Acta Haematologica Polonica

Studies on platelet function during the first year of ibrutinib treatment in patients with chronic lymphocytic leukemia

Authors: Bożena Sokołowska, Dariusz Jawniak, Aleksandra Nowaczyńska, Agnieszka Szymczyk, Ewa Wąsik-Szczepanek, Marek Hus

SHORT COMMUNICATION

Studies on platelet function during first year of ibrutinib treatment in patients with chronic lymphocytic leukemia

Bożena Sokolowska[1], Dariusz Jawniak[1], Aleksandra Nowaczyńska[1], Agnieszka Szymczyk[2*], Ewa Wąsik-Szczepanek[1], Marek Hus[1]

[1]Department of Hematooncology and Bone Marrow Transplantation, Medical University of Lublin, Lublin, Poland

[2]Department of Clinical Transplantology, Medical University of Lublin, Lublin, Poland

*Address for correspondence: Agnieszka Szymczyk, Department of Clinical Transplantology, Medical University of Lublin, Chodźki 7, 20–093 Lublin, Poland, e-mail: agnieszka.szymczyk.med@wp.pl

Introduction

Ibrutinib, an inhibitor of Bruton's tyrosine kinase (BTK), exhibits a significant activity in chronic lymphocytic leukemia (CLL). Although the treatment is generally well tolerated, it has been proven that ibrutinib demonstrates an increased bleeding risk compared to standard chemotherapy. Complications have ranged from minor mucocutaneous bleeding to life-threatening hemorrhage [1].

Wang et al. [2], in their meta-analysis of randomized controlled trials (4,288 patients), concluded that ibrutinib treatment was associated with a significantly higher risk of bleeding (both overall and major bleeding) in patients with B cell malignancies, especially in CLL. The main reason for these bleeding events is impaired platelet function especially due to a reduction of collagen-mediated platelet aggregation [3].

The present study aimed to evaluate platelet function before and during the first year of ibrutinib therapy in patients with CLL.

Material and methods

We examined 16 patients with CLL (six females and 10 males, median age 66 years, range 48–82). The characteristics of the patients are set out in supplementary material, Table S1. The patients were treated in an early access program for ibrutinib in Poland. All patients started with 420 mg ibrutinib, although later in one patient the dose was reduced to 140 mg due to atrial fibrillation: a 66-year-old female patient after three lines of chemotherapy [CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone), FC (fludarabine and cyclophosphamide), and BR (rituximab, bendamustine)]. The first episode of paroxysmal atrial fibrillation (AF) occurred after three weeks of ibrutinib whole-dose therapy. Ibrutinib was stopped for two weeks, and the patient received beta blockers (sotalol) with good effect. Then the dose of ibrutinib was reduced to one tablet a day (140 mg). Simultaneously, due to the recurrence of paroxysmal AF, anticoagulation therapy (AC) was introduced. Patients received in the beginning Pradaxa 2 × 150 mg, than 1 × 110 mg, than Xarelto 15 mg, periodically vitamin K antagonist (VKA) 1/2 mg daily and finally Xarelto 15 mg daily again. The type of anticoagulation was changed because of minor bleeding complications (grade 1–2). In all groups of patients, platelet aggregation studies were performed in whole blood samples using multiplate technology, in which platelets were activated by an adenosine diphosphate (ADP) test, an arachidonic acid (ASPI) test, a collagen (COL) test, and a TRAP-6 (TRAP) test. Platelet function was evaluated at four time points: prior to ibrutinib treatment (point 1); after one month (point 2); after 2–4 months (point 3); and after one year of treatment (point 4). The control group (CG) included 20 healthy volunteers, six males and 14 females with a median age of 44 years (range 26–59).

This study was approved by the Ethics Committee of the Medical University of Lublin (KE No. 0254/159/2014). All patients provided written informed consent to participate in the study.

Results

Statistically significant impaired platelet function ($p < 0.05$) was detected in the treatment group in all the tests and at all time points compared to the CG. The COL test revealed exacerbation of platelet defects during the course of the treatment. The COL test value was reduced after 12 months of treatment compared to that before the treatment [COL test 1: median (range): 33 U (6–70), COL test 4: 11 U (0–22). The test value of the fourth time point

2

was increased, which indicated some improvement in ADP-induced, arachidonic acid-induced, and TRAP-induced platelet aggregation: ADP test 1: 27 U (0–58), ADP test 4: 34 U (3–84); ASPI test 1: 45 U (21–101), ASPI test 4: 56 U (2–123); TRAP test 1: 56 U (28–95), TRAP test 4: 86 U (15–140). This tendency was most pronounced in the TRAP test, but was not sufficiently high to reach statistical significance. Platelet function during the course of therapy is shown in Figure 1.
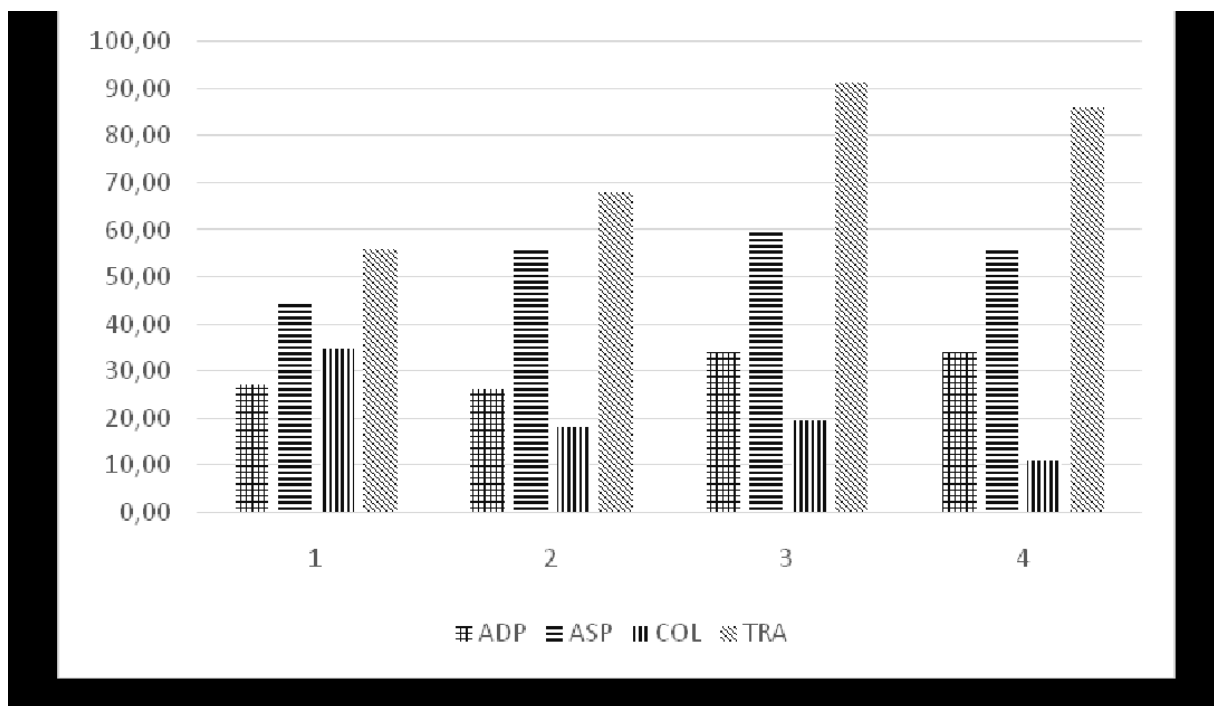

Figure 1. Platelet function during course of therapy with ibrutinib; 1 — platelet function prior to ibrutinib therapy, 2 — platelet function after one month of therapy, 3 — platelet function after 2–4 months of therapy, 4 — platelet function after one year of therapy; ADP — platelets activated by adenosine diphosphate; ASP — platelets activated by arachidonic acid; COL — platelets activated by collagen; TRA — platelets activated by TRAP-6

Bleeding complications [grade 1 according to Common Terminology Criteria for Adverse Events (CTCAE)] were observed in five patients (31%) and occurred during the first 3-6 months of ibrutinib therapy. We did not observe any major bleeding complications or any thrombotic complications. Nose bleeding occurred in two patients (12.5%), while three patients experienced skin petechiae (18.7%). Grade 1 thrombocytopenia was detected in three of five patients immediately before ibrutinib therapy (52 G/L, 74 G/L, 85 G/L). The median

platelet count in the group of patients without bleeding complications before ibrutinib therapy and after one year of therapy was 141 G/L (106–204) and 166.5 G/L (126–209), respectively. However, in the group of patients with bleeding complications, the median platelet count before therapy and after one year of therapy was low: 85 G/L (52–171) and 132 G/L (87–188), respectively (Supplementary Table I).

Supplementary Table I. Characteristics of study group

Characteristics		All patients (n = 16)	Patients with bleeding events (n = 5)	Patients without bleeding events (n = 11)	p-value
Median age [range] (years)		65.0 [58–81]	51.0 [48–75]	66.0 [48–81]	$p < 0.05$
Male/female		10/6	2/3	8/3	$p > 0.05$
Clinical stage according to Rai classification	Early (0–II)	13	2	11	$p < 0.05$
	Advanced (III–IV)	3	3	0	
Cytogenic abnormalities	17p deletion	2 (n = 7)	0 (n = 2)	2 (n = 5)	$p > 0.05$
	11p deletion	2 (n = 7)	1 (n = 2)	1 (n = 5)	
CD 38 positive (cut-off 30%)		4 (n = 5)	3 (n = 3)	1 (n = 2)	$p > 0.05$
ZAP 70 positive (cut-off 20%)		3 (n = 4)	2 (n = 2)	1 (n = 2)	$p > 0.05$
Lines of treatments (number)		2	3	2	$p > 0.05$
Median platelet count at beginning of therapy		136	85	141	$p > 0.05$
Median platelet count after 12 months of therapy		163	132	166.5	$p > 0.05$
Median hemoglobin at		12.7	12.3	13.1	$p > 0.05$

beginning of therapy				
Median hemoglobin after 12 months of therapy	13.6	12.6	13.8	$p > 0.05$
Median WBC at beginning of therapy	14.7	13.3	20.9	$p > 0.05$
Median WBC after 12 months of therapy	9.1	7.4	10.4	$p < 0.05$
Median PCT at beginning of therapy	0.14	0.13	0.14	$p > 0.05$
Median PCT after 12 months of therapy	0.2	0.16	0.2	$p < 0.05$
Response to treatment (n)	Responding (CR or PR)	13	3	10	$p > 0.05$
	Non-responding (SD or PD)	3	2	1	

WBC — white blood cell count; PCT — platelet crit; CR — complete remission; PR — partial remission; SD — stable disease; PR — progression of disease

Comparison between the groups of patients with and without bleeding complications showed statistically significant differences immediately after 12 months of ibrutinib treatment. Patients with bleeding complications showed lower values of ADP test than those without bleeding complications (29 U vs. 52 U, $p < 0.05$). Additionally, after 12 months of therapy, white blood cell (WBC) count was lower in patients with bleeding complications than in those without bleeding episodes: 7.4 versus 10.4 ($p < 0.05$). The last difference was related to platelet crit (PCT), the value of which was lower in patients with bleeding complications than in those without such episodes: 0.16 versus 0.20 ($p < 0.05$). Both these values were within the normal range.

Parametric tests were used for statistical analysis. Statistical inference was performed at a confidence level of $\alpha < 0.05$.

Discussion

Ibrutinib is indicated for the treatment of patients with mantle cell lymphoma (MCL) and CLL who have received at least one prior therapy, and for patients with CLL and 17p deletion.

Regarding ibrutinib characteristics, grade 3 or higher bleeding events have occurred in up to 3–4% of patients [4]. We did not observe these types of bleeding events in our group of patients. We noted only mild bleeding events i.e. nose bleeding and skin petechiae in five patients (31%).

Lipsky et al. [5] observed that cumulative bleeding events plateaued at six months, which suggests that the risk of bleeding decreases with continued therapy.

The mechanism responsible for the occurrence of bleeding events is not well understood. Bruising and petechiae are often observed when the platelet number is low or when platelet function is impaired [5, 6].

Ibrutinib acts as a BTK inhibitor. In 2015, Rigg et al. [7] conducted a series of *in vitro* studies on platelet activation which revealed that BTK inhibition caused spreading and aggregation of platelets. Their results showed that irreversible inhibition of BTK with ibrutinib *in vitro* decreases human platelet activation, phosphorylation of BTK, P-selectin exposure, and platelet aggregation under shear flow conditions.

Kamel et al. [8] reported defects in collagen-induced and ADP-induced platelet responses when ibrutinib was added *ex vivo* to patients' samples. As BTK plays a central role in glycoprotein VI signaling, ibrutinib by inhibiting BTK may cause collagen-induced defects of platelet function. However, there is no available explanation for the defects of ADP-dependent platelet activation observed during ibrutinib therapy [8]. Previously, Pulte et al. [9] reported that platelet response to ADP is inhibited by ADP-ase CD39 on lymphocytes. By using multiplate technology, we concluded that collagen-induced and ADP-induced platelet aggregation was impaired in all patients immediately before ibrutinib therapy. We found the same results using other agonists such as ASPI and TRAP-6.

We inferred that CLL per se has the potential to impair platelet function. The same observation was reported by Lipsky et al. [5] who showed decreased response to collagen and ADP, not only in CLL patients on ibrutinib therapy, but also in CLL patients who did not receive this drug. During the course of ibrutinib treatment, we observed exacerbation of platelet defects as measured by the COL test.

A similar observation was reported by Alberelli et al. [10] in a group of nine patients with CLL treated with ibrutinib. Eight patients showed abnormalities of collagen-induced platelet aggregation after the initiation of ibrutinib treatment. On the other hand, a group of five patients showed a significant improvement of ADP-induced platelet aggregation. The

authors concluded that ibrutinib treatment in patients with CLL causes a mild bleeding phenotype, most probably due to platelet dysfunction.

In our patients, after one year of ibrutinib therapy, the platelet count increased in both patients with and patients without bleeding complications. Despite the worsening of collagen-induced platelet aggregation, platelet aggregation measured by the three other agonists adenosine diphosphate (ADP), arachidonic acid, and TRAP was improved and platelet count increased, and perhaps because of these all changes, only transient bleeding complications were observed. However, in patients with bleeding complications, the ADP test showed that after 12 months of treatment, the improvement of ADP-induced platelet aggregation was statistically significantly lower than that in patients without bleeding complications. This could imply that platelet function remained impaired and may have caused a higher risk of bleeding complications.

Dmitrieva at al. [11] investigated platelet functional activity in group of 50 chronic lymphocytic leukemia (CLL) patients treated with ibrutinib. They found similar observations i.e. in the beginning, impaired platelet response to ADP caused by CLL, then impaired collagen-induced aggregation caused by ibrutinib which shifts the balance to bleeding. During ibrutinib treatment, bleeding tendency was decreased mainly because of the improved response to ADP [11].

In 2016, Kazianka et al. [12] reported that ristocetin-induced platelet aggregation (RIPA) might be a useful indicator to predict and monitor bleeding tendency in CLL patients treated with ibrutinib. However, in 2018, Alberelli et al. [13] studied a group of 68 CLL patients on ibrutinib therapy and proved that RIPA is not affected in ibrutinib-treated CLL patients and cannot be used to identify patients at a higher risk of bleeding. Dmitrieva et al. [11] confirmed that platelet aggregation with ADP and ristocetin, as well as platelet count, were the best indicators of bleeding.

Sometimes patients on ibrutinib therapy due to atrial fibrillation require anticoagulation (AC) for primary and secondary prevention. Such patients should be followed closely. Direct oral anticoagulants (DOAC) are preferred over a VKA because of the lower risk of major bleeding events and because of the favorable stroke risk-benefit profile [14]. Our abovementioned patient received DOAC simultaneously with a reduced dose of ibrutinib. According to Raz et al., when ibrutinib was used in combination with DOAC, most of the bleeding was grade 1–2, so the safety profile of concurrent treatment of DOAC and ibrutinib

seemed tolerable [15]. In our patient receiving DOAC and ibrutinib simultaneously, we only observed minor (grade 1–2) bleeding events.

Conclusions

We detected impaired platelet aggregation by multiplate technology in all the studied patients with CLL. The impairment of collagen-induced platelet aggregation was exacerbated during ibrutinib treatment. Interestingly, ADP-induced, arachidonic acid-induced, and TRAP-induced platelet aggregation tended to improve during the treatment.

Only 31% of ibrutinib-treated patients showed some mild bleeding complications at the beginning of the treatment. Platelet function in these patients remained impaired even after 12 months of therapy.

Conflicts of interest
None.

Ethics
The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; uniform requirements for manuscripts submitted to biomedical journals.

References

1. Shatzel JJ, Olson SR, Tao DL, et al. Ibrutinib-associated bleeding: pathogenesis, management and risk reduction strategies. J Thromb Haemost. 2017; 15(5): 835–847, doi: 10.1111/jth.13651, indexed in Pubmed: 28182323.
2. Wang J, Zhao A, Zhou H, et al. Risk of bleeding associated with ibrutinib in patients with B-cell malignancies: a systematic review and meta-analysis of randomized

controlled trials. Front Pharmacol. 2020; 11: 580622, doi: 10.3389/fphar.2020.580622, indexed in Pubmed: 33658926.

3. Stephens DM, Byrd JC. How I manage ibrutinib intolerance and complications in patients with chronic lymphocytic leukemia. Blood. 2019; 133(12): 1298–1307, doi: 10.1182/blood-2018-11-846808, indexed in Pubmed: 30642919.

4. Brown JR, Moslehi J, Ewer MS, et al. Incidence of and risk factors for major haemorrhage in patients treated with ibrutinib: an integrated analysis. Br J Haematol. 2019; 184(4): 558–569, doi: 10.1111/bjh.15690, indexed in Pubmed: 30506764.

5. Lipsky AH, Farooqui MZH, Tian X, et al. Incidence and risk factors of bleeding-related adverse events in patients with chronic lymphocytic leukemia treated with ibrutinib. Haematologica. 2015; 100(12): 1571–1578, doi: 10.3324/haematol.2015.126672, indexed in Pubmed: 26430171.

6. Levade M, David E, Garcia C, et al. Ibrutinib treatment affects collagen and von Willebrand factor-dependent platelet functions. Blood. 2014; 124(26): 3991–3995, doi: 10.1182/blood-2014-06-583294, indexed in Pubmed: 25305202.

7. Rigg RA, Aslan JE, Healy LD, et al. Oral administration of Bruton's tyrosine kinase inhibitors impairs GPVI-mediated platelet function. Am J Physiol Cell Physiol. 2016; 310(5): C373–C380, doi: 10.1152/ajpcell.00325.2015, indexed in Pubmed: 26659727.

8. Kamel S, Horton L, Ysebaert L, et al. Ibrutinib inhibits collagen-mediated but not ADP-mediated platelet aggregation. Leukemia. 2015; 29(4): 783–787, doi: 10.1038/leu.2014.247, indexed in Pubmed: 25138588.

9. Pulte D, Olson KE, Broekman MJ, et al. CD39 activity correlates with stage and inhibits platelet reactivity in chronic lymphocytic leukemia. J Transl Med. 2007; 5: 23, doi: 10.1186/1479-5876-5-23, indexed in Pubmed: 17480228.

10. Alberelli MA, Innocenti I, Sica S, et al. PO-54 — clinical and laboratory characterization of platelet dysfunction caused by ibrutinib treatment in patients with chronic lymphocytic leukemia. Thromb Res. 2016; 140(Suppl 1): S196, doi: 10.1016/S0049-3848(16)30187-6, indexed in Pubmed: 27161742.

11. Dmitrieva EA, Nikitin EA, Ignatova AA, et al. Platelet function and bleeding in chronic lymphocytic leukemia and mantle cell lymphoma patients on ibrutinib. J Thromb Haemost. 2020; 18(10): 2672–2684, doi: 10.1111/jth.14943, indexed in Pubmed: 32511880.

12. Kazianka L, Drucker C, Skrabs C, et al. Ristocetin-induced platelet aggregation for monitoring of bleeding tendency in CLL treated with ibrutinib. Leukemia. 2017; 31(5): 1117–1122, doi: 10.1038/leu.2016.316, indexed in Pubmed: 27909342.

13. Alberelli MA, Innocenti I, Autore F, et al. Ibrutinib does not affect ristocetin-induced platelet aggregation evaluated by light transmission aggregometry in chronic lymphocytic leukemia patients. Haematologica. 2018; 103(3): e119–e122, doi: 10.3324/haematol.2017.179044, indexed in Pubmed: 29242303.

14. Santoro RC, Falbo M, Levato L, et al. Dabigatran in ibrutinib-treated patients with atrial fibrillation and chronic lymphocytic leukemia: experience of three cases. Blood Coagul Fibrinolysis. 2021; 32(2): 159–161, doi: 10.1097/MBC.0000000000000981, indexed in Pubmed: 33273263.

15. Raz MA, Arnason J, Bairey O, et al. The risk of bleeding in patients receiving ibrutinib combined with novel direct oral anticoagulants. Br J Haematol. 2020; 189(2): e31–e33, doi: 10.1111/bjh.16422, indexed in Pubmed: 32150642.