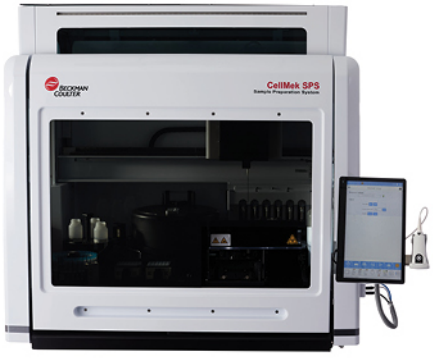

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REFERENCES

1. Schanz J, Tuchler H, Sole F, et al. New comprehensive cytogenetic scoring system for primary myelodysplastic syndromes (MDS) and oligoblastic acute myeloid leukemia after MDS derived from an international database merge. *J Clin Oncol*. 2012;30:820-829.
2. Lugthart S, Groschel S, Beverloo HB, et al. Clinical, molecular, and prognostic significance of WHO type inv(3)(q21q26.2)/t(3;3)(q21;q26.2) and various other 3q abnormalities in acute myeloid leukemia. *J Clin Oncol*. 2010;28:3890-3898.
3. Soderholm J, Kobayashi H, Mathieu C, Rowley JD, Nucifora G. The leukemia-associated gene MDS1/EVI1 is a new type of GATA-binding transactivator. *Leukemia*. 1997;11:352-358.
4. Tefferi A, Idossa D, Lasho TL, et al. Mutations and karyotype in myelodysplastic syndromes: TP53 clusters with monosomal karyotype, RUNX1 with trisomy 21, and SF3B1 with inv(3)(q21q26.2) and del (11q). Blood. *Cancer J*. 2017;7:658.
5. Rogers HJ, Vardiman JW, Anastasi J, et al. Complex or monosomal karyotype and not blast percentage is associated with poor survival in acute myeloid leukemia and myelodysplastic syndrome patients with inv(3)(q21q26.2)/t(3;3)(q21;q26.2): a Bone Marrow Pathology Group study. *Haematologica*. 2014;99:821-829.
6. Halaburda K, Labopin M, Houhou M, et al. AlloHSCT for inv(3)(q21;q26)/t(3;3)(q21;q26) AML: a report from the acute leukemia working party of the European society for blood and marrow transplantation. *Bone Marrow Transplant*. 2018;53:683-691.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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A multicenter real-life study on anticoagulant treatment with direct oral anticoagulants in patients with Ph-negative myeloproliferative neoplasms

To the Editor:

Patients with Ph-negative (Ph-neg) myeloproliferative neoplasms (MPNs) are at increased risk of thrombotic events. Despite the significant increase in knowledge of pathogenesis, risk stratification and effective biologic treatments of Ph-neg MPNs, arterial thrombosis and venous thromboembolism (VTE) (in particular atypical site VTE) are the main reasons of morbidity and mortality in patients with MPNs.[1] Due to the morbidity and mortality of these complications, antiplatelet and/or anticoagulant agents are commonly administered as primary and/or secondary prophylaxis.[2] In Ph-neg MPNs patients, vitamin K antagonists (VKAs) provide a high level of protection against VTE recurrences, although reported rates of both recurrent thrombosis and major bleeding remain higher compared to the general population.[3] The direct oral anticoagulants (DOACs) dabigatran, rivaroxaban, apixaban, and edoxaban are currently approved for the treatment and/or secondary prophylaxis of VTE and ischemic stroke prevention in atrial fibrillation (AF) in the general population.[4] In cancer patients, recent prospective clinical trials compared edoxaban, rivaroxaban and apixaban vs low-molecular-weight heparin (LMWH) demonstrating the non-inferiority of DOACs, but unfortunately only 10% of enrolled patients were affected by hematological malignancies. Although data on DOACs efficacy and safety in Ph-negative MPNs patients are yet limited,[5] DOACs may represent a valuable alternative, given the reduced bleeding risk and low incidence of VTE recurrences and/or stroke. In MPN patients, indication to cytoreductive treatment derived from guidelines suggested to start treatment only in high-risk patients. However, no evidences of the benefits of cytoreductive treatments in preventing recurrent thrombosis after MPN-related atypical site venous thrombosis (i.e. splanchnic vein thrombosis, SVT) have been reported so far. Thus, in general, a life-long anticoagulant treatment is suggested after a first VTE episode within the course of MPN. We report our experience on the administration of DOACs in a real-world setting of Ph-neg MPNs patients affected by VTE or AF. We evaluated consecutive Ph-negative MPNs patients who started DOACs from 2014 to 2019 for VTE or non-valvular AF. Patients were followed at three Italian Hematologic centers:

University Hospital "Sapienza" in Rome, University Hospital "Paolo Giaccone" in Palermo and "S.M. Goretti" Hospital in Latina. We included patients with a diagnosis of polycythemia vera (PV), essential thrombocythemia (ET), primary or secondary myelofibrosis (MF) and MPNs-unclassified (MPN-U). All MPN patients received a baseline prognostic stratification: PV patients according to cardiovascular risk factors, MF patients according to the international prognostic scoring system (IPSS) and ET patients were stratified according to the international prognostic score for thrombosis in ET (IPSET). The DOACs were started as front line anticoagulant treatment or after a previous treatment with VKA and the dosage was administered according to VTE and AF guidelines, and adjusted for liver and kidney function or body weight. The DOAC treatment was maintained all over the study and none of the patients changed the type of drug or dosage. For this study we evaluated DOACs safety and efficacy: as regards the safety, we recorded the bleeding events defined according to the International Society of Thrombosis and Hemostasis (ISTH) as major, minor and clinically relevant non-major (CRNM). Also, DOACs efficacy was evaluated in terms of recurrences of thromboembolic events: in the presence of specific symptoms, thromboembolic events were evaluated with recommended imaging techniques (computed tomography, Doppler ultrasound). Since the DOACs start, the patients were checked every 3 months for blood count, renal and liver function. We considered also the baseline comorbidities, in particular the concomitant presence of hypertension, renal failure, diabetes, cardiopathies, previous cancer, neurological diseases and chronic pulmonary diseases. All data were recorded in a dedicated database, including patients' characteristics, comorbidities, concomitant medication, thrombotic and hemorrhagic adverse events. Patients signed informed consent to the therapy and to the use of medical information for scientific purposes. Data were expressed as mean ± SD (normally distributed data), median and interquartile range (IR) (non-normally distributed data), or as percentage frequencies. Seventy-one patients were enrolled in the study. The patients' characteristics are reported in Table 1. Administered DOACs were: rivaroxaban in 26 (37%) cases, apixaban in 21 (29%), edoxaban in 14 (20%) and dabigatran in 10 (14%). Forty-six out of 71 patients started DOACs as frontline anticoagulant treatment (15 patients were already on DOACs therapy before MPN diagnosis); 25/71 patients were shifted from a previous VKA treatment. The main reasons for a switch from VKA to DOACs were: instability of the INR (TTR <70%) in 12 patients; adverse events (hypersensitivity with cutaneous rush) related to therapy in four patients; difficulties in carrying out regular monitoring check in nine patients. Thirty-five patients were affected by non-valvular AF: at MPN diagnosis, 10 patients started antiplatelet treatment as primary prophylaxis. In 20 cases, DOACs were started as frontline anticoagulant treatment (8/20 patients were already on DOACs before MPN diagnosis); in 15 patients DOACs were shifted from a previous VKA therapy with a median time of VKA length of 32 months (range 11-92 months). The median follow-up duration of DOACs was 12 months (range 8.7-26 months). We did not record thrombotic complications, major or CRNM bleeding. One minor bleeding (epistaxis in an ET patient), not requiring DOAC discontinuation because of a spontaneous resolution, occurred. Thirty-six patients presented VTE: 26/36 were of typical sites and 10/36 were of atypical sites (including splanchnic venous thrombosis and cerebral sinus vein

TABLE 1 Patients' characteristics

	AF	VTE
71 patients	35	36
Age (years) at MPN diagnosis (median)	72 (67-79)	67 (65-77)
Age (years) at DOACs start (median)	74 (67-79)	70 (65-77)
MPN type		
PV	10	15
ET	13	15
MF	9	4
MPN-U	3	2
Risk stratification		
PV		
Low	3	2
High	7	13
ET (IPSET)		
Low	2	2
Intermediate	2	5
High	9	8
MF (IPSS)		
Low	2	0
Intermediate -1	4	2
Intermediate -2	3	2
High	0	0
Driver mutation		
JAK2V617F	26	28
CALR	2	0
MPL	1	1
Negative	6	7
MPN treatment		
Hydroxycarbamide	20	30
Ruxolitinib	8	3
6-mercaptopurine	1	0
Interpheron-alpha	1	0
Anagrelide	0	1
Phlebotomy	1	0
Observation	4	2
Comorbidities		
Hypertension	18	14
Cardiopathy and arrhythmias	10	7
Diabetes	6	2
Renal impairment	1	3
Respiratory disease	2	2
History of neoplasm	0	4
No comorbidities	13	9
1 comorbidity	10	9
2 comorbidities	7	7
3 comorbidities	3	10

TABLE 1 (Continued)

	AF	VTE
4 comorbidities	1	1
5 comorbidities	1	0

Abbreviations: AF, atrial fibrillation; ET, essential thrombocythemia; IPSET, international prognostic score for thrombosis in ET; IPSS, international prognostic scoring system; MF, myelofibrosis; MPN-U, myeloproliferative neoplasm-unclassified; PV, polycythemia vera; VTE, venous thromboembolism.

thrombosis). At MPN diagnosis, four patients started antiplatelet treatment for concomitant cardiopathy. In 26 cases, DOACs were started as frontline anticoagulant treatment (7/26 patients were already on DOACs before MPN diagnosis); in 10 patients, DOACs were shifted from a previous VKA therapy with a median time of VKA length of 12 months (range 9-63 months). The median follow-up duration of DOACs was 15 months (range 12-30.7 months). Three patients presented a mild thrombocytopenia with platelet count ranging from 80×10^9/L to 100×10^9/L at DOACs start. We did not record thrombotic complications during DOACs therapy in VTE population. One patient with MF discontinued DOACs for thrombocytopenia $<50 \times 10^9$/L. In the 10 patients, previously treated with VKA, six thrombotic events were observed during VKAs therapy: four recurrences of splanchnic vein thrombosis in three patients with primary MF and one with PV (all the patients were on cytoreductive therapy and one-fourth of patients presented mild thrombocytopenia with a platelet count of 80×10^9/L at the VTE recurrence); two deep vein thrombosis in two patients with ET (not in cytoreductive therapy and with a platelet count $>500 \times 10^9$/L). All these patients presented at least two comorbidities. During DOACs administration, no major or CRNM bleedings were reported. During VKAs, one CRNM bleeding was reported: an intramuscular hematoma of the femoral quadriceps in a MF patient with VTE and no comorbidities. Growing evidence supports the use of DOACs for cancer associated VTE, especially in patients with solid tumors, but the best anticoagulant treatment in MPN patients with highly thrombogenic features is still a matter of discussion.[6] DOACs resulted a safe and effective treatment in our real-life casuistic. Furthermore, considering that the majority of our patients presented at least two comorbidities (29 patients presented ≥ two comorbidities) and 10 patients presented VTE in atypical sites, DOACs seem a suitable option also for frail MPN subjects at increased thrombotic risk. Finally, 11 patients were treated with the JAK2 inhibitor ruxolitinib: the association seemed safe and effective even in this combination, suggesting that co-administration of DOACs and ruxolitinib is feasible. In conclusions, our results proved that in Ph-negative MPNs patients, anticoagulant treatment with DOACs is well tolerated, apparently safe, without recurrences of thrombotic events and significant bleeding complications even in high risk patients. Although the limitations due to the retrospective nature, these data are in line with the currently limited literature so far reported. Under the perspective of a long-term anticoagulant treatment during MPNs, DOACs seem a feasible option even in second line. A prospective clinical trial is required to confirm these findings.

DISCLOSURE OF INTERESTS

The authors declare no potential conflict of interest.

DATA AVAILABILITY STATEMENT

All the data are deposited in specific database. The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Hultcrantz M, Björkholm M, Dickman PW, et al. Risk for arterial and venous thrombosis in patients with myeloproliferative neoplasms: A population-based cohort study. *Ann Intern Med*. 2018;168(5):317-325.
2. Rungjirajittranon T, Owattanapanich W, Ungprasert P, Siritanaratkul N, Ruchutrakool T. A systematic review and meta-analysis of the prevalence of thrombosis and bleeding at diagnosis of Philadelphia-negative myeloproliferative neoplasms. *BMC Cancer*. 2019;19(1):184.
3. De Stefano V, Ruggeri M, Cervantes F, et al. High rate of recurrent venous thromboembolism in patients with myeloproliferative neoplasms and effect of prophylaxis with vitamin K antagonists. *Leukemia*. 2016;30:2032-2038.
4. Kearon C, Akl EA, Ornelas J, et al. Antithrombotic therapy for VTE disease: CHEST guideline and expert panel report. *Chest*. 2016;149:315-352.

5. Curto-Garcia N, Doyle AJ, Breen KA, et al. Outcomes of patients receiving direct oral anticoagulants for myeloproliferative neoplasm-associated venous thromboembolism in a large tertiary centre in the UK. *Br J Haematol*. 2020;189(3):e79-e81.
6. Arachchillage DR, Laffan M. Pathogenesis and Management of Thrombotic Disease in Myeloproliferative Neoplasms. *Semin Thromb Hemost*. 2019;45(6):604-611.

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BCR-ABL1 tyrosine kinase inhibitor-associated thyroid dysfunction: A review of cases reported to the FDA Adverse Event Reporting System and published in the literature

To the Editor:

Chronic myeloid leukemia (CML) is characterized by the presence of the Philadelphia chromosome (Ph+) producing a constitutively active tyrosine kinase, BCR-ABL1.[1] BCR-ABL1 tyrosine kinase inhibitors (TKIs) are the mainstay treatment for patients with CML. Currently, there are five BCR-ABL1 TKIs approved by the U.S. Food and Drug Administration (FDA), including imatinib, dasatinib, nilotinib, bosutinib, and ponatinib, most of which have indications beyond CML. Thyroid dysfunction, specifically hypothyroidism and hyperthyroidism, is a well-known adverse event observed with many TKIs.[2] Among the BCR-ABL1 TKIs, hypothyroidism, hyperthyroidism, and thyroiditis were identified in clinical trials with dasatinib and nilotinib, whereas a distinct risk of hypothyroidism in thyroidectomy patients on levothyroxine was identified with imatinib in the postmarket setting. The latter was observed in studies conducted by DeGroot and colleagues, in which thyroidectomized patients receiving imatinib required increased thyroid hormone replacement, while patients with intact thyroid glands receiving imatinib remained euthyroid.[3] The FDA approved prescribing information for imatinib with this risk in 2008.

We evaluated postmarketing adverse event reports from the FDA Adverse Event Reporting System (FAERS) and published in the literature from the product approval date through September 10, 2019, to investigate an association between BCR-ABL1 TKI exposure and thyroid dysfunction as a potential class effect. A confirmed case of thyroid dysfunction was supported by laboratory evidence or initiation of thyroid treatment. An unconfirmed case of thyroid dysfunction did not provide this level of evidence. We further characterized the cases as new-onset or worsening thyroid dysfunction as indicated by modification of thyroid medication regimen or worsening thyroid function tests. The severity of thyroid dysfunction was graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.[4]

We identified 326 unique cases of thyroid dysfunction from FAERS (n = 239) and the literature (n = 87) associated with imatinib (n = 112), dasatinib (n = 41), nilotinib (n = 126), bosutinib (n = 8), and ponatinib (n = 39) use. We applied the World Health Organization-Uppsala Monitoring Centre (WHO-UMC) causality assessment and determined causality as probable in five and possible in all others. Descriptive characteristics of the cases are summarized in Table 1. Overall, 74% (240/326) described hypothyroidism, 20% (67/326) hyperthyroidism, and 6% (19/326) did not specify the type of thyroid dysfunction. Among 122 cases that reported sufficient clinical information to assess baseline thyroid function, new-onset and worsening thyroid dysfunction occurred in 83% (n = 101) and 17% (n = 21) of cases, respectively.

Half of the cases reported a time to onset, which varied widely. Among these cases, 54% (88/162) of thyroid dysfunction occurred within the first nine months of BCR-ABL1 TKI initiation. This may reflect gradual progression of drug-induced thyroid toxicity. Alternatively, inadequate monitoring requirements for thyroid function tests may have limited earlier detection of thyroid dysfunction.[5]

All forms of thyroid disorders may be associated with significant morbidity and mortality if unsuspected and untreated.[6] Among cases that reported severity grading information, 83% (156/189) were CTCAE grade 2 thyroid dysfunction. Most cases required intervention with thyroid replacement or antithyroid therapy, and patients were able to continue BCR-ABL1 TKI therapy at the same or reduced dose. One fatal case of hyperthyroidism reported with imatinib therapy occurred in a patient with pre-existing hyperthyroidism, but the report was missing sufficient detail to adequately assess the cause. Clinical management of thyroid dysfunction depends on severity; however, as observed in our case series, cases of low severity tolerated continuing BCR-ABL1 TKI therapy with appropriate management, such as levothyroxine supplementation or anti-thyroid directed therapy.

We assessed any contributory role between BCR-ABL1 TKIs and thyroid dysfunction by considering competing causes, temporality, existence of positive rechallenge and dechallenge, and potential biologic mechanisms of toxicity. All cases had a compatible temporal relationship and a suspected biologic mechanism. Cases deemed probable included one positive rechallenge case with imatinib and those cases without known confounding factors. Most cases were deemed to have possible causality due to missing data, presence of potential confounding, or a history of thyroid dysfunction. All cases that did not report thyroidectomy were presumed to have an intact thyroid. It is noteworthy that four cases of worsening thyroid dysfunction resulted in increased levothyroxine requirements after imatinib initiation in patients with pre-existing hypothyroidism and an intact thyroid. This supports an imatinib-induced exacerbation of hypothyroidism in patients with an intact thyroid.