Leukemia (2016) **30,** 2032–2038

www.nature.com/leu

ORIGINAL ARTICLE

High rate of recurrent venous thromboembolism in patients with myeloproliferative neoplasms and effect of prophylaxis with vitamin K antagonists

V De Stefano[1,25], M Ruggeri[2], F Cervantes[3], A Alvarez-Larrán[4], A Iurlo[5], ML Randi[6], E Elli[7], MC Finazzi[8], G Finazzi[8], E Zetterberg[9], N Vianelli[10], G Gaidano[11], E Rossi[1], S Betti[1], I Nichele[2], D Cattaneo[5], M Palova[12], MH Ellis[13], R Cacciola[14], A Tieghi[15], JC Hernandez-Boluda[16], E Pungolino[17], G Specchia[18], D Rapezzi[19], A Forcina[20], C Musolino[21], A Carobbio[22], M Griesshammer[23], E Sant'Antonio[24], AM Vannucchi[24] and T Barbui[22,25]

The optimal duration of treatment with vitamin K antagonists (VKA) after venous thromboembolism (VTE) in patients with Philadelphia-negative myeloproliferative neoplasms (MPNs) is uncertain. To tackle this issue, we retrospectively studied 206 patients with MPN-related VTE (deep venous thrombosis of the legs and/or pulmonary embolism). After this index event, we recorded over 695 pt-years 45 recurrences, venous in 36 cases, with an incidence rate (IR) of 6.5 per 100 pt-years (95% confidence interval (CI): 4.9–8.6). One hundred fifty-five patients received VKA; the IR of recurrent thrombosis per 100 pt-years was 4.7 (95% CI: 2.8–7.3) on VKA and 8.9 (95% CI: 5.7–13.2) off VKA ($P = 0.03$). In patients receiving VKA, the IR of recurrent thrombosis per 100 pt-years was 5.3 (95% CI: 3.2–8.4) among 108 patients on long-term VKA and 12.8 (95% CI: 7.3–20.7) after discontinuation among the 47 who ceased treatment ($P = 0.008$), with a doubled risk of recurrence after stopping VKA (hazard ratio: 2.21, 95% CI: 1.19–5.30). The IR of major bleeding per 100 pt-years was 2.4 (95%: CI: 1.1–4.5) on VKA and 0.7 (95% CI: 0.08–2.5) off VKA ($P = 0.08$). In conclusion, in MPN patients with VTE recurrent thrombosis is significantly reduced by VKA and caution should be adopted in discontinuation; however, the incidence of recurrence on treatment remains high, calling for clinical trials aimed to improve prophylaxis in this setting.

Leukemia (2016) **30,** 2032–2038; doi:10.1038/leu.2016.85

INTRODUCTION

Thrombosis is the main burden of Philadelphia-negative myeloproliferative neoplasms (MPNs), particularly of polycythemia vera (PV) and essential thrombocythemia (ET); additionally, patients are prone to the long-term risk of fibrotic or leukemic transformation.[1] Thromboses involve venous vessels in about one-third of cases in both disorders. In prospective trials, the overall rate of major venous thromboembolism (VTE) was 0.7–1.3 per 100 pt-years in PV patients[2–4] and 0.5–1.2 per 100 pt-years in ET patients.[2–6] This incidence is higher than that of the general population of Western countries, where the annual incidence of major VTE is between 0.1 and 0.2 per 100 pt-years.[7]

In MPN patients, the rate of recurrent thrombosis after either arterial or venous thrombosis is 6–7.6 per 100 pt-years.[8,9] Patients with MPN and previous thrombosis are labeled as a high-risk group; cytoreduction is warranted to reduce the probability of recurrence in this group.[10–12] The modalities of long-term antithrombotic treatment for secondary prophylaxis in these patients have been scarcely explored. In non-MPN patients, anticoagulation with VKA or with direct oral anticoagulants (DOACs) is recommended, with indefinite anticoagulation suggested for patients with unprovoked first VTE or for those patients with permanent risk factors, such as cancer.[13] This recommendation is supported by the current guidelines and by most experts in the case of MPN patients with splanchnic venous thrombosis or recurrent events, but it appears weaker and more uncertain in the case of MPN patients with a previous thrombosis at the usual sites.[10–12] The lack of firm evidence in this setting is mirrored by a recent survey that showed a marked heterogeneity in the opinion of hematologists regarding the duration of VKA treatment after VTE.[14] Finally, in MPN patients, there is concern for a higher potential for bleeding,[1] making the decision regarding the mode and duration of antithrombotic treatment even more challenging.

To tackle such perplexities, we investigated the clinical course after a previous deep venous thrombosis (DVT) of the legs and/or a pulmonary embolism (PE) in a multicenter retrospective cohort of MPN patients to gain information about the rate of recurrent thrombosis in such a population and about the efficacy and safety of VKA in this setting.

[1]Institute of Hematology, Catholic University, Roma, Italy; [2]Ospedale San Bortolo, Vicenza, Italy; [3]Hospital Clínic, IDIBAPS, Barcelona, Spain; [4]Hematology Department, Hospital del Mar, Barcelona, Spain; [5]Oncohematology Division, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milano, Italy; [6]A.O. Universitaria, Padova, Italy; [7]Hematology Division, A.O. San Gerardo, Monza, Italy; [8]Hematology Division, A.O. Papa Giovanni XXIII, Bergamo, Italy; [9]Department of Hematology and Vascular Disorders, Skane University Hospital, Lund, Sweden; [10]Università di Bologna, Bologna, Italy; [11]Department of Translational Medicine, Università del Piemonte Orientale, Novara, Italy; [12]University Hospital of Olomouc, Olomouc, Czech Republic; [13]Meir Medical Center, Hematology Institute, Kefar Sava, Israel; [14]A.O. Universitaria, Catania, Italy; [15]Arcispedale Santa Maria Nuova - IRCCS, Reggio Emilia, Italy; [16]Hospital Clinico, Valencia, Spain; [17]A.O. Ospedale Niguarda Ca' Granda, Milano, Italy; [18]A.O. Universitaria, Bari, Italy; [19]A.O. Santa Croce e Carle, Cuneo, Italy; [20]IRCCS Ospedale San Raffaele, Milano, Italy; [21]A.O. Universitaria, Messina, Italy; [22]FROM Research Foundation, A.O. Papa Giovanni XXIII, Bergamo, Italy; [23]J. W. Klinikum, Minden, Germany and [24]Center for Research and Innovation of Myeloproliferative Neoplasms, A.O.U. Careggi, University of Florence, Firenze, Italy. Correspondence: Professor T Barbui, FROM Research Foundation, Ospedale Papa Giovanni XXIII, Piazza OMS, 1, Bergamo 24127, Italy.
E-mail: tbarbui@asst-pg23.it
[25]These authors contributed equally to this work.
Received 28 December 2015; revised 2 March 2016; accepted 30 March 2016; accepted article preview online 26 April 2016; advance online publication, 13 May 2016

SUBJECTS AND METHODS

Study patients

A retrospective study was conducted across 23 centers within the European Leukemia Network on patients with a diagnosis of MPN according to the WHO 2008 criteria,[15] after the approval of the ethics committees (primary approval by the central ethics committees of the coordinating center was obtained on 2 October 2014).

The participating centers were asked to select from their consecutive patients with MPN those who had suffered from a VTE objectively documented from January 2005, including DVT of the limbs, PE, thrombosis of the cerebral and splanchnic veins (hepatic, portal, mesenteric and splenic veins) and thrombosis of the retinal vein, and who had received a course of VKA or DOACs. A diagnosis of VTE was accepted only if it was confirmed by objective methods according to current clinical practice, as previously reported,[8] and was defined as a positive result using techniques such as angiography, ultrasonography, computerized tomography or nuclear magnetic resonance. PE was defined as a positive pulmonary angiogram, a ventilation–perfusion scan or computerized tomography scan indicating a high probability of PE. Retinal vein thrombosis was defined by fluoroangiography or fundus examination. Patients with superficial vein thrombosis and those who received VKA or DOACs for atrial fibrillation or any for medical reason other than VTE were excluded from the study.

Data were collected in an electronic system. The total number of medical files was reported by each center by data input into an electronic database developed to record all study data after de-identification of the patients with an alphanumeric code to protect personal privacy.

For each patient, the following information was recorded: demographic data, WHO diagnosis, location of thrombosis, method of objective diagnosis, presence of microvascular disturbances or constitutional symptoms, mutational profile, results of the laboratory investigation for thrombophilia, full blood count at diagnosis and at thrombosis and presence of constitutional risk factors (that is, history of previous thrombosis before the index event, smoking habit, hypertension and dyslipidemia, diabetes). Moreover, the presence of circumstantial risk factors at the time of any episode of VTE, such as surgery, pregnancy, puerperium (until 6 weeks from delivery), oral contraceptive intake, hormone replacement therapy, trauma, leg cast and prolonged bed immobilization (>10 days), and long travel (>8 h), was also recorded; in the absence of the previously mentioned risk factors, VTE was considered unprovoked. Finally, data regarding cytoreductive or antithrombotic treatment after VTE, the duration of the treatment and the reasons for discontinuation of the treatment were recorded.

Outcomes

The aim of the present study was to determine the rate of recurrent thrombosis in the patients recruited in the general database who had a DVT of the legs, with or without PE, as an index event.

Venous or arterial thrombotic events that occurred after the index event were recorded only if objectively documented. Objectively established contralateral DVT of the legs and PE were computed as recurrences. The following manifestations objectively proven were also defined as recurrences: DVT of the arm, occlusion of cerebral or abdominal veins, thrombosis of the great saphenous vein of the leg not involved in the first manifestation and objectively diagnosed with ultrasonography. Finally, arterial recurrences included ischemic stroke, transient ischemic attack, acute myocardial infarction, unstable angina pectoris and peripheral arterial thrombosis.

Major bleeding was defined according to the criteria of the International Society on Thrombosis and Haemostasis[16] when it was fatal and/or was symptomatic in a critical area or organ, such as intracranial bleeding, intraspinal bleeding, intraocular bleeding, retroperitoneal bleeding, intrarticular bleeding, pericardial bleeding, intramuscular bleeding with compartment syndrome and/or bleeding that led to a reduction of 2 g/dl or more in the hemoglobin concentration and/or necessitated transfusion of two or more blood units.

Statistical methods

For continuous variables, the median and the 5th–95th percentiles or range are provided. The annual incidence of recurrent thrombosis was calculated by dividing the number of events by the total number of patient-years. Differences in the proportions were estimated using the Fisher's exact test (statistical significance threshold set at $P < 0.05$). The estimate of the association of continuous variables at the time of the index event (that is, age and hematological parameters) with the probability of future thrombosis was performed using a receiver operating characteristic analysis. The probability of recurrence as a function of time was estimated using the Kaplan–Meier method by analyzing the interval between the initial thrombosis and a recurrent thrombotic event (uncensored observations), or the duration of time until death, or the time elapsed until the patient's final visit to the center (censored observations). The probability of recurrence was compared between the groups using the log-rank test (statistical significance threshold set at $P < 0.05$), and the relative risk of recurrence was estimated as a hazard ratio (HR) with a 95% confidence interval using a Cox proportional hazards regression model.

RESULTS

Clinical and laboratory features of the patient cohort

Overall, 436 patients with VTE were recruited, including 206 patients with VTE at usual sites, 181 patients with splanchnic vein thrombosis and 35 patients with cerebral vein thrombosis (Supplementary Table 1). For the aims of the present investigation, we selected the 206 patients who had suffered from DVT of the legs and/or PE.

The clinical and laboratory features of the study cohort are reported in Table 1. The majority of the patients (81.6%) were over 60 years of age at the time of the index event. PV or ET was diagnosed in 96 and 87 patients, respectively, accounting for 88.8% of the cases; myelofibrosis was diagnosed in the remaining patients. Only a minority of patients had a complete mutational profile; however, a large majority of them carried the *JAK2V617F* mutation, rendering unlikely the presence of other mutations that are usually mutually exclusive. Microvascular disturbances, defined as erythromelalgia, transient ocular attacks, pulsatile headache, dizziness and tinnitus, were present in 21.3% of the patients, almost exclusively (40 of 44) with diagnosis of PV or ET; constitutional symptoms defined as pruritus, fatigue, night sweats, fever, weight loss and pain in the limbs were present in 23.5% of the patients, the majority (59%) of whom had PV.

Thrombophilia was present in 44.8% of the 39 tested patients, in agreement with current knowledge: 3 with a deficiency of protein C or protein S, 12 with factor V Leiden and/or prothrombin G20210A, 1 with increased levels of factor VIII, 11 with increased levels of homocysteine and 11 with antiphospholipids (lupus anticoagulant and/or anticardiolipin antibodies and/or anti-beta-2-glycoprotein I antibodies).

A provoking circumstance of the VTE index event was recorded in 54 patients: comorbidity in 18 (cancer in 12, infection in 2, nephrotic syndrome in 2, liver disease in 1 and autoimmune disease in 1), prolonged immobilization in 11, surgery in 10, trauma with or without fracture in 9, oral contraception in 2, pregnancy in 2, hormone replacement therapy in 1 and a long journey in 1.

Antithrombotic treatments after the VTE index event

Long-term treatment after the VTE index event is reported in Table 2. All patients received a course of low molecular weight heparin as acute treatment. The majority of the patients received VKA (155/206, 75.7%, associated with aspirin in 9.2% of cases), while very few patients received DOACs (3.3%) as long-term treatment after the VTE index event. Among the 155 patients receiving VKA, ongoing treatment was continued in 108 patients (69.7%) for a median follow-up time of 2.5 years (range 1 month–9.7 years), and treatment was discontinued in 47 patients (30.3%) after a median time of 2.6 years (range 1 month–8.1 years). The reasons for discontinuation of treatment are reported in Supplementary Table 2. The majority of the patients discontinued treatment based on a medical decision (33 of 47, 70.2%), having ended the period of antithrombotic prophylaxis judged appropriate by the care physician. The duration of VKA treatment exceeded 6 months in almost all of the cases (32/33), and the duration of VKA treatment exceeded 1 year in one-third of the cases (10/33). Major bleeding was the cause of discontinuation in two cases.

Table 1. Clinical and laboratory features of the study cohort.

Male/Female, N (%)	*92/114 (44.7/55.3)*
Age at VTE index event, years—median (range)	72 (28–90)
< 40 years, *N* (%)	9 (4.4)
40–60 years, *N* (%)	29 (14.0)
⩾ 60 years, *N* (%)	168 (81.6)
Diagnosis, N (%)	
Polycythemia vera	96 (46.6)
Essential thrombocythemia	87 (42.2)
Myelofibrosis	23 (11.2)
Years from diagnosis to index thrombosis, median (range)	2.28 (0.0–28.4)
Diagnosis at the time of index thrombosis, *n* (%)	48 (23)
Hb g/dl at the VTE index event, median (range)	15.0 (8.3–23.1)
Hematocrit % at the VTE index event, median (range)	0.45 (0.25–0.70)
WBC count $\times 10^9$/L at the VTE index event, median (range)	8.65 (4.69–35.79)
Platelet count $\times 10^9$/L at the VTE index event, median (range)	597 (98-1645)
JAK2V617F mutation, N/N tested (%)	170/198 (85.8)
CALR mutations, *N/N* tested	12/34 (35.3)
MPL mutations, *N/N* tested	2/37 (5.4)
Triple negative, *N/N* tested	4/25 (16.0)
Exon 12 mutations, *N/N* tested	1/17 (5.9)
Microvascular disturbances, *N* (%)	44 (21.3)
Constitutional symptoms, *N* (%)	48 (23.5)
Type of index thrombosis, N (%)	
Deep venous thrombosis of the legs	119 (57.8)
Deep venous thrombosis of the legs+pulmonary embolism	42 (20.4)
Pulmonary embolism	45 (21.8)
Diagnostic method, N (%)	
Ultrasound	114 (55.3)
Computerized tomography	58 (28.2)
Scintigraphy	4 (1.09)
Angiography	12 (5.8)
Not reported	18 (8.7)
Unprovoked thrombosis, *N* (%)	152 (73.6)
Risk factors for VTE index	
History of thrombosis, *N* (%)	74 (35.9)
Presence of at least one vascular risk factor, *N* (%)	117 (56.8)
Smoking habit	30 (14.5)
Hypertension	96 (46.6)
Dyslipidemia	27 (13.1)
Diabetes	20 (9.7)
Presence of thrombophilia, *N/N* tested (%)	39/87 (44.8)
Inherited thrombophilia,[a] *N/N* tested (%)	16/87 (18.3)

[a]Deficiency of protein C, protein S, factor V Leiden and/or prothrombin G20210A. Other causes of thrombophilia include increased levels of factor VIII or homocysteine and the presence of antiphospholipids.

Table 2. Long-term antithrombotic treatments after the index thrombosis according to cytoreductive therapy.

	Total (%)	*Cytoreduction*[a]	
		Yes	*No*
VKA	136 (66.5)	125 (69.1)	11 (44.0)
VKA+ASA	19 (9.2)	17 (9.4)	2 (8.0)
ASA	11 (5.3)	9 (5.0)	2 (8.0)
Heparin	19 (9.2)	14 (7.7)	5 (20.0)
DOACs	7 (3.3)	7 (3.9)	0 (0.0)
No antithrombotic treatment	14 (6.5)	9 (5.0)	5 (20.0)
Total (%)	206 (100)	181	25

Abbreviations: ASA, aspirin; DOACs, direct oral anticoagulants; VKAs, vitamin K antagonists. [a]Hydroxyurea in 151/181 cases (83.4%).

Table 3. Overall incidence of recurrent thrombosis and bleeding after the index thrombosis.

	Events, n (%)	*Incidence rate, % pt-years (95% CI)*
Thrombotic events	45 (21.8)	6.5 (4.7–8.7)
Venous thrombosis	36 (17.5)	5.2 (3.6–7.2)
DVT +/− pulmonary embolism	30 (14.5)	
Splanchnic vein thrombosis	3 (1.5)	
Superficial vein thrombosis	3 (1.5)	
Arterial thrombosis	9 (4.4)	1.3 (0.6–2.4)
Major bleeding	12 (5.8)	1.7 (0.9–3.0)

Abbreviations: CI, confidence interval; DVT, deep venous thrombosis.

Incidence of recurrent thrombosis

The overall observation time recorded after the VTE index event was 695 years (median 3, range 1 month–9.7 years). Forty-five patients had a recurrent thrombosis after the index event (21.8%). In 36 cases (80%), the recurrence occurred in the venous district; and, in 9 cases, the recurrence involved arterial vessels (ischemic stroke, $n = 4$; acute myocardial infarction, $n = 2$; unstable angina, $n = 1$; peripheral artery thrombosis, $n = 1$; and transient ischemic attack, $n = 1$).

Ischemic stroke was the cause of death in one patient. The incidence rate of either recurrent arterial and venous thrombosis or recurrent VTE was 6.5 per 100 pt-years (95% CI 4.9–8.6) and 5.2 per 100 pt-years (95% CI 3.8–7.1), respectively (Table 3). The incidence rate of recurrent thrombosis was 5.3 per 100 pt-years (95% CI 3.2–8.3), 6.8 per 100 pt-years (95% CI 4.1–10.5) and 13.3 per 100 pt-years (95% CI 4.9–29.0) in PV, ET and myelofibrosis, respectively. The cumulative probability of recurrent thrombosis at 1 year, 3 years and 5 years was 10.1% (95% CI 5.8–14.4), 18.0% (95% CI 12.0–24.2) and 27.4% (95% CI 19.5–35.3), respectively. The cumulative probability of recurrent VTE at 1 year, 3 years and 5 years was 7.9% (95% CI 3.9–11.9), 13.9% (95% CI 8.4–19.4) and 22.4% (95% CI 14.8–30.0), respectively (Figure 1). The distribution of putative predictors of recurrent thrombosis was substantially similar among the patients who had a recurrence and those who did not. Patients with recurrences suffered from microvascular disturbances more frequently than those without recurrences (37.8 versus 17%, $P = 0.003$). No differences were found between the two groups regarding age, gender, MPN diagnosis, blood counts, frequency of constitutional symptoms, frequency of the JAK2*V617F* mutation and constitutional or circumstantial risk factors for thrombosis (Supplementary Table 3). Previous diagnosis of cancer at the time of the index thrombosis did not influence the rate of recurrent thrombosis, which was recorded only in 1 of the 12 patients who had cancer as provoking factor of the first thrombotic event. Stopping smoking is warranted by the current guidelines.[10] Accordingly, current smoking habit was present only in a minority of patients (30 of 206, 14.5%); recurrent thrombosis occurred in 9 of 30 current smokers (30%) and in 36 of 176 non-smokers (24.6%, $P = 0.24$). Recurrent thrombosis involved arterial vessels in three current smokers (10%) and in six non-smokers (3.4%) ($P = 0.12$). Finally, the receiver operating characteristic analysis did not identify any associations between recurrent thrombosis and continuous variables in the patients with VTE at the time of the index event: age (area under the curve (AUC) 0.546, $P = 0.32$), values of Hb (AUC 0.518, $P = 0.69$), hematocrit (AUC 0.531, $P = 0.49$), WBC (AUC 0.513, $P = 0.79$) and platelet count (AUC 0.510, $P = 0.83$).

Effect of VKA treatment on the incidence rate of recurrent thrombosis

The total observation time of the patients who received VKA after the index event without discontinuation was 352 pt-years, and the total observation time of the patients who never received VKA or DOACs after the index event was 154 pt-years. Among the patients who received VKA after the index event for a limited period of time, the total observation time was 52 pt-years on VKA and 125 pt-years after discontinuation (Table 4). Seven patients received DOACs for a total of 12 pt-years, which were not computed in the analysis; accordingly, one patient on DOACs had recurrent VTE, and the event was not computed. The overall incidence rate of recurrent thrombosis was 4.7 per 100 pt-years (95% CI 2.8–7.3) on VKA and 8.9 per 100 pt-years (95% CI 5.7–13.2) off VKA ($P=0.03$); the incidence rate of recurrent VTE was 3.7 per 100 pt-years (95% CI 2.0–6.1) on VKA and 7.1 per 100 pt-years (95% CI 4.3–11.0) off VKA ($P=0.04$) (Table 4). A sensitivity analysis confirmed that the incidence rates of recurrent thrombosis and of recurrent VTE per 100 pt-years were significantly lower among the patients who continued VKA than among those who discontinued VKA (Table 4). Patients who discontinued VKA had no recurrent thrombosis while on treatment but, after stopping VKA, the cumulative probability of recurrent thrombosis significantly increased, and the overall risk of novel events was significantly higher than in patients receiving long-term VKA (HR, 1.95, 95% CI 1.05–4.05) (Figure 2, upper panel). The analysis by treatment showed that the cumulative probability of recurrent thrombosis at 1 year, 3 years and 5 years on VKA after VTE or after discontinuation of VKA was 7.8% (95% CI 2.6–13.0), 14.7% (95% CI 7.2–22.2) and 21.1% (95% CI 11.0–31.2), respectively, in patients who continued VKA and was 20.4% (95% CI 8.4–32.4), 32.6% (95% CI 17.7–47.5) and 42.3% (95% CI 24.8–60.3), respectively, in patients who discontinued VKA, with double the risk of recurrent thrombosis after withdrawal of treatment (HR, 2.21, 95% CI 1.19–5.30) (Figure 2, lower panel).

The incidence rate of arterial thrombosis per 100 pt-years was double in patients off VKA compared with those on VKA (1.7, 95% CI 0.5–4.1, versus 0.9, 95% CI 0.2–2.4), but the difference was not significant ($P=0.34$) likely because of the small number of events.

Effect of cytoreductive treatment on the incidence rate of recurrent thrombosis

Cytoreduction (mostly hydroxyurea) was administered to 181 patients (87.8% of the cohort) and was associated with an antithrombotic treatment in almost all of the cases (Table 2). Therefore, no reliable analysis could be attempted to compare the efficacy for preventing recurrent thrombosis of prophylactic strategies based on cytoreduction, VKA alone or the association of both treatments. Three recurrent VTEs over 47 pt-years occurred in patients receiving VKA in the absence of cytoreduction, with no significant difference in the incidence rate of recurrent thrombosis per 100 pt-years observed in patients receiving both VKA and cytoreduction (6.3, 95% CI 1.3–18.6 and 4.4, 95% CI 2.5–7.2, respectively, $P=0.57$).

Overall, recurrent thrombosis occurred in 6 of the 25 patients not receiving cytoreduction after the index event; hematocrit $>45\%$ and/or WBC count $>10\times10^9$/L and/or a platelet count of $>400\times10^9$/L was recorded at the time of the recurrent thrombosis in four cases. Similar blood values indicating a poor control of cell proliferation were observed at the time of the recurrence in 27 of the remaining 39 patients (69.2%) who had recurrent thrombosis during cytoreduction.

Incidence of major bleeding

Ten major bleeding events (gastrointestinal, $n=3$; muscle, $n=3$; CNS, $n=1$; eye, $n=1$; epistaxis, $n=1$; and unspecified site, $n=1$) occurred in patients on VKA. Two major bleeding events (1 eye and 1 gastrointestinal) occurred in patients receiving VKA associated with aspirin. Two additional major bleeding events (1 gastrointestinal and 1 unspecified site) occurred in two patients off VKA who were receiving s.c. low molecular weight heparin and aspirin, respectively. One patient died after massive gastric bleeding during VKA treatment.

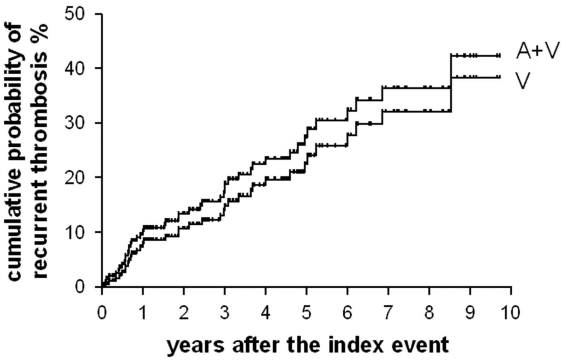

Figure 1. Cumulative probability of recurrent arterial and venous (A+V) or venous (V) thrombosis in the overall cohort, irrespective of the treatment after the index thrombosis.

Table 4. Influence of long-term VKA treatment and discontinuation on the rate of incident thrombosis and bleeding in VTE patients

	Vitamin K antagonists[a]		*P*	*Vitamin K antagonists*[b]		*P*
	Yes (N = *155*)	*No* (N = *44*)[c]		*Not discontinued* (N = *108*)	*Discontinued* (N = *47*)	
Pt-years	404	279		352	125[d]	
Thrombosis, N[c]	19	25	0.03	19	16	0.008
Incidence rate	4.7	8.9		5.3	12.8	
% pt-yrs (95% CI)	2.8–7.3	5.72–13.2		3.2–8.4	7.3–20.7	
Venous thrombosis, N	15	20	0.04	15	12	0.03
Incidence rate	3.7	7.1		4.2	9.6	
% pt-yrs (95% CI)	2.0–6.1	4.3–11.0		2.3–7.0	4.9–16.7	
Major bleeding, N	10[e]	2	0.08	6[e]	4	0.32
Incidence rate	2.4	0.7		1.7	3.2	
% pt-yrs (95% CI)	1.1–4.5	0.08–2.5		0.6–3.7	0.8–8.1	

Abbreviations: CI, confidence interval; DOACs, direct oral anticoagulants; VKA, vitamin K antagonist; VTE, venous thromboembolism. [a]Analysis by intention-to-treat. [b]Analysis by treatment. [c]The patients ($n=7$), the pt-years ($n=12$) and the recurrence ($n=1$) on DOACs are not computed in the total. [d]Pt-years after discontinuation of VKA. [e]Two patients bled during treatment with VKA+antiplatelet agents.

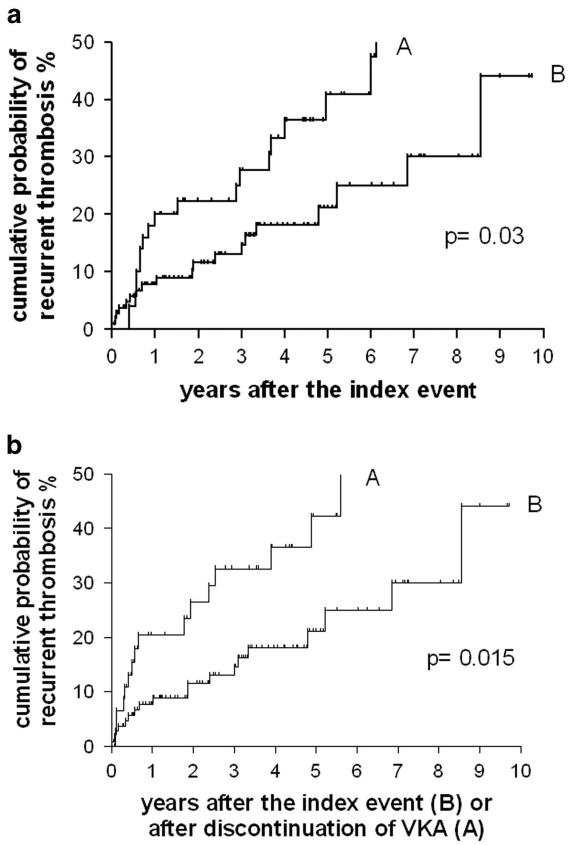

Figure 2. Cumulative probability of recurrent thrombosis in patients who discontinued VKA after index thrombosis (curve A) or did not (curve B). Analysis by intention-to-treat (**a**) and by treatment (**b**).

The incidence of major bleeding was 2.4 per 100 pt-years (95% CI 1.1–4.5) on VKA and 0.7 per 100 pt-years (95% CI 0.08–2.5) off VKA ($P = 0.08$) (Table 4). The cumulative probability of major bleeding during VKA treatment (intention-to-treat analysis) at 1 year, 3 years and 5 years was 2.8% (95% CI 0.9–5.6), 6.5% (95% CI 2.1–10.9) and 8.9% (95% CI 2.5–15.3), respectively. The incidence of major bleeding per 100 pt-years on VKA or on VKA combined with antiplatelet agents was 2.2 (95% CI 0.9–4.4) and 3.8 (95% CI 0.4–13.8) ($P = 0.50$), respectively.

Development of hematological transformation and non-MPN cancer

Hematological transformation occurred in 19 patients, transformation to myelofibrosis in 18 cases and transformation to acute myeloid leukemia in 1 case (Supplementary Table 4).

Six of them had recurrent thrombosis (arterial in 2 cases and venous in 4 cases). Recurrent thrombosis did not predict hematological transformation (odds ratio for transformation 1.7, 95% CI 0.6–4.9, $P = 0.37$).

Non-MPN cancer developed during the follow-up in 15 patients, and the sites of malignancy were lung ($n = 4$), stomach ($n = 2$), colon ($n = 2$), skin (basalioma, $n = 1$), duodenum ($n = 1$), bone marrow (myeloma, $n = 1$), parathyroid ($n = 1$), prostate ($n = 1$) and bladder ($n = 1$) (Supplementary Table 4). After discontinuation of VKA, recurrent thrombosis occurred in four patients with cancer of the lung, stomach, colon and prostate. Cancer could have been the triggering event provoking thrombosis, having been diagnosed in the patient with prostate cancer 3 months before thrombosis and in the remaining cases 1.5–2 years after thrombosis. However, in this cohort, recurrent thrombosis was not associated with a diagnosis of non-MPN cancer (odds ratio for cancer 1.0, 95% CI 0.3–3.2, $P = 1.0$).

DISCUSSION

The results of randomized trials in non-MPN patients indicate that treatment with conventional-intensity VKA (target INR 2.5) reduces recurrent VTE by approximately 90%.[13,17] After anticoagulation is stopped, recurrent VTE develops in at least 30% of patients who receive 3–6 months of anticoagulation after their first episode.[18] This rate did not change after extending anticoagulation for up to 24 months.[17] Extended anticoagulant therapy was associated with an approximately 2.6-fold increase in major bleeding.[13]

Therefore, assessing the optimal duration of anticoagulant therapy after an episode of VTE is still challenging. In fact, the recent introduction of DOACs significantly reduced the burden of bleeding by approximately 40% but did not eliminate it entirely.[19] In patients with an unprovoked first VTE or for those with permanent risk factors, such as cancer, oral anticoagulation for an indefinite duration is suggested.[13]

In the case of MPN patients with splanchnic venous thrombosis, the current guidelines and most experts recommend indefinite anticoagulation because of the life-threatening potential of recurrence in this anatomical site;[10–12] however, in MPN patients with DVT of the legs this recommendation remains uncertain because the data are derived from small groups of patients. Moreover, the high potential for bleeding in MPN patients[1] could discourage physicians from prescribing intensive anticoagulation in patients with VTE at usual sites. A previous study was conducted by the GIMEMA Italian centers in a retrospective cohort of 494 patients with PV ($n = 235$) or ET ($n = 259$) and with a previous thrombosis, 114 with VTE at usual sites (that is, DVT of the legs and/or PE). In this cohort, thrombosis recurred in 166 patients (33.6%), with an incidence of 7.6 per 100 pt-years and preferentially involving the same arterial or venous district affected in the first event. Multivariable analysis showed that cytoreduction non-significantly reduced the recurrence in patients with a first venous event (HR 0.66, 95% CI 0.38–1.13), but antiplatelet or VKA agents effectively prevented recurrence (HR 0.42, 95% CI 0.22–0.77 and HR 0.32, 95% CI 0.15–0.64, respectively). Both strategies showed acceptable safety profiles, with a similar major bleeding incidence with or without aspirin, while the association of antiplatelet agents plus VKA increased major bleedings compared with antiplatelet agents or VKA alone (2.8 per 100 pt-years versus 0.8 per 100 pt-years and 0.9 per 100 pt-years, respectively).[8]

Recently, the Spanish Group GEMFIN investigated a cohort of 150 MPN patients receiving VKA after a first thrombosis, 75 of them were diagnosed with DVT of the legs and/or PE. In this series, the overall rate of recurrent thrombosis was 6.0 per 100 pt-years (4.5 on VKA and 12.0 off VKA, $P < 0.0005$); in the subgroup of patients with DVT and/or PE, the incidence rate of recurrent VTE was 3.4 per 100 pt-years on VKA and 9.4 per 100 pt-years off VKA ($P = 0.016$). There was no significant difference in hemorrhagic events based on whether the patients were on VKA or not on VKA (1.8 versus 1.5 per 100 pt-years, respectively).[9]

In the present paper, we recruited a homogeneous group of 206 patients with DVT of the legs and/or PE, 155 of them receiving VKA after the index event. The incidence rate of recurrent thrombosis was 6.5 per 100 pt-years, with 80% of the recurrent events affecting the venous circulation, which was consistent with previous observations. We found no significant predictor of recurrent thrombosis among the constitutional or circumstantial risk factors associated with the patients or with the first event. This could be explained by the fact that the majority of the patients (81.6%) were more than 60 years old, and older age could be such a strong risk factor that it obscures the role of other predictors. The influence of the age stratification on the putative risk factors for recurrent thrombosis has been already reported in

a cohort of MPN patients with thrombosis where leukocytosis at the time of the first event and thrombophilia resulted significantly associated with the risk of recurrence only in patients aged < 60 years.[8] The role of microvascular disturbances as clinical features associated with the risk of recurrent thrombosis is biologically uncertain and will require further confirmation in future studies.

VKA treatment was confirmed to be highly effective in preventing either overall recurrent thrombosis or recurrent VTE, confirming and extending, in a large patient cohort, the results of previous investigations.[8,9] The overall incidence rate of recurrent thrombosis was 4.7 per 100 pt-years on VKA and 8.9 per 100 pt-years off VKA ($P = 0.03$); consistently, the incidence rate of recurrent VTE was 3.7 per 100 pt-years on VKA and 7.1 per 100 pt-years off VKA ($P = 0.04$). A sensitivity analysis by treatment showed that the incidence rate of recurrent VTE was 4.2 per 100 pt-years among the patients who continued VKA and 9.6 per 100 pt-years among patients after discontinuation of VKA ($P = 0.03$). Additionally, discontinuation was associated with a 2.2-fold increased risk of novel thrombotic events over time.

In spite of their high-risk score due to the history of thrombosis, a small group of patients did not receive cytoreduction according to the decision of their care physicians. However, the number of patients ($n = 25$), the total pt-years ($n = 101$) and the number of recurrent thrombosis ($n = 6$) recorded in this group did never exceed 15% of the values of the total cohort, so that the overall results are not significantly influenced (data not shown).

In this cohort of patients with VTE, recurrent thrombosis involved arterial vessels in 20% of the cases. This is consistent with the well-established increased risk of subsequent symptomatic arterial cardiovascular events observed in non-MPN patients, strongly suggesting that arterial and venous thrombosis may share common mechanisms or risk factors and may have a common origin in abnormalities of various blood constituents.[20]

We were unable to demonstrate a significant effect of VKA in preventing arterial thromboses after a VTE event, likely because of a lack of sample power. Further studies are needed to investigate this specific issue as well as the possible efficacy of adding aspirin to VKA to prevent arterial events in this setting.

The incidence of major bleeding was 2.4 per 100 pt-years on VKA and 0.7 per 100 pt-years off VKA. The bleeding risk was tendentially increased in patients receiving both VKA and antiplatelet agents in respect to those receiving VKA alone, as previously reported.[8] In patients with MPN, the overall incidence of major hemorrhages (both intracranial and gastrointestinal) ranged between 0.3 and 0.8 per 100 pt-years.[1] The incidence of major gastrointestinal bleeding has been reported to be between 0 and 0.3 per 100 pt-years in the absence of aspirin treatment. However, in the cohorts for which aspirin was administered to more than 70% of the patients, the incidence of major gastrointestinal bleeding was between 0.3 and 1.2 per 100 pt-years.[1] The 2.4 per 100 pt-years rate of major bleeding during VKA treatment is slightly higher than the probabilities reported by recent trials among non-MPN patients receiving VKA treatment as a control arm with respect to treatment with DOACs, which were between 1.2 and 2.2 per 100 pt-years.[21–24]

Interestingly, an indirect comparison of the probabilities or recurrent thromboses reported in non-MPN patients recruited in the previously mentioned trials and receiving VKA suggests a higher thrombotic potential in MPN patients than in non-MPN patients. In fact, in the present study, the cumulative probability of recurrent thrombosis at 1 year of VKA treatment is 7.8%, definitely higher than the probabilities of recurrent thrombosis on VKA reported in the recent trials, which were between 1.8 and 3.5%.[21–24] On the other hand, the cumulative probability of recurrent thrombosis after discontinuation of VKA observed in our cohort was 42% at 5 years from the withdrawal of treatment, which was higher than the 29.1% rate observed at 5 years in the non-MPN patients.[18,25]

The retrospective nature of our study recruiting patients from tertiary referral centers may have led to selection bias. However, the overall distribution of the cases with DVT of the legs and/or PE mirrors the 30–40% rate of PE observed in non-MPN patients with VTE in the population-based studies,[26,27] in the prospective study cohorts[18] and in the randomized clinical trials.[19,23,24] Moreover, putative risk factors for recurrent VTE such as history of thrombosis, unprovoked first thrombosis, male sex and thrombophilia were equally distributed among the patients with recurrences and those without, so that it is unlikely that any bias had an unbalanced distribution. Finally, our findings are consistent with previous studies on this issue.[8,9] Therefore, generalization of our results to the overall population of the MPN patients can be considered.

In conclusion, VKAs are effective in reducing the burden of recurrent thrombosis in MPN patients with a previous VTE, and indefinite anticoagulation could be an appropriate option. The importance of cytoreduction was highlighted by the observation that two-thirds of recurrent thromboses occurred in patients with hypercythemia in patients not receiving cytoreduction as well as in those who had not reached hematological response to cytoreductive treatment. However, the incidence of recurrence remains high despite antithrombotic treatment on a background of cytoreduction, suggesting the need for clinical trials aimed at improving prophylaxis in this setting. Furthermore, the relatively higher hemorrhagic risk may be possibly ameliorated with the introduction of DOACs in the future.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

TB and AMV were supported by a grant from Associazione Italiana per la Ricerca sul Cancro (AIRC, Milano) 'Special Program Molecular Clinical Oncology 5×1000' to AGIMM (AIRC-Gruppo Italiano Malattie Mieloproliferative). VDS was supported by an unrestricted grant from Bruno Farmaceutici Foundation.

REFERENCES

1 Patrono C, Rocca B, De Stefano V. Platelet activation and inhibition in polycythemia vera and essential thrombocythemia. *Blood* 2013; **121**: 1701–1711.
2 Landolfi R, Marchioli R, Kutti J, Gisslinger H, Tognoni G, Patrono C *et al.* Efficacy and safety of low-dose aspirin in polycythemia vera. *N Engl J Med* 2004; **350**: 114–124.
3 Marchioli R, Finazzi G, Landolfi R, Kutti J, Gisslinger H, Patrono C *et al.* Vascular and neoplastic risk in a large cohort of patients with polycythemia vera. *J Clin Oncol* 2005; **23**: 2224–2232.
4 Marchioli R, Finazzi G, Specchia G, Cacciola R, Cavazzina R, Cilloni D *et al.* Cardiovascular events and intensity of treatment in polycythemia vera. *N Engl J Med* 2013; **368**: 22–33.
5 Harrison CN, Campbell PJ, Buck G, Wheatley K, East CL, Bareford D *et al.* Hydroxyurea compared with anagrelide in high-risk essential thrombocythemia. *N Engl J Med* 2005; **353**: 33–45.
6 Gisslinger H, Gotic M, Holowiecki J, Penka M, Thiele J, Kvasnicka HM *et al.* Anagrelide compared with hydroxyurea in WHO- classified essential thrombocythemia: the ANAHYDRET Study, a randomized controlled trial. *Blood* 2013; **121**: 1720–1728.
7 Goldhaber SZ. Venous thromboembolism: epidemiology and magnitude of the problem. *Best Pract Res Clin Haematol* 2012; **25**: 235–242.
8 De Stefano V, Za T, Rossi E, Vannucchi AM, Ruggeri M, Elli E *et al.* Recurrent thrombosis in patients with polycythemia vera and essential thrombocythemia: incidence, risk factors, and effect of treatments. *Haematologica* 2008; **93**: 372–380.
9 Hernández-Boluda JC, Arellano-Rodrigo E, Cervantes F, Alvarez-Larrán A, Gómez M, Barba P *et al.* Oral anticoagulation to prevent thrombosis recurrence in polycythemia vera and essential thrombocythemia. *Ann Hematol* 2015; **94**: 911–918.
10 Barbui T, Barosi G, Birgegard G, Cervantes F, Finazzi G, Griesshammer M *et al.* Philadelphia-negative classical myeloproliferative neoplasms: critical concepts

and management recommendations from European LeukemiaNet. *J Clin Oncol* 2011; **29**: 761–770.
11 Kreher S, Ochsenreither S, Trappe RU, Pabinger I, Bergmann F, Petrides PE *et al*. Prophylaxis and management of venous thromboembolism in patients with myeloproliferative neoplasms: consensus statement of the Haemostasis Working Party of the German Society of Hematology and Oncology (DGHO), the Austrian Society of Hematology and Oncology (ÖGHO) and Society of Thrombosis and Haemostasis Research (GTH e.V.). *Ann Hematol* 2014; **93**: 1953–1963.
12 Tefferi A, Barbui T. Polycythemia vera and essential thrombocythemia: 2015 update on diagnosis, risk-stratification and management. *Am J Hematol* 2015; **90**: 162–173.
13 Kearon C, Akl EA, Comerota AJ, Prandoni P, Bounameaux H, Goldhaber SZ *et al*. Antithrombotic therapy for VTE disease: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest* 2012; **141** (2 Suppl): e419S–e4194.
14 Ellis MH, Lavi N, Vannucchi A, Harrison C. Treatment of thromboembolic events coincident with the diagnosis of myeloproliferative neoplasms: a physician survey. *Thromb Res* 2014; **134**: 251–254.
15 Tefferi A, Vardiman JW. Classification and diagnosis of myeloproliferative neoplasms: the 2008 World Health Organization criteria and point-of-care diagnostic algorithms. *Leukemia* 2008; **22**: 14–22.
16 Schulman S, Kearon C. Subcommittee on Control of Anticoagulation of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis Definition of major bleeding in clinical investigations of antihemostatic medicinal products in non-surgical patients. *J Thromb Haemost* 2005; **3**: 692–694.
17 Boutitie F, Pinede L, Schulman S, Agnelli G, Raskob G, Julian J *et al*. Influence of preceding length of anticoagulant treatment and initial presentation of venous thromboembolism on risk of recurrence after stopping treatment: analysis of individual participants' data from seven trials. *BMJ* 2011; **342**: d3036.
18 Prandoni P, Noventa F, Ghirarduzzi A, Pengo V, Bernardi E, Pesavento R *et al*. The risk of recurrent venous thromboembolism after discontinuing anticoagulation in patients with acute proximal deep vein thrombosis or pulmonary embolism. A prospective cohort study in 1,626 patients. *Haematologica* 2007; **92**: 199–205.
19 van Es N, Coppens M, Schulman S, Middeldorp S, Büller HR. Direct oral anticoagulants compared with vitamin K antagonists for acute venous thromboembolism: evidence from phase 3 trials. *Blood* 2014; **124**: 1968–1975.
20 Prandoni P. Links between arterial and venous disease. *J Intern Med* 2007; **262**: 341–350.
21 EINSTEIN Investigators, Bauersachs R, Berkowitz SD, Brenner B, Buller HR, Decousus H, Gallus AS *et al*. Oral rivaroxaban for symptomatic venous thromboembolism. *N Engl J Med* 2010; **363**: 2499–2510.
22 EINSTEIN–PE Investigators, Büller HR, Prins MH, Lensin AW, Decousus H, Jacobson BF, Minar E *et al*. Oral rivaroxaban for the treatment of symptomatic pulmonary embolism. *N Engl J Med* 2012; **366**: 1287–1297.
23 Agnelli G, Buller HR, Cohen A, Curto M, Gallus AS, Johnson M *et al*. Oral apixaban for the treatment of acute venous thromboembolism. *N Engl J Med* 2013; **369**: 799–808.
24 Hokusai-VTE Investigators, Büller HR, Décousus H, Grosso MA, Mercuri M, Middeldorp S, Prins MH *et al*. Edoxaban versus warfarin for the treatment of symptomatic venous thromboembolism. *N Engl J Med* 2013; **369**: 1406–1415.
25 Paul A Kyrle. How I manage recurrent deep-vein thrombosis. *Blood* 2015; **27**: 696–702.
26 Oger E. Incidence of venous thromboembolism: a community-based study in Western France EPI-GETBP Study Group. Groupe d'Etude de la Thrombose de Bretagne Occidentale. *Thromb Haemost* 2000; **83**: 657–660.
27 Naess IA, Christiansen SC, Romundstad P, Cannegieter SC, Rosendaal FR, Hammerstrøm J. Incidence and mortality of venous thrombosis: a population-based study. *J Thromb Haemost* 2007; **5**: 692–699.

Supplementary Information accompanies this paper on the Leukemia website (http://www.nature.com/leu)