Peripheral Artery Oclusive Disease Among Patients With Chronic Myeloid Leukemia Receiving Tyrosine Kinase Inhibitors: A Cross-Sectional Case-Control Study

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ABSTRACT:
BACKGROUND: There were some reports of peripheral artery occlusive disease (PAOD) associated with nilotinib usage in chronic myeloid leukemia (CML). These complications in other tyrosine kinase inhibitors are revealed as unknown.

MATERIALS AND METHODS: We determined the prevalence of PAOD in patients with CML as compared with matched-control population by cross-sectional case-control study. Peripheral artery occlusive disease was screened by ankle-brachial index (ABI).

RESULTS: In total, 78 CML and 156 matched-control patients were included. The median age was 55 years. In all, 61 (78.2%) were on imatinib and 13 (16.7%) were on nilotinib, whereas 4 patients (5.2%) were on dasatinib. Prevalence of low ABI (<0.9) was 9.0%, and nilotinib users had the highest prevalence of low ABI of 30.7%. All cases with low ABI were not shown to be clinically overt of PAOD. There were well-balanced characteristics between cases of CML and matched control except in higher levels of hypercholesterolemia in the control. Interestingly, CML had more amounts of pathologic ABI than the control (odds ratio: 2.09, 95% confidence interval: 0.71-6.21), and diagnosis of diabetes found it to be independent of the risk of PAOD.

CONCLUSIONS: Peripheral artery occlusive disease was higher among patients with CML than the control, especially in patients who had diabetes.

KEYWORDS: Chronic myeloid leukemia, tyrosine kinase inhibitors, peripheral artery occlusive disease

Introduction
Tyrosine kinase inhibitors (TKIs) are standard therapy in chronic myeloid leukemia (CML) individuals, especially for second-generation TKIs, and these inhibitors include nilotinib and dasatinib which pursue more cytogenetic and molecular response compared with imatinib.¹⁻⁵ Although the efficacy of newer TKIs has been proved, the association of TKIs with long-term complications has been of concern. There were a few studies among nilotinib-receiving patients who experienced peripheral artery occlusive disease (PAOD) and cardiovascular diseases.⁶⁻⁷ Most of these were case reports and case series. Atherosclerotic risk modifications are essential for the prevention of these complications, and some of them require angioplasty. Nilotinib increases the risk of PAOD more than imatinib by 14.6 times, and a 10-year probability of being free from PAOD for imatinib and nilotinib was found to be 100% and 67%, respectively.⁸ However, these studies had a small scale of population and lack of metabolic profiles and ankle–brachial index (ABI) for PAOD screening. Most of the previous studies on PAOD in CML individuals have revealed arterial occlusion to be clinically overt and recommended that the patients who had cardiovascular risk factors, for example, diabetes, hyperlipidemia, and hypertension, be screened for PAOD. This complication also brought about a decline in the quality of life and was related to many comorbidities.

In this study, we performed the cross-sectional case-control study to compare the prevalence of PAOD between patients with CML who were receiving TKIs and non-CML patients. All PAOD-related parameters were collected, and PAOD screening with ABI was performed.

Materials and Methods
This was a single-center, cross-sectional, case-control study of patients who were diagnosed with CML and were receiving...
TKIs at Chiang Mai University Hospital between February 2014 and December 2014 in comparison with a control population in a 1:2 ratio with age, sex, and diabetes status matched. The study protocol was approved by the Institutional Review Board of Chiang Mai University and was conducted according to the Declaration of Helsinki. All patients provided written informed consent.

Patients and control population
All the study participants were adults (age >18 years) diagnosed with the chronic phase of CML and patients in Chiang Mai University Hospital. The clinical variables and the PAOD-related risk factors were recorded. Both the case and the control groups were screened to obtain ABI with the VaSera VS-1500N instrument (Fukuda Denshi Co Ltd, Tokyo, Japan). Ankle-brachial index values lower than 0.9 were classified into pathologic outcomes.9 In cases of pathologic ABI, duplex ultrasound scan of lower legs or computed tomography angiogram was performed for confirming the diagnosis of PAOD.

Statistical analysis
According to previous reports on PAOD prevalence in patients with CML, the prevalence was found to be around 20%,10,11 and for the general population, it reached 5%.12 With the 2-sided significance level of .05 with at least 80% power, the planned sample size for the 1:2 ratio consisted of 76 patients and 152 patients for the case group and the control groups, respectively. The subgroups were compared using χ² or Fisher exact test for categorical data and Mann-Whitney U test for quantitative data. Logistic regressions were applied to any significant values for multivariate analysis. A stratified Cox proportional hazards model was used to generate the odds ratio (OR) and 95% confidence interval (CI). The software used to obtain all the analytical values was SPSS for Mac version 20.

Results
Baseline characteristics
Between February 2014 and December 2014, the study enrolled 78 patients with CML with 156 control population as the planned 1:2 ratio. In our center, imatinib was first-line treatment of patients with CML. Nilotinib will be provided as the planned 1:2 ratio. In our center, imatinib was first-line treatment of patients with CML, Patients receiving nilotinib had the highest prevalence of abnormal ABI of 30.8%, whereas patients receiving imatinib and dasatinib had prevalence of 4.9% and 0%, respectively. Nilotinib-using patients had augmented risk of PAOD, according to the ABI screening, compared with imatinib (OR: 8.59; 95% CI: 1.64–44.89; P = .004). For the multivariate analysis, there was only the independent factor of hemoglobin A₁c (HbA₁c) over 7 g/dL (OR: 2.41; 95% CI: 1.11–5.25; P = .026) which was positively associated with low ABI. All the 7 patients were asymptomatic and also achieved major molecular response (MR1.5) by real-time quantitative polymerase chain reaction. The median age was 70 (50–86) years. Four were used to nilotinib usage with a median duration of 62 (55–67) months. Vascular imaging was performed, but only 2 patients who received nilotinib had significant stenosis of lower limb arteries (Table 1S). Because all of the patients did not have limb ischemia clinically, the vascular surgeons settled on conservative methods with atherosclerosis risk factor modification and closed monitoring.

PAOD prevalence compared with control population
Age, sex, and diabetes status of the control population were well matched with the patients with CML, but the control group had higher LDL-C and lower HDL-C levels (Table 3, Figure 2). However, the patients with CML, eventually, were found to have more prevalence of PAOD with OR of 2.09 (95% CI: 0.71–6.21; P = .181) by ABI.

Discussion
This study was the first to report prevalence of PAOD in patients with CML in Thailand, which was 9% by ABI. The gold standard of PAOD detection is the use of angiogram; however, that method is invasive, carries the risk of contrast-induced nephropathy, and might be exposed to anaphylactoid
Table 1. Clinical variables and PAOD-related risk factors in patients with CML.

| VARIABLES                              | CASE, TOTAL | IM, N=61 (%) | NIL, N=13 (%) | DAS, N=4 (%) | P VALUE |
|----------------------------------------|-------------|--------------|--------------|-------------|---------|
| Age, y, median (range)                 | 55 (21-86)  | 54 (21-83)   | 68 (25-86)   | 60 (52-81)  | .053    |
| Male gender                            | 41 (52.6)   | 33 (54.1)    | 6 (46.2)     | 2 (50)      | .868    |
| BMI, kg/m², median (range)             | 22.8 (14.4-31.3) | 23.4 (17.3-31.3) | 22.2 (14.4-28.8) | 21.0 (19.5-25.5) | .218    |
| Previous medical illness               |             |              |              |             |         |
| Hypertension                           | 16 (20.5)   | 13 (21.3)    | 3 (23.1)     | —           | .888a   |
| Diabetes mellitus                      | 10 (12.8)   | 7 (11.5)     | 3 (23.1)     | —           | .267a   |
| Dyslipidemia                           | 21 (26.9)   | 13 (21.3)    | 6 (46.2)     | 2 (50)      | .108    |
| Concurrent medications                 |             |              |              |             |         |
| Antihypertensive                       | 9 (11.5)    | 8 (13.1)     | 1 (7.7)      | —           | .587a   |
| Antiplatelet                           | 2 (2.6)     | 2 (3.3)      | —            | —           |         |
| Lipid-lowering agents                  | 4 (5.1)     | 3 (4.9)      | 1 (7.7)      | —           | .688a   |
| Blood chemistry, median                |             |              |              |             |         |
| FPG, mg/dL                             | 96 (77-222) | 95 (80-165)  | 90 (77-222)  | 100 (98-111)| .475    |
| HbA₁C, g/dL                            | 5.5 (4.2-10.5) | 5.4 (4.2-9.4) | 5.7 (4.6-10.5) | 5.4 (4.7-5.8)| .133    |
| Triglycerides, mg/dL                   | 107 (39-2371) | 105 (39-2371) | 124 (56-228) | 130.5 (79-273)| .752    |
| Total cholesterol, mg/dL               | 166 (81-318) | 154 (81-297) | 206 (137-318) | 223 (217-230)| <.001   |
| LDL-C, mg/dL                           | 99.5 (25-233) | 92 (25-185)  | 135 (80-233) | 157.5 (152-168)| <.001   |
| HDL-C, mg/dL                           | 51 (23-92)  | 51 (23-92)   | 58 (47-91)   | 48 (31-56)  | .037    |
| Metabolic syndrome                     | 15 (19.2)   | 12 (19.7)    | 2 (15.4)     | 1 (25)      | .897    |
| Duration of TKI, mo, median (range)    | 80 (3-233)  | 89.6 (1.9-194) | 46.7 (2.9-67) | 22.1 (4.7-45.3) | <.001  |
| Line of treatment                      |             |              |              |             | <.001   |
| First line                             | 61 (78.2)   | 61 (100)     | —            | —           |         |
| Second line                            | 13 (16.7)   | 13 (100)     | —            | —           |         |
| Third line                             | 4 (5.1)     | —            | —            | 4 (100)     |         |

Abbreviations: BMI, body mass index; CML, chronic myeloid leukemia; DAS, dasatinib; FPG, fasting plasma glucose; HbA₁C, hemoglobin A₁C; HDL-C, high-density lipoprotein cholesterol; IM, imatinib; LDL-C, low-density lipoprotein cholesterol; NIL, nilotinib; PAOD, peripheral artery occlusive disease; TKI, tyrosine kinase inhibitor.

aWhen comparing only the IM group with the NIL group.

Table 2. Seven cases of CML with pathologic ABI (<0.9).

| SUBJECT* | VASCULAR IMAGING | GENDER/AGE | DM/HT/DLP | TKIS, MG/D, DURATION |
|----------|------------------|------------|-----------|----------------------|
| 006      | CTA: normal      | F/50       | —/—/—    | IM-400, 116 mo       |
| 035      | Ultrasound Doppler: normal | M/59 | +/+/-    | IM-400, 119 mo       |
| 072      | Ultrasound Doppler: normal | M/58 | —/+/+    | IM-400, 42 mo        |
| 012      | Ultrasound Doppler: positiveb | F/73 | —/+/+    | NIL-800, 62 mo       |
| 043      | CTA: positiveb   | M/86       | —/—/—    | NIL-800, 67 mo       |
| 010      | Ultrasound Doppler: normal | F/55 | —/+/-    | NIL-800, 55 mo       |
| 091      | Ultrasound Doppler: normal | M/70 | +/-/—    | NIL-800, 62 mo       |

Abbreviations: CML, chronic myeloid leukemia; CTA, computed tomography angiography; DLP, dyslipidemia; DM, diabetes mellitus; F, female; HT, hypertension; IM, imatinib; M, male; NIL, nilotinib; TKIs, tyrosine kinase inhibitors.

− indicates nondiagnosed; + indicates diagnosed.

*All subjects were asymptomatic and MMR (major molecular response, MR1.5 by real-time quantitative polymerase chain reaction method).

*Occlusion or significant stenosis of lower leg arteries.
reaction with infusional contrast media. Less invasive procedures have been invented in various methods, with one of the most generally used being ABI. Peripheral artery occlusive disease screening by ABI has given values lower than 0.9, with sensitivity in the range of 15% to 79%, specificity in the range of 83.3% to 99%, and accuracy in the range of 72.1% to 89.2%. The prevalence of PAOD in this study was less than that reported by previous reports which predominantly measured the same by ABI, and the average was 20%. This might be explained by the difference in ethnicities, body mass indexes, cardiovascular risk factors, and numbers of populations in the various studies. For the risk of PAOD among 3 kinds of TKIs, nilotinib uncovered an OR of 8.59 (95% CI: 1.64-44.89; \( P = .004 \)) which harmoniously correlates to the findings of previous studies which reported about 10.3-fold to 14.6-fold higher risk of PAOD in the case of nilotinib compared with imatinib. Recently, a review of PAOD screening in patients using only nilotinib showed abnormal ABI or pulse wave velocity in 19.3%, which strongly implicates nilotinib therapy as being proatherogenic. Moreover, results of pathologic ABI have reported up to 3.2-fold increase in cardiovascular morbidities in type 2 diabetes, and we also found that the only independent risk for PAOD was \( HbA1c \) being more than 7 g/dL (OR: 2.41; 95% CI: 1.11-5.25; \( P = .026 \)). This finding reveals the close relationship between PAOD and diabetes mellitus, too. Some of the previous reports of PAOD in CML in comparison with this study are presented in Table 4.

With reference to earlier PAOD screening development, stiffness of vasculature was one of the interests. The arterial stiffness usually appears before vascular obstruction. This is because the inflammatory process causes intima media degradation, increase in collagen, calcification, and proteolytic enzymes, resulting in elastin damage and arterial occlusion. However, the method of measuring arterial stiffness is not widely used because of inconclusive data for cutoff level and the procedure not being available in most of the centers in our country.

The effect of protein kinase inhibition on endothelium and myocardium has been studied in patients with metastatic renal cell carcinoma who used sunitinib and sorafenib as TKIs for the therapeutic scheme. These studies showed evidence of vasculopathies with circulatory disturbance. As far as nilotinib is concerned, it not only inhibited KIT kinase and PDGFR kinase, which are actions similar to imatinib, but also inhibited the discoidin domain receptor 1 (DDR1) which resulted in plaque formation and atherosclerosis in mouse models. The cellular mechanism of DDR1 inhibition also enhanced the

Table 3. Peripheral artery occlusive disease prevalence compared with control population.

| DATA                  | CASE (N = 78) | CONTROL (N = 156) | P VALUE |
|----------------------|---------------|-------------------|---------|
| Age, y               | 55 (21-86)    | 54 (21-83)        | .342    |
| Male gender          | 52.6          | 52.6              | 1.000   |
| BMI, kg/m²           | 22.8 (14.4-31.3) | 23.7 (15.4-40.4) | .085    |
| Previous illness     |               |                   |         |
| Hypertension         | 20.5          | 21.8              | .822    |
| Diabetes mellitus    | 12.8          | 12.8              | 1.000   |
| Dyslipidemia         | 26.9          | 25.0              | .751    |
| Blood chemistry, mg/dL |             |                   |         |
| FPG                  | 96 (76-222)   | 99 (79-231)       | .249    |
| Triglycerides        | 107 (39-2371) | 108 (37-603)      | .835    |
| Total cholesterol    | 166 (81-318)  | 204 (51-363)      | .178    |
| LDL-C                | 99.5 (25-233) | 138 (56-289)      | .018    |
| HDL-C                | 51 (23-92)    | 48 (17-92)        | .014    |

Abbreviations: BMI, body mass index; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

*Values are expressed as median (range) or percentages.
possibility of diabetes mellitus with evidence of elevated serum amylase and beta cells indicative of dysfunction of the pancreas. Nilotinib may exert proatherogenic and antiangiogenic effects on endothelial cells. Arterial stenosis resulting from the proatherogenesis and the antiangiogenesis of the drug may inhibit the repair mechanism of recanalization and reperfusion. Recently, a review on vascular adverse events (VAEs) in TKI-treated patients with CML also reported the frequency of VAEs in nilotinib users as being in the range of 1% to 29%, including PAOD in 1% to 20%, and it also found the number of patients developing VAEs increasing over time.

To the best of our knowledge, this study was the first case-matched control design study on PAOD risk factor assessment in CML. Interestingly, matched control of age and diabetes had greater LDL-C and lower HDL-C levels, and the PAOD prevalence in CML individuals appeared to be higher for ABI among various kinds of TKIs patients with in CML. Hypercholesterolemia might not be a PAOD predictive risk, or TKIs may play a greater role in vascular inflammation than higher levels of cholesterol in circulation. However, the type of TKI was not found to have an influence on PAOD risk when analyzed using multivariate analysis.

Limitations

This was a cross-sectional case-control, single-institution study that had a small sample size. Because of the limited resources and reimbursement issues in our center, imatinib was the only first choice for therapy in patients with chronic phase CML, with the second line of treatment and the third line of treatment being scheduled on nilotinib and dasatinib, respectively. There was inequality in the number of patients between the 3
kinds of TKIs, which might have caused misleading in the
interpretation of results. This trial was not analysed on sex and
aged effects causing PAOD, menopause or andropause might be
related factors, number of subjects was inadequate power.
Larger number of subjects should be provided to address these outcomes.

Conclusions
The investigation regarding prevalence of PAOD using ABI
found that it was higher among patients with CML than in the
control population. Patients with CML receiving TKIs
and who had diabetes have a higher chance of developing
PAOD and should be carefully monitored for this complica-
tion. Peripheral artery occlusive disease screening might be
made pretreatment schema for patients with CML who have
levels of HbA1c higher than 7 g/dL, especially in cases
where nilotinib is planned to be included in the long-term
treatment.

Acknowledgements
The authors thank all of the patients who participated in this
study and collaborators from the Division of Cardiology,
Department of Internal Medicine, Chiang Mai University,
Thailand, who provided the facilities.

Author Contributions
TR, AT, ER, CC-A, SH, AP, WW, SG, and LN conceived
and designed the experiments; agree with manuscript results
and jointly developed the structure and arguments for
the paper; made critical revisions and approved final version;
and reviewed and approved the final manuscript. TR, AP, WW,
and LN analyzed the data. TR, AT, ER, and LN wrote the first
draft of the manuscript. TR, AT, ER, AP, and LN contributed
to the writing of the manuscript.

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