%).

Our meta-analysis has a number of limitations. First, only case-control and cohort studies have been published, and the application of formal meta-analytic methods to observational studies is controversial, since bias implicit in the study design may misrepresent the strength of associations within the data [12]. In addition, we considered studies with different end-points and with different durations of follow up. Moreover, the therapeutic strategies were highly heterogeneous among studies, and different compounds with different dosing regimens were used. Unfortunately, separate analysis of these therapeutic regimens was not feasible due to the insufficient information provided in the selected studies. Another limitation is in the lack of standardized definitions of bleeding severity among selected studies, which mandates some caution when interpreting the reported figures. This in particular applies to the definition of PPH. PPH is traditionally defined as any blood loss from the genital tract during delivery above 500ml [13,14,38], but other definitions also required the presence of clinical signs of blood loss [39]. Furthermore, we could not obtain data related to the entire duration of the puerperium; firstly because of the wide variability in follow up durations among the selected studies, secondly because in most studies data on anticoagulant treatment and on related complications after the delivery were not provided. Similarly, the criteria used to diagnose recurrent VTE were not reported in most studies, and it is possible that some heterogeneity exists in the adjudication criteria that were applied. Moreover, we had insufficient information to identify early recurrences that may have been due to thrombosis extension or to inadequate anticoagulation. Finally, we acknowledge the existence of studies conducted with other anticoagulant agents including danaparoid [40] and fondaparinux . However treatment with the heparinoid is not standard of practice and is not listed in the recommended strategies in the most important guidelines [ACCP, RCOG], in addition in our search we identified one study on the use of danaparoid in pregnant patients who were intolerant to heparin (also in case of previous HIT), but in this article we could not distinguish data from patients treated for an acute episode of VTE or for the prophylaxis of VTE, so we decided to not include the study in the meta-analysis; whereas none of the studies with fondaparinux was eligible for inclusion in our study because none had more than 10 included patients.

In conclusion, this is to our knowledge the first systematic review and meta-analysis specifically aimed to estimate the incidence of recurrent VTE and bleeding during anticoagulant treatment for pregnancy-related VTE. The results of this study suggest that anticoagulant drugs during pregnancy are effective in the prevention of recurrent VTE, with an acceptable safety profile. Most events, both bleeding and recurrences, occurred during the first week of treatment. We could not identify the optimal therapeutic regimen among the different approaches used in the selected studies.

Table 1: Baseline characteristics of the study populations

Author	Patients (number)	Age	Gestational age	BMI	DVT	PE
Aburahma1999 [19]	24	Mean 24 (17-39) y	1 first trimester 5 second trimester 18 third trimester	nr	24	nr
Bahlmann 2000 [20]	12	nr	26.1±6.2	nr	12	0
Barillari 2007 [21]	38	nr	nr	nr	nr	nr
Blanco-Molina 2010 [22]	173	mean 31±6 y	nr	nr	135	38
Clark 2008 [23]	17	nr	median 19 w (7-34w)	nr	nr	nr
Daskalakis 1997 [24]	18	27-43 y	7-38 w	nr	18	0
Donnelly 2012 [25]	25	nr	nr	nr	21	4
Jacobsen 2003 [26]	20	mean 31.8±5.19 y	4 first trimester 6 second trimester 11third trimester	nr	19	2
Mitic 2011 [27]	87	mean 29.7±4.86	mean 22 w± 10.2w	nr	82	5
Narin 2008 [28]	35 18 gruop I 17 group II	Mean 28.4±3.5 y group I mean 30.0±5.2 y groupII	mean 29.3 w ±4.4w group I mean 26.5±6.5w group II	nr	35	0
Nelson-Piercy 2011 [29]	247	mean 30.1y*	nr	mean 27.7*	nr	nr
O'Connor 2010 [30]	34	nr	nr	nr	nr	nr
Parent 2007 [31]	39	mean 32.5 y	nr	nr	20	19
Rodie 2002 [32]	29	nr	nr	nr	23	6
Roshani 2011[33]	13	Mean 32	nr	nr	8	5
Rowan 2003 [34]	13	nr	nr	nr	nr	nr
Ulander 2002 [35]	31 10 group I 21 group II	31.0±5.7 y group I 31.6 ±4.3 y group II	27.±8.5 w group I 21 ±9.8 w group II	23.4±4.7 group I 25. ±4.6 group II	31	0
Voke 2007 [36]	126	median 32(16-42) y	31 first trimester 37 second trimester 58 third trimester	median 26 (19-43)	78	48
Total 18 studies	**981**				**506**	**127**

BMI Body Mass Index, DVT deep vein thrombosis, PE Pulmonary Embolism, y years, w weeks, nr not reported
*data regarding 252 patients treated with therapeutic tinzaparin

Table 2: Treatment regimens for the acute phase of venous thromboembolism

Author	LMWH	Dose of LMWH	UFH	Dose of UFH	WARFARIN
Aburahma 1999 [19]	0	-	24	11 pt i.v. according to aPTT (target 1.5-2.5 times normal); 13 pt i.v. bolus then 5000-10000 U s.c. every 8-12 hours	0
Bahlmann 2000 [20]	1 dalteparin	5000 IU s.c 3 times day	11	According to aPTT (target 1.5-2.0 times normal)	0
Barillari 2007 [21]	38 nadroparin	100 IU/Kg bid	0	-	0
Blanco-Molina* 2010 [22]	154	Mean 187±51 IU/Kg/day	16	Nr	0
Clark 2008 [23]	2 enoxaparin	100-110 mg/kg bid	15	6 pt s.c.	0
Daskalakis 1997 [24]	0	-	18	According to aPTT (target 2.0 times normal)	0
Donnelly 2012 [25]	25	nr	0	-	0
Jacobsen 2003 [26]	20 dalteparin	initially 100 IU/Kg bid then according to anti Xa levels (0.5-1.0 U/ml)	0	-	0
Mitic 2011 [27]	84 83 nadroparin 1 dalteparin	100IU/Kg bid	3	nr	0
Narin 2008 [28]	0	-	35	According to aPTT (target 1.5-2.5 times normal)	0
Nelson-Piercy 2011 [29]	247 tinzaparin	median 13000 IU (3500-28000)†	0	-	0
O'Connor 2010 [30]	23	nr	11	nr	0
Parent 2007 [31]	39 tinzaparin	18119 UI/Kg/day	0	-	0
Rodie 2002 [32]	29 enoxaparin	1mg/Kg bid	0	-	0
Roshani 2011¶[33]	9 nadroparin 2 dalteparin	100IU/Kg bid or 200IU/Kg od	1	nr	0
Rowan 2003 [34]	13 enoxaparin	1mg/Kg bid	0	-	0
Ulander 2002 [35]	21 dalteparin	Based on anti Xa (target 1-1.5 U/ml)	10	According to aPTT (70-100s)	0
Voke 2007 [36]	115	83 pt od 39pt bid	11	nr	0
Total	**822**		**155**		**0**

Table 3: Treatment regimens for the long term treatment period

Author	LMWH	Dose	UFH	Dose	WARFARIN	Duration therapy
Aburahma1999 [19]	0	-	24	5000-10000 U s.c. every 8-12 h	0	6-8 weeks after delivery
Bahlmann 2000 [20]	2	500-600 IU/h i.v.	10 8 s.c. 2 i.v.	5000-7000 Ux3 s.c.	0	nr
Barillari 2007 [21]	38	80-100IU/Kg once	0		0	3 months after delivery
Blanco-Molina* 2010 [22]	133	Mean 173 ±59 IU/Kg/day	0	-	36	nr
Clark 2008 [23]	3	70 mg/Kd bid 17000 IU od	14 s.c.	9000-13000 IU every 8-12 h	0	nr
Daskalakis 1997 [24]	18	6150 anti Xa IU od	0	-	0	1 month after delivery
Donnelly 2012 [25]	25	nr	0	-	0	nr
Jacobsen 2003 [26]	20	According to anti Xa levels (0.5-1.0 U/ml)	0	-	0	nr
Mitic 2011 [27]	85	Intermediate dosage	2	nr	0	nr
Narin 2008 [28]	35	1 mg/Kg bid group I 1.5 mg/Kg od group II	0	-	0	nr
Nelson-Piercy 2011 [29]	247	median 13000 IU (3500-23100)†	0	-	0	nr
O'Connor 2010 [30]	23	nr	11	nr	nr	nr
Parent 2007 [31]	39	According to anti Xa levels	0		0	2210 w
Rodie 2002 [32]	29	According to anti Xa levels (0.4-1.0 U/ml)	0	-	0	Median 6w (1-33w)
Roshani 2011 [33]	9 nadroparin 2 dalteparin	100IU/Kg bid or 200IU/Kg od	1	nr	0	nr
Rowan 2003 [34]	13	Some patients according to anti Xa levels	0	-	0	nr
Ulander 2002 [35]	31	100 UI/Kg bid for 2 weeks then od according to anti Xa levels (0.7U/ml and 0.5-0.6 at the end of pregnancy)	0	-	0	nr
Voke 2007 [36]	118	Some patient according to antiXa levels	4	nr	0	nr
Total	**874**		**66**		**36**	

LMWH low molecular weight heparin; UFH unfractionated heparin; IU International units; od once daily; bid bis in die; h hours; pt: patients; i.v: intravenous; s.c: subcoutaneous; nr: not reported; w weeks

*data reported only for 169 patients

† data regarding 252 patients treated with therapeutic tinzaparin

Table 4: Adverse events

Author	Ante-partum bleeding	Ante-partum recurrent VTE	Other antepartum adverse events	Peri-partum bleeding (first 24 h after delivery)	Other Post-partum bleeding	Other post partum adverse events	FU
Aburahma1999 [19]	1 retroperitoneal bleeding in acute phase*	2 PE one fatal in acute phase*on UFH	nr	nr	nr	nr	61 Months (18-102)
Bahlmann 2000 [20]	nr	4 (2 PE): 3 in acute phase on UFH	nr	nr	nr	nr	nr
Barillari 2007 [21]	0	0	0	0	1 epystaxis	nr	1 year after delivery
Blanco-Molina 2010 [22]	3 major in acute phase*: 2 on UFH; 1 on LMWH	2 PE during LMWH after the acute phase*	nr	nr	nr	nr	3 months after VTE
Clark 2008 [23]	0	1 PE in acute phase* on LMWH	nr	1 wound hematoma, 1 minor bleeding	1 clinically relevant non major bleeding; 1 major	nr	nr
Daskalakis 1997 [24]	0	0	1 abortion (at 10°w)	0	nr	0	nr
Donnelly 2012 [25]	nr	nr	nr	0 bleeding > 1500 ml	nr	nr	nr
Jacobsen 2003 [26]	0	0	1 intrauterine death (at 37° w)	1 atonic post partum bleeding, 1 wound hematoma	nr	0	nr
Mitic 2011 [27]	3 minor	1 DVT in acute phase* on LMWH	4 skin reactions on nadroparin 1 fetal loss (at 8°w)	2 clinically relevant non major bleedings 2 minor bleedings 1 wound hematoma	0	0	nr
Narin 2008 [28]	1 vaginal bleeding 1 hematuria , and 1 clinically relevant vaginal on LMWH	0	1 abortion (at 9 w)	0	0	0	4-8-w after delivery in 57% of pt
Nelson-Piercy 2012 [29]	4‡ unspecified	5 (4 PE) °	nr	196 bleedings <500 ml 31 bleedings >500 ml<1000 3 bleeding s> 1000 ml †	nr	2 stillbirths 1 terminatio n §	nr
O'Connor 2010 [30]	2 unspecified	nr	nr	Nr	4 unspecified	nr	nr
Parent 2007 [31]	1 hematuria 2 vaginal bleeding 1 major on LMWH	0	1 fetal death 1 abortion 1 eclampsia with thrombocytopenia (gestational age not specified)	1 wound hematoma	nr	nr	nr
Rodie 2002 [32]	0	0	2 Skin reactions (on enoxaparin)	3 atonic post-partum bleeding, 1 wound hematoma	nr	0	nr
Roshani 2011 [33]	0	0	nr	2 major bleedings 2 bleedings <1000cc	1	nr	nr
Rowan 2003 [34]	0	0	nr	Nr	nr	nr	nr
Ulander 2002 [35]	1 hematuria	1 during LMWH after the acute phase*	1 premature delivery (at 23° w)	3 abnormal bleeding s> 1000 ml; 1 wound	nr	nr	nr

				hematoma			
Voke 2007 [36]	7 minor	0	2 intrauterine deaths (at 38° and 23° w), 1 miscarriage (at 12 w), 1 abortion (at 12° w)	6 bleedings> 1000 ml; 2 bleedings >500 ml<1000 ml	2 major bleeding; 4 clinically relevant not major bleeding	1 neonatal death	nr
Total	**28:** **5 major** **1 clinically relevant non major** **16 minor** **6 unspecified**	**16 °:** **11 PE; <u>1 fatal</u>**	**17:** **6 skin reactions** **4 abortion** **1 miscarriage** **4 intrauterine deaths** **1premature delivery** **1 eclampsia**	**260:** **205 minor bleedings,** **41 clinically relevant non major bleedings,** **14 major bleedings**	**14:** **3 major;** **5 clinically relevant;** **5unspecified; 1 minor**	**1 neonatal death** 2 stillbirths § 1 terminatio n §	

VTE venous thromboembolism; FU follow-up; h hours; nr not reported; UFH unfractionated heparin; w weeks; pt patients

* Acute phase: the first week after the diagnosis of TEV

‡ data regarding 254 patients treated with therapeutic tinzaparin

° in 2 cases recurrent VTE occurred after tinzaparin cessation

† data regarding 229 patients treated with therapeutic tinzaparin

§ data regarding 262 patients in therapeutic group

Table 5: Bleeding complication rates

	Incidence (%)	95% CI
All ante-partum bleeding complications	3.28	2.10-4.72
Ante-partum major bleeding	1.41	0.60-2.41
Acute ante-partum major bleeding*	1.00	0.40-2.00
Post-partum major bleeding (< 24 hours)	1.90	0.80-3.60
Puerperium major bleeding†	1.2	0.30-2.5

*Occurred during the first week after the introduction of anticoagulant therapy

†Occurred after the first 24 hours after delivery

Figure 1: studies selection

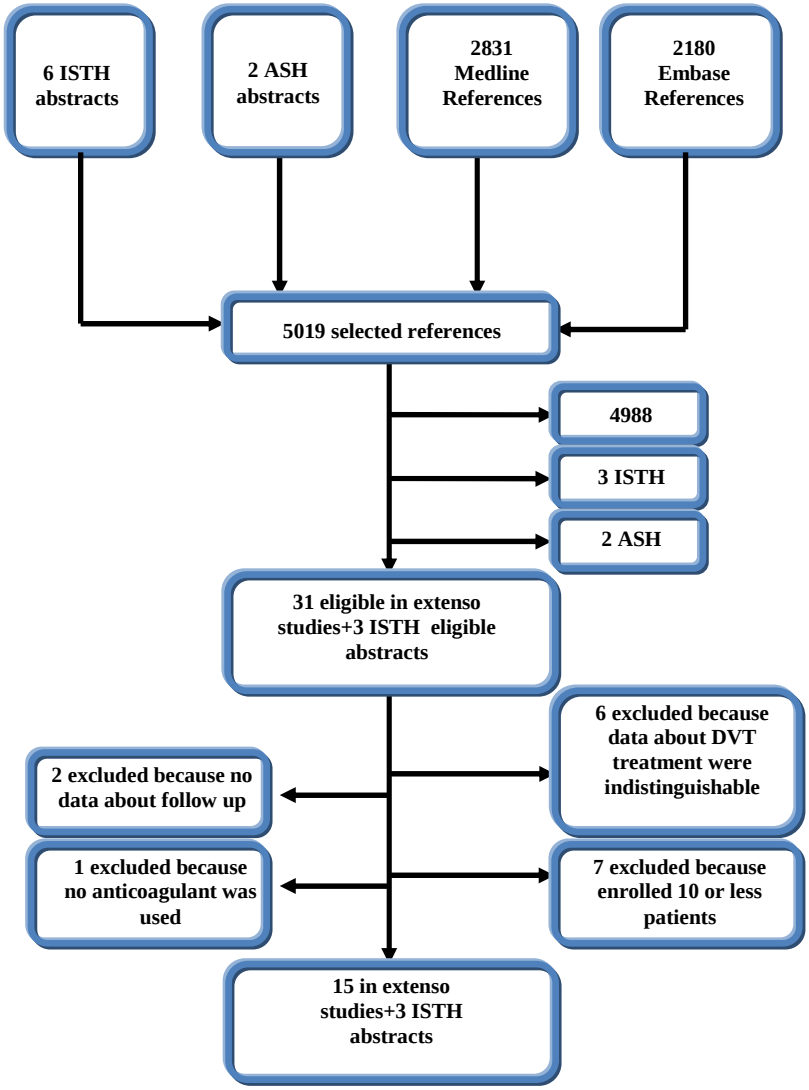
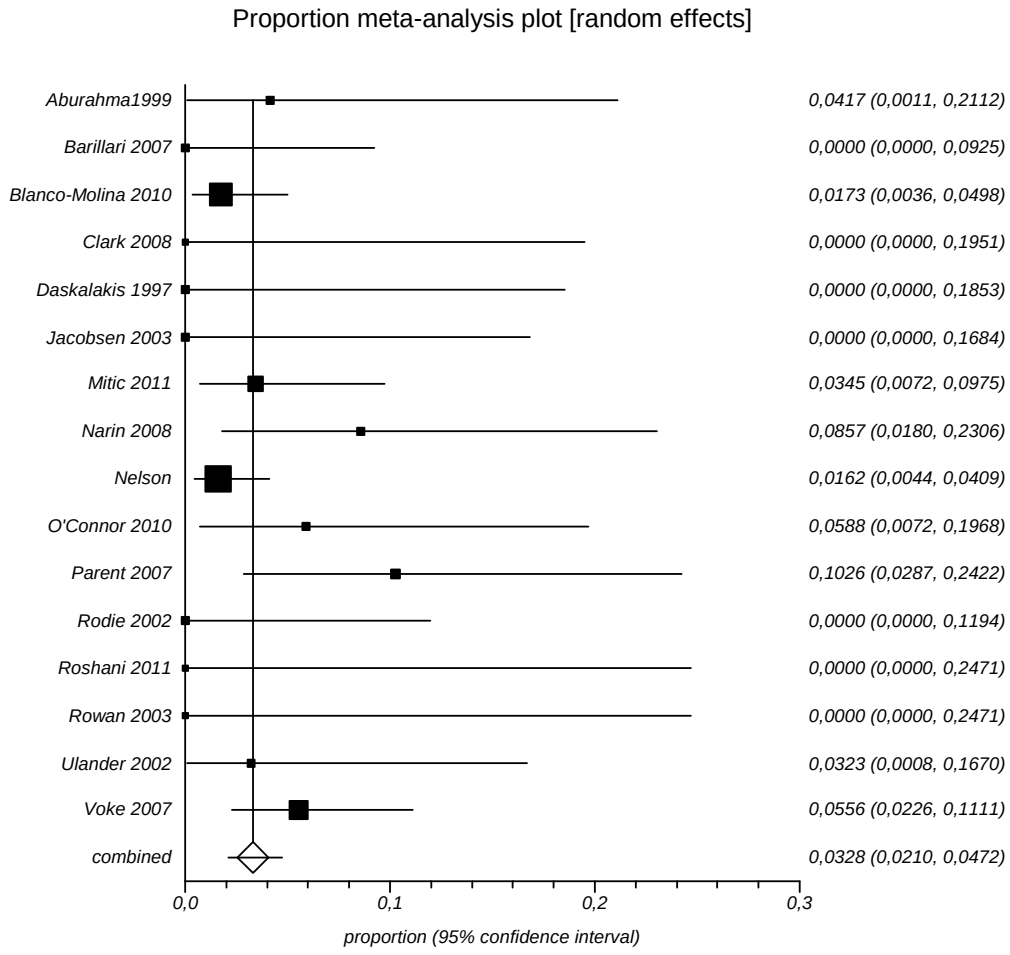
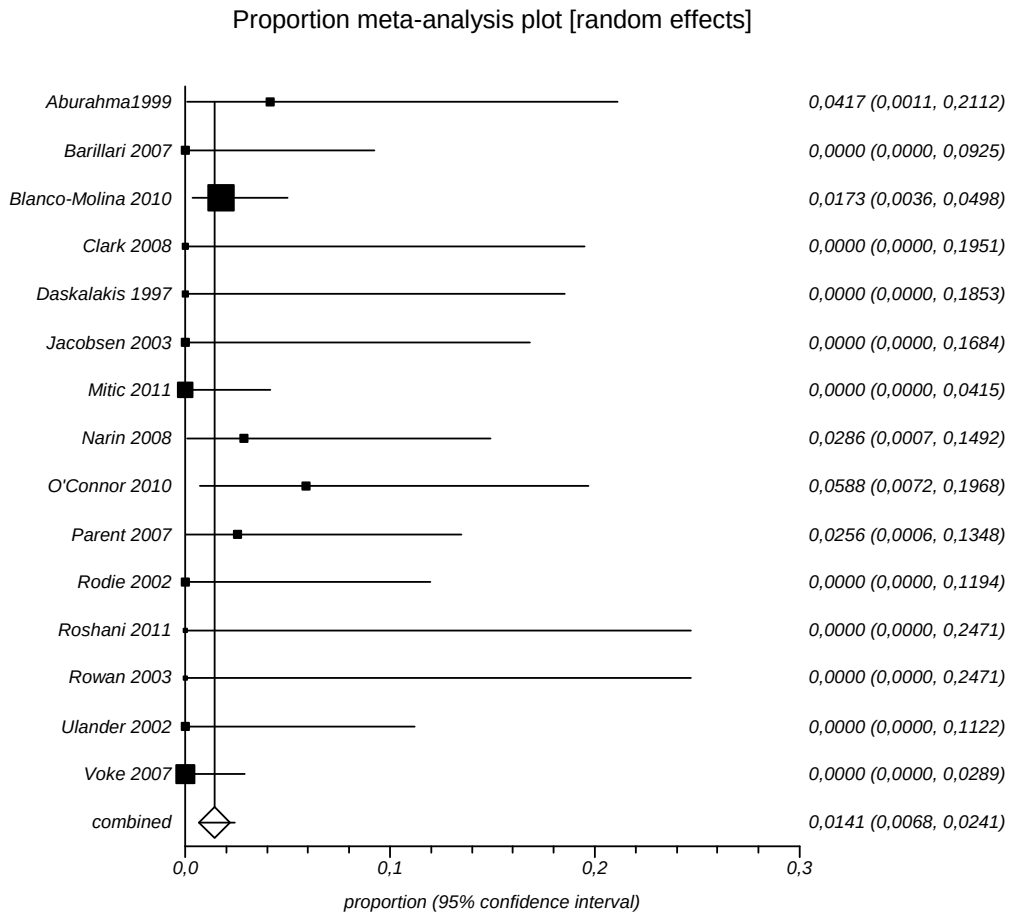


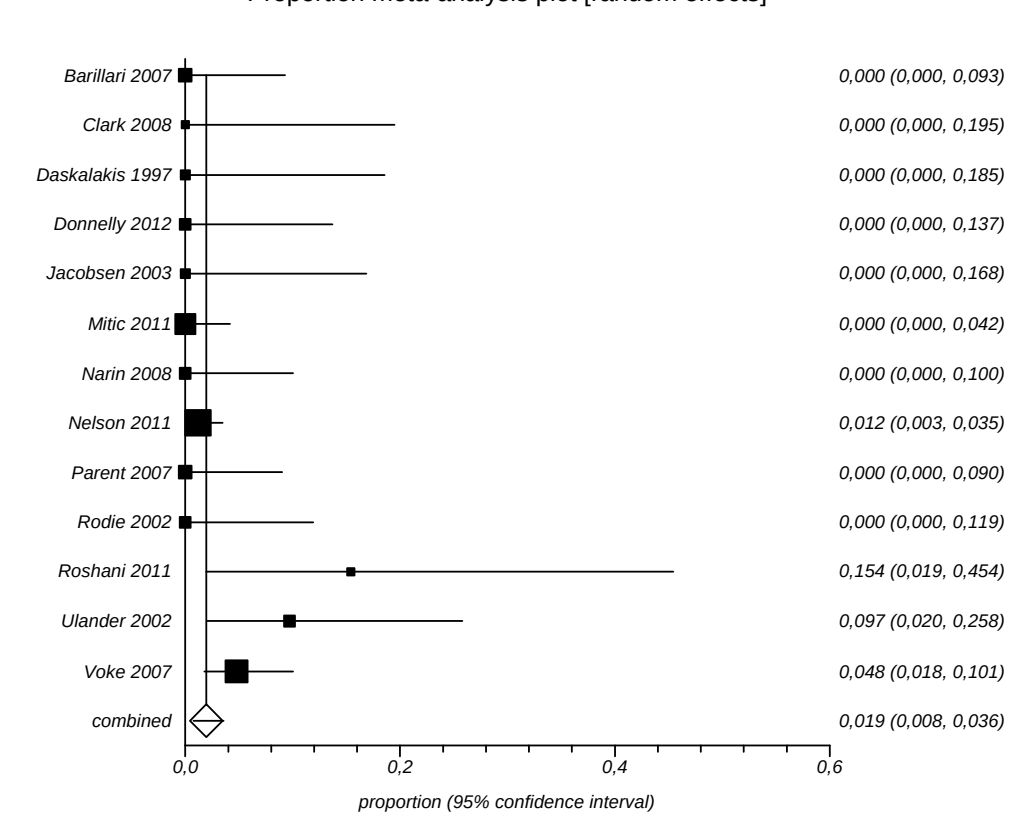
Figure 2
All antepartum bleedings [**WMD 3.28% (95% CI:2.10-4.72;I^2 : 14.6%)]**



Major antepartum bleedings **[WMD 1.41 % (95% CI:0.60-2.41;I^2:0%]**



Major bleeding in the first 24 hours postpartum **[WMD 1.00 % (95% CI:0.4-2.0%;I^2:0%]**



Major post-partum bleedings after 24 hours è **[WMD (1.2%, 95% CI:0.30-2.50%; I^2:0%)]**

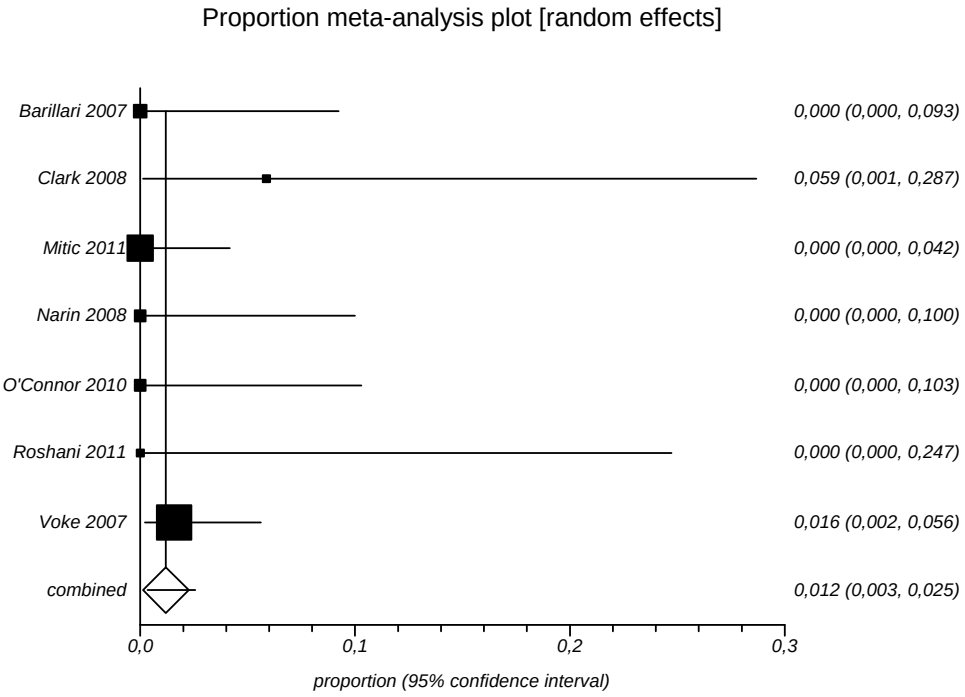
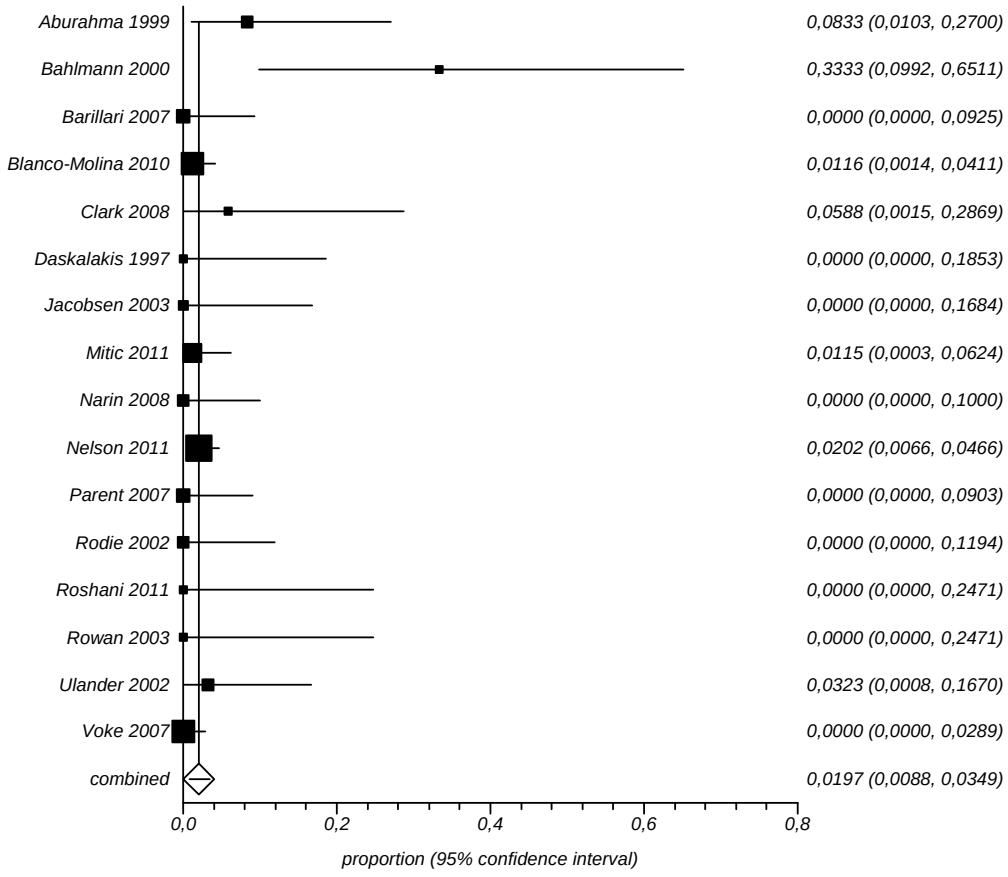


Figure 3: Ante-partum recurrent **[VTE WMD 1.97% (95% CI:0.88-3.49% I^2: 39.5%)]**



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Terapia anticoagulante orale

La terapia con inibitori della vitamina K (AVK) è indicata nella prevenzione primaria e secondaria di eventi trombotici arteriosi e venosi; in particolare tali farmaci vengono utilizzati frequentemente nella prevenzione di embolie sistemiche in pazienti portatori di protesi valvolari cardiache o in pazienti affetti da fibrillazione atriale e nella terapia di eventi tromboembolici venosi.[28]

La fibrillazione atriale è la più comune alterazione del ritmo cardiaco e rappresenta un importante fattore di rischio per lo sviluppo di ictus ischemico. La sua frequenza aumenta in modo proporzionale all'età, raggiungendo una prevalenza del 10% circa nei soggetti con più di 80 anni[29]. E' ormai noto che pazienti portatori di protesi valvolari meccaniche cardiache necessitino di un terapia anticoagulante a lungo termine[30]; infatti il rischio di complicanze emboliche o trombosi di valvola si attesta attorno al 12% e al 22% all'anno rispettivamente in caso si tratti di una protesi tipo St. Jude in posizione aortica o mitralica[31].

Gli AVK sono stati per molto tempo l'unica terapia anticoagulante orale disponibile in commercio; esercitano la loro azione anticoagulante interferendo con la conversione della vitamina K nel suo epossido, molecola fondamentale nel processo di carbossilazione di alcuni fattori della coagulazione (fattore II, VII, IX e X), definiti pertanto vitamina K dipendenti.

In tutto il mondo il AVK più utilizzato è rappresentato dal warfarin; questo presenta due forme enantiomeriche S- ed R-warfarin[32]. La forma S è un antagonista della vitamina K

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3-5 volte più potente rispetto alla forma R[33]. Entrambi gli enantiomeri subiscono un forte metabolismo epatico prima dell'escrezione. Il CYP2C9 è il principale enzima responsabile della metabolizzazione dell'S-warfarin ciò rende gli SNPs (single nucleotide polymorphisms) in questo enzima particolarmente importanti nel determinare la variabilità della terapia con questo farmaco. Tra questi gli SNPs con una maggiore frequenza allelica nella popolazione caucasica sono il CYP2C9*2 (rs1799853, R144C) e il CYP2C9*3 (rs4362691, I359L) che portano alla formazione di un enzima con una ridotta capacità di idrossilare l'S-warfarin, diversi studi hanno mostrato come queste varianti alleliche portano alla necessità di modificare la dose del farmaco[34]. L'R-warfarin è metabolizzato dai citocromi appartenenti alle isoforme CYP1A2 e CYP3A, con una prevalenza della forma 3A[35]. L'attività enzimatica della famiglia di citocromi 3A è dovuta alle isoforme 3A4 e 3A5 che hanno la medesima specificità di substrato. Esiste una considerevole variabilità nell'attività enzimatica del CYP3A4 e diversi polimorfismi sono stati finora identificati per questo gene, tuttavia fino ad ora le basi molecolari alla base di tale variabilità non sono ancora state perfettamente comprese. Il CYP3A5 costituisce il 17-50% del CYP3A presente al livello epatico[36]. Per questo enzima è stata identificata una variante allelica detta CYP3A5*6 che porta ad un difetto dello splicing con conseguente drastica riduzione dell'attività enzimatica. Per questo motivi il polimorfismo CYP3A4*5 rappresenta, nella popolazione caucasica la principale causa di variabilità per la famiglia del citocromo CYP3A.

Anche la P-glicoproteina (p-GP) sembra contribuire alla variabilità nella risposta la warfarin. La P-GP appartiene alla classe delle proteine trasportatrici adenosine triphosphate-binding cassette (ABC), di cui costituisce l'isoforma B1, espressa in

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[34] Takahashi H, Echizen H. Pharmacogenetics of CYP2C9 and interindividual variability in anticoagulant response to warfarin. Pharmacogenomics J 2003; 3: 202–214

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diversi tessuti come intestino, fegato, rene, barriera emato-encefalica e la placenta e che agisce come una pompa di efflusso per diverse molecole lipofiliche che sono frequentemente substrato del CYP3A[37]. Una volta assorbito, il warfarin agisce a livello epatico, dove inibisce la vitamina K epossido reduttasi[38]. Almeno in teoria quindi una riduzione dell'attività della P-GP potrebbe portare ad un aumento dei livelli del farmaco a livello delle cellule bersaglio aumentando la sensibilità di alcuni pazienti per il warfarin. Finora sono stati identificati diversi polimorfismo per il gene ABCB1 (ad esempio: rs4362691, rs4362691, rs4362691), che sembrano essere importanti nel determinare variazioni nella biodisponibilità de farmaci substrato per questo trasportatore, anche se i risvolti farmacologici e clinici di questi SNPs non sono stati ancora completamente chiariti [39].

Nei pazienti trattati con warfarin viene monitorato il tempo di protrombina (prothrombin time - PT) che viene espresso come rapporto internazionale normalizzato (international normalised ratio - INR), un indice che dà la misura della somma delle attività dei fattori di coagulazione dipendenti dalla vitamina K II, VII e X. Le variazioni interindividuali nella risposta la warfarin e il basso indice terapeutico sono due tra i principali fattori che rendono difficoltosa la terapia con questo farmaco. Il sesso, l'età, malattie epatiche (che riducono la sintesi dei fattori di coagulazione), stati ipermetabolici (che aumentano la clearance dei fattori di coagulazione), insufficienza cardiaca (che altera la distribuzione del warfarin) sono tutti fattori in grado di ridurre la sensibilità per il farmaco che quindi richiedono la riduzione del dosaggio[40]. Al contrario l'eccessivo

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introito alimentare di vitamina K potrebbe richiedere un aumento del dosaggio del farmaco.

Inoltre studi clinici hanno dimostrato che in centri specializzati la stabilità di valori di INR, espressa come percentuale di tempo in range terapeutico, si attesta in media attorno al 60% e che esistono fattori associati ad una maggiore stabilità dei valori di INR come ad esempio l'assenza di scompenso cardiaco o di diabete[41].

Esiste la possibilità che vi sia anche una predisposizione genetica alla risposta al warfarin che potrebbe influenzare la stabilità del trattamento, tuttavia nessuno studio ha fino ad ora valutato questo effetto sulla stabilità a lungo termine.

Il warfarin è stato oggetto di numerosi studi compiuti allo scopo di identificare i fattori alla base dell'ampia variabilità interindividuale nel dosaggio a questo farmaco, ma solo per quanto riguarda il fabbisogno iniziale relativo alle prime settimane di terapia. Da questi studi sono emersi diversi algoritmi sia comprendenti variabili genetiche che non genetiche allo scopo di stabilire un dosaggio ottimale per il warfarin in ogni paziente[42].

Gli SNPs nei geni di VKORC1 e CYP2C9 sono tra le variabili più informative in diversi algoritmi utilizzati per definire il dosaggio del warfarin.

Tali evidenze comunque non escludono la possibilità che SNPs in altri geni possano contribuire alle differenze interindividuali nel dosaggio del warfarin anche a lungo termine.

E' stato quindi condotto uno studio volto ad identificare i fattori genetici che possono essere associati ad una maggiore o minore stabilità del warfarin nella fase cronica del trattamento, definita come il periodo successivo al primo mese di terapia. In particolare obiettivo dello studio è quello di stabilire se esiste un'associazione tra un particolare

[41] Witt DM, Delate T, Clarck NP, et al. Outcomes and predictors of very stable INR control during chronic anticoagulation therapy. Blood 2009;114:952-6

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SNP e/o una combinazione di differenti SNPs con la stabilità dei valori di INR, definita come tempo trascorso in range terapeutico (TTR)[43].

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ASSOCIATION BETWEEN ABCG2 AND ABCB1 GENES AND WARFARIN STABILITY: A CASE-CONTROL STUDY[44]

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Warfarin is the most prescribed oral anticoagulant worldwide and is largely used for the primary and secondary prevention of thromboembolic events[1]. The management of warfarin therapy is complex and requires a regular monitoring of the international normalized ratio (INR)[2]. Even in specialized centers, only about 60% of INR values are within the therapeutic range, with a consequent increased risk of developing thrombotic or haemorrhagic complications[3]. Several variables have been associated with INR stability, in particular age, sex, hepatic disorders, cardiac failure, concomitant drugs and low intake of vitamin K[2,4], however several authors suggest that genetic variant in both pharmacokinetic that pharmacodynamic genes play a key role in warfarin response.

Warfarin is predominantly metabolized by cytochrome P-450 (CYP) 2C9[5] and produces pharmacodynamic effects by inhibiting vitamin K epoxide reductase complex subunit 1 (VKORC1). Several pharmacogenetic studies have previously shown that polymorphic alleles within CYP2C9 and VKORC1 genes are related to warfarin dose requirement[6], conversely, only few data are available on the association between gene polymorphisms and warfarin stability. Warfarin pharmacokinetic is influenced also by intestinal transporter such as P-glycoprotein (P-GP) coding by ABCB1 genes[7], a potent efflux pump for a wide variety of lipophilic compounds[8,9]. P-GP have a large substrates overlapping with Breast Cancer Resistance Protein (BCRP), other intestine transporters, coding by ABCG2 gene. Individuals carrying SNPs in both ABCB1 and ABCG2 have low levels of protein expression[10], that, at least for ABCB1, resulting in increased bioavailability and higher plasma warfarin concentrations[11]. So far, BCRP (coded by ABCG2) role in warfarin disposition has never been investigated; however a contribution may be well hypothesized, considering the large overlapping of substrates with P-glycoprotein[12-15].

The aim of our study was to assess whether genetic polymorphisms in important genes for warfarin pharmacokinetic and pharmacodynamic such as ABCB1, ABCG2, CYP2C9, and VKORC1 are associated with warfarin stability in patients on long-term therapy.

Methods.

From November 2010 to November 2011, all patients on long term warfarin therapy for atrial fibrillations (AF) or presence of mechanical heart valves (MHV) and with unstable warfarin therapy followed at the Anticoagulation Clinic of the Ospedale di Circolo, Varese, Italy were enrolled. Patients with stable warfarin therapy and matched for: age, sex, warfarin dose, indication and duration of anticoagulant therapy, concomitant drugs, alcohol use, smoking habit, weighted index of comorbidity (WIC)[16] and the mean vitamin K intake with diet, were enrolled as controls.

[44] Accepted for publication by Thrombosis Research

Warfarin stability was measured as the time spent in therapeutic range (TTR), estimated by linear interpolation between successive INR measurements, using methods published by Rosendaal[17]. In a 6-month period a TTR ≤55% defined unstable patients and a TTR ≥85% defined stable patients. Exclusion criteria were: patients on warfarin for less than 6 month; temporary stop of anticoagulant therapy during the 6-month period; renal (Glomerular Filtration Rate ≤ 90 mL/min/1.73 m^2) or hepatic (transaminases elevation upper normal limit) failure and inadequate compliance in following medical prescriptions on anticoagulant treatment[18]. The following information was collected for all patients: age, sex, TTR, warfarin dose at the time of enrolment, indication and duration of anticoagulant therapy, concomitant drugs, alcohol use, smoking habit, weighted index of comorbidity[19] and the mean vitamin K intake with diet by a food frequency questionnaire[20]. The Institutional Review Board approved the study, and all eligible patients provided written informed consent.

Genotyping was performed by Real Time PCR on an Applied Biosystems GeneAmp 9700 PCR System (ABI, Foster City, California) using a pre-designed genotyping assay (Applied Biosystems, Foster City, California, USA). The candidate gene polymorphisms are listed in **Table 1**.

Continuous variables are expressed as mean±standard deviation (SD); categorical data are given as counts and percentages. Each polymorphism was tested to ensure that it fitted Hardy–Weinberg equilibrium. The associations between categorical variables were assessed by Fisher's exact test. The odds ratios (OR) with the corresponding 95% confidence interval (CI) were calculated.

To explore the potential effects of interactions between genes, we identified SNPs combinations by a logistic additive model. Frequencies of different SNPs combinations were estimated using the implementation of the EM algorithm coded into the aplo.stats package[21]. The most frequent SNPs combinations was selected as the references category and rare SNPs combinations (frequency <3%) were pooled together in a group. Association between SNPs combinations and TTR were accomplished using Fisher's exact test.

Table 1. SNPs considered in the study.

Gene	Full name	SNPs	Genotype	SNPs effects
ABCB1	ATP-binding cassette, sub-family B (MDR/TAP), member 1	rs1128503	C1236T	Reduced gene expression [10]
		rs1045642	C3425T	Reduced gene expression [10]
ABCG2	ATP-binding cassette, sub-family G (breast cancer resistance protein), member 2	rs2231142	C421A	Reduced transport function and drug efflux [10]
CYP2C9	Cytocrome P450, family 2, subfamily C, member 9	rs1057910	A1075C	Reduced catalytic activity [6]
		rs1799853	C8633T	Reduced catalytic activity [6]

CYP3A5	Cytocrome P450, family 3, subfamily a, member 5	rs776746	G6986A	Reduced catalytic activity [28]
VKORC1	Vitamin K epoxide reductase complex subunit 1	rs7294	G3673A	Increased gene expression [27]
		rs9934438	C6484T	Reduced gene expression [6]

Results.

Patients and controls had similar age, gender distribution and indication for anticoagulation. BMI, warfarin weekly dose and weighted comorbidity index were also similar between the two groups (**Table 2**). The mean value of TTR was 47.5±6.1% in cases and 93.7±5.3% in controls. Cases were on warfarin for a mean of 88 months, controls for a mean of 68 months. There was no association between SNPs (genotype and combinations) and age, gender, BMI, treatment durations, warfarin dose or numbers of concomitant drugs.

ABCG2 421C/A genotype was significantly more prevalent in unstable patients than in controls (36,4% vs 9,1%, $p<0.016$ resulting in a corresponding odds ratio of 5.7 (95% CI 1.4-22.8). No association was found between TTR and SNPs in both CYP that VKORC1 genes (**Table 3**).

Using a logistic additive model, we identified 4 SNPs combinations for SNPs in ABCB1 and ABCG2 genes (named from 1 to 4), 4 SNPs combinations for SNPs in CYP3A5 and CYP2C9 (named from 5 to 8), and 4 for SNPs in VKORC1 (named from 9 to 12) with a frequency greater than 3% (**Table 4**). SNPs combinations 3 (TTA) was only present in unstable patients, resulting in an OR of 30,7 (95% CI 2.3-53.0) ($P<0.001$) for a low TTR.

No association was found between TTR and all other SNPs combinations (**Table 4**).

Table 2. Characteristics of the subjects included in the study. Values are means ± DS. M, male; F, female; BMI, body max index; a.f., atrial fibrillations; hvp., heart valve prosthesis.

	Total	**TTR>85%**	**TTR<55%**
number	66	33	33
age (years)	76.8±6.6	76.6±6.5	75.6±6.7
gender M/F	34/32	17/16	17/16
BMI	26.4±4.1	26.3±4.0	26.5±4.3
Disease (af/hvp)	54/12	27/6	27/6
Warfarin dose (mg/sett)	25.2±11.2	24.1±10.4	26.3±13
TTR (%)	70.7±23.9	47.54±6.1	93.66±5.3
Concomitant drugs	4.8±2.5	4.48±2.4	5.15±2.6
Mean of weighted index of comorbidity	0.87	0.85	0.91

Table 3. SNPs Frequency and correlations with warfarin stability.

Gene	SNP	Genotype	TTR >85%	TTR <55%	OR (95% CI)	P-value
ABCB1	rs1128503	C/C	9 (27.3%)	3 (9.1%)	3.1 (0.9-11.7)	0.130
		C/T	20 (60.6%)	20 (60.6%)		
		T/T	4 (12.1%)	10 (30.3%)		
ABCB1	rs1045642	C/C	8 (24.2%)	3 (9.1%)	3.1 (0.9-11.7)	0.130
		C/T	21 (63.6%)	20 (60.6%)		
		T/T	4 (12.1%)	10 (30.3%)		
ABCG2	rs2231142	C/C	30 (90.9%)	21 (63.6%)	5.7 (1.4-22.8)	**<0.016**
		C/A	3 (9.1%)	12 (36.4%)		
		C/C	0 (0%)	0 (0%)		
CYP2C9	rs1799853	C/C	19 (57.6%)	26 (78.8%)	0.4 (0.1-1.1)	0.111
		C/T	14 (42.4%)	7 (21.2%)		
		T/T	0 (0%)	0 (0%)		
CTP2C9	rs1057910	A/A	26 (78.8%)	28 (84.8%)	0.7 (0.2-2.3)	0.751
		A/C	7 (21.2%)	5 (15.2%)		
		C/C	0 (0%)	0 (0%)		
CYP3A5	rs776746	G/G	28 (84.8%)	27 (81.8%)	0.3 (0.01-8.8)	1.000
		A/G	4 (12.1%)	6 (18.2%)		
		A/A	1 (3.0%)	0 (0.0%)		
VKORC1	rs7294	G/G	11 (33.3%)	11 (33.3%)	0.4 (0.06-2.0)	0.426
		G/A	17 (51.5%)	20 (60.6%)		
		A/A	5 (15.2%)	2 (6.1%)		
VKORC1	rs9934438	C/C	6 (18.2%)	4 (12.1%)	1.8 (0.5-6.2)	0.532
		C/T	22 (66.7%)	21 (63.6%)		
		T/T	5 (15.2%)	8 (24.2%)		

Table 4. SNPs combinations frequencies for gene coding for warfarin transport (panel A), metabolism (panel B) and target (Panel C) and correlations with range TTR.

Panel A

	ABCB1 C1236T	**ABCB1 C3435T**	**ABCG2 C421A**	**Total**	**TTR >85%**	**TTR <55%**	**O.R. (95% CI)**	**P**
1	C	C	C	0,43	0,50	0,34	1.00	-
2	T	T	C	0,42	0,41	0,45	2.1 (0.7-6.8)	0.200
3	T	T	A	0,08	0,00	0,13	30.7 (2.3-53.0)	**<0.001**
4	C	T	C	0,03	0,03	0,04	2.0 (0.2-26.3)	0.521
rare	*	*	*	0,05	0,06	0,04	2.6 (0.2-27.0)	0.492

Panel B

	CYP2C9 C430T	**CYP2C9 A1075C**	**CYP3A5 A6986G**	**Total**	**TTR >85%**	**TTR <55%**	**O.R. (95% CI)**	**P**
1	C	A	G	0,65	0,61	0,74	1.00	-
2	T	A	G	0,16	0,19	0,10	0.4 (0.12-1.3)	0.133
3	C	A	A	0,09	0,09	0,08	0.9 (0.27-3.1)	0.882
4	C	C	G	0,09	0,09	0,07	0.7 (0.16-2.8)	0.591
rare	*	*	*	0,01	0,02	0,00	1.0 (0.1-3.6)	0.698

Panel C

	VKORC1 G3673A	**VKORC1 C7484T**	**Total**	**TTR >85%**	**TTR <55%**	**O.R. (95% CI)**	**P**
1	G	T	0,41	0,30	0,42	1.00	-
2	A	C	0,27	0,22	0,30	0.6 (0.2-1.8)	0.382
3	G	C	0,21	0,29	0,20	0.5 (0.2-1.0)	0.066
4	A	T	0,12	0,15	0,09	0.4 (0.2-1.1)	0.315

Discussion.

The main finding of our study is the association between ABCG2 C421A SNP and warfarin stability. To our best knowledge this is the first report of a significant association between warfarin stability and individual genotype.

Several studies reported that genetic variants may contribute to the variability in warfarin response, and algorithms containing CYP2C9 and VKORC1 polymorphisms have been developed for estimating warfarin loading doses[5,22]. Conversely, only few data are available on the association between gene polymorphisms and warfarin stability, and recent studies, concluding that no association existed between the CYP2C9 and VKORC1 genotypes and therapy stability[5,23]. These results are consistent with our

study, which confirms that CYP2C9 and VKORC1 variants are not associated with warfarin stability (Table 3). However, we also explored the role of common and well characterized SNPs in ABCB1 and ABCG2 genes[24], which are relatively less studied genes implicated in warfarin pharmacokinetics.

The most intriguing finding of our study was the significantly higher frequency of ABCG2 421A variant allele in subjects with low TTR as compared to stable patients (Table 3). The ABCG2 gene encodes an efflux pump, and the presence of the SNP C421A reduces the activity of this transporter. Impairment of warfarin transport could affect the warfarin stability in different ways. One hypothesis is that the worsening of the carrier activity increases the risk of interaction with other substrates with affinity for the pump. On the other hand *in vitro* BCRP is able to carry vitamin K[15], thus a second hypothesis is that reduced efficiency of the pump leads to changes in the vitamin K disposition, in turn affecting warfarin TTR[2,4].

It is widely accepted that complex gene-gene interactions exist and that the role of genetic differences in drug response are usually multifactorial, involving the contribution of multiple genes and gene variants. We therefore decided to explore the potential effects of gene-to-gene interactions. Using a logistic additive model, we identified SNPs combinations for studied SNPs (Table 2). We identified a significant association between low TTR and the TTA SNPs combinations formed by SNPs in genes coding for warfarin transport (namely ABCB1 and ABCG2). On the contrary, the SNPs combinations formed by SNPs in CYP2C9 and VKORC1 were not associated with TTR. The TTA SNPs combinations contains ABCB1 3435T, 1236T and ABCG2 421A alleles, all associated with impairment of warfarin transport activity thus supporting our hypothesis that reduction in warfarin efflux result in reduced TTR due to increases potential for interaction with other transporter substrates and/or alterations in vitamin K intake.

The main limitation of our study is the small number of patients that does not allow to assess the prevalence of SNPs in anticoagulated patients. Moreover, we cannot exclude that additional genetic mutations not assessed in our study could also interfere with warfarin stability. The small sample size might also impact on the results of the statistical analysis, possibly leading to effects just due to chance. Nonetheless, in our opinion this is unlikely since the association between the ABCG2 gene SNP C421A is biologically plausible (as discussed above) and it has been confirmed by different approaches in data analysis, namely conventional comparison of genotypes (Table 3, $P = 0.016$) and SNP combination frequencies (Table 4, $P<0.001$); in particular, the level of significance of the latter result is well beyond the conventional 0.05 threshold for significance. Another limitation is the possible confounders in our study, indeed it is well-known that several patient characteristics influence warfarin stability[3,25]. To minimize this risk we matched cases and controls, our results shown that the distribution of major risk factors for warfarin instability was well balanced between the two groups thus reducing the risk confounders.

In conclusion, the present study suggests that patient's carriers of SNP in warfarin transporter protein alone or in combination may be at higher risk of unstable anticoagulant therapy. The results are only hypothesis-generating, and the association between the ABCG2 gene SNP C421A and stability of the response to warfarin must be confirmed into an appropriate prospective longitudinal study. Nonetheless, they may have a relevant clinical impact since patients with low TTR are at increased risk of developing thrombotic or hemorrhagic complications[2] and it could be important if individuals with a genetic predisposition to an unstable response to warfarin could be identified before starting therapy. These

patients could be either monitored more closely or could beneficiate from alternative anticoagulant therapies, such as the oral direct inhibitors[2,26].

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CONCLUSIONI

In questo progetto di dottorato si è cercato di studiare alcune condizioni inusuali che possono essere associate alla sviluppo di eventi cardiovascolari.

Valutando pazienti affetti da trait talassemico abbiamo potuto concludere che questa condizione risulta essere protettiva, a differenza di quello che è stato segnalato per le forme di talassemia intermedia.

Occupandoci di tireotossicosi abbiamo evidenziato come sia presente uno stato di ipercoagulabilità mediato dall'azione degli ormoni tiroidei, allo stato attuale non è ancora possibile definire quanto clinicamente questa condizioni si associ allo sviluppo di eventi trombotici; sarebbero quindi necessari studi mirati anche per definire l'eventuale necessità di terapia profilattica antitrombotica da attuare in corso di ipertiroidismo.

Ci siamo quindi occupati della gravidanza, una condizioni associata ad un aumentato rischio trombotico, in cui non era ben chiaro quale fosse la terapia anticoagulante migliore. Siamo riusciti a radunare una casistica ragguardevole, dimostrando che l'eparina a basso peso molecolare è efficace, è associata ad un rischio trombotico ed emorragico sovrapponibile a quello che si verifica in pazienti non gravide e soprattutto non è correlata a rischi evidenti per il feto.

Nell'ultima parte della trattazione ci siamo soffermati sulla ricerca di alcuni fattori che potessero giustificare la presenza di una grave instabilità della terapia anticoagulante con AVK in particolari soggetti. Abbiamo individuato come la presenza di alcuni SNPs sia un indice predittivo di tale condizione. Questo fa sì che si possano individuare persone che, a causa del loro patrimonio genetico, non possano beneficiare di una terapia anticoagulante condotta con AVK, ma al contrario dovrebbero essere sottoposti ad una altro tipo di terapia come ad esempio ad una terapia con i nuovi anticoagulanti orali (inibitori diretti del fattore X e II della coagulazione). Questi nuovi farmaci

presentando un profilo farmacocinetico e farmacodinamico più regolare potrebbero garantire una maggiore stabilità della coagulazione, tuttavia sarebbero utili studi sugli effetti dei SNPs anche su questi.