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Analysis of imatinib for therapeutic drug monitoring in patients with adjuvant and neoadjuvant therapy for gastrointestinal stromal tumours using UPLC-MS/MS

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Abstract

In China, 1100 ng/mL is used as the recommended threshold for imatinib Cssmin in therapeutic drug monitoring (TDM) for metastatic recurrence/unresectable gastrointestinal stromal tumors (GIST) patients. However, there are few studies on characteristics of imatinib Cssmin for adjuvant therapy after complete resection of GIST and neoadjuvant therapy. Consider individual differences of GIST patients, analysis of imatinib in patients with adjuvant and neoadjuvant treatment using a quantitative ultra-performance liquid chromatography coupled with tandem massspectrometry (UPLC-MS/MS) method was conducted in this study. This developed method showed good selectivity and reliability. 25 blood samples collected from October 2019 to October 2021 in 16 patients with adjuvant therapy and 9 patients with neoadjuvant therapy were determined. In the neoadjuvant treatment group, all patients initially received 400 mg/d imatinib. The range of the imatinib steady-state plasma concentration (Cssmin) was 1083-4722 ng/mL. In the adjuvant treatment group, the imatinib Cssmin was in the range of 584-2692 ng/mL after patients given at a dosage of 200, 300, 400 and 600 mg/d. There is no statistical difference in Cssmin between two groups after receiving 400 mg/d imatinib (p=0.402). Among all patients given 400 mg/d imatinib, the Cssmin was significantly correlated with gender (P=0.016).

1 Introduction

Gastrointestinal stromal tumor (GIST) is a relatively rare tumor, but it is the most common stromal tumor of the gastrointestinal tract with complicated biological manifestations. It can occur anywhere from the esophagus to the rectum.[1] The development of this tumor is related to increased tyrosine kinase activity caused by multiple genetic mutations, such as mast/stem cell growth factor receptor kinase (c-Kit) and platelet-derived growth factor (PDGF).[2] GIST is not sensitive to conventional chemotherapy with its efficacy less than 5%.[3]Meanwhile, the efficacy of radiotherapy has not been clinically proven.[4] Even with complete surgical resection of the primary tumor, a high recurrence rate, higher than 50% within 2 years, still is observed.[5]

Imatinib mesylate is one of orally small molecule tyrosine kinase inhibitors.[6] BCR-ABL fusion protein and the tyrosine kinases for PDGF and c-Kit can be selectively inhibited by this drug.[7,8] Therefore, imatinib was developed as first-line standard therapy in patients with unresectable, metastatic, or recurrent GIST.[9] Adjuvant imatinib therapy has dramatically reduced relapse rate, mortality and prolonged the overall survival rates of patients with GISTs.[10,11] However, there are still some GIST patients with large tumors or tumor located in special areas, such as esophagogastric junction, duodenum, rectum), which may cause difficulties in surgical resection or require combined multiple organ resection, and even lead to permanent lifestyle changes of patients.[12] For such patients, preoperative imatinib neoadjuvant therapy can effectively shrink the tumor,

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strive for the opportunity of complete resection, protect the surrounding organs, and reduce the difficulty of surgery.[13]

Despite the apparent clinical activity of imatinib, there is a high drug resistance rate in patients with GIST treated with imatinib, and 10% to 15% of patients have primary drug resistance to imatinib. Patients with effective imatinib therapy in the past may also have secondary drug resistance after imatinib treatment.[14,15] Meanwhile, patients taking standard doses of imatinib have significantly different response. Adverse reactions included diarrhea, edema, muscle cramps still be observed as the dosage of 400 mg/d imatinib is generally withstood for the bulk of patients.[16] Clinical efficacy has been shown to be related to trough plasma levels (C_{ssmin}) in patients with chronic myelogenous leukemia (CML) in chronic phase or advanced unresectable GIST.[17,18] Thus it is necessary to implement TDM under imatinib treatment. The threshold of 1100 ng/mL has been perceived to be an important indicator of higher response rates for advanced unresectable GIST by some prospective clinical trials.[18,19] In China, 1100 ng/mL is used as the recommended threshold for imatinib C_{ssmin} for metastatic recurrence/unresectable GIST patients by Chinese Society of Clinical Oncology.[20] However, there are few studies on the threshold of imatinib C_{ssmin} for adjuvant imatinib treatment following by complete turmor resection and neoadjuvant treatment. Consider individual differences of GIST patients with advanced unresectable GIST and adjuvant treatment or preoperative imatinib neoadjuvant treatment, whether imatinib imatinib C_{ssmin} threshold of 1100 ng/mL can be used to guide adjuvant imatinib therapy or preoperative imatinib neoadjuvant therapy remains to be discussed.

Xia et al. studied the profile of C_{ssmin} of imatinib at different doses. A broad ranges of the C_{ssmin} (421-7493 ng/mL, 1103-3775 ng/mL, 2303-5017 ng/mL) were observed in patients given 400, 600 and 800 mg/d imatinib, respectively.[17] The high variability in imatinib C_{ssmin} manifests that C_{ssmin} may be affected by a variety of factors, such as mainly resulted from genetic, demographic, and environmental factors. In addition, some of adverse reactions have been proven dose-related.[14] Considering TDM of imatinib in clinic has not been fully promoted in China, it is necessary and urgent to investigate related factors of imatinib C_{ssmin} in Chinese GIST patients to improve therapeutic effect.

The aim of this study was to develop an UPLC-MS/MS method for analysis of imatinib C_{ssmin} in human plasma. Then it was applied for routine TDM in GIST patients with adjuvant imatinib therapy and preoperative imatinib neoadjuvant therapy.

2 Material and methods

2.1 Chemicals and Reagents

Imatinib (No. YM-160604) and apatinib (internal standard, IS, No. 668160505) were kindly supplied by Jiangsu Hengrui Medicine Co., Ltd. (Jiangsu, China). Formic acid and ammonium acetate were obtained from Mreda Technology Inc. (Dallas, TX, USA). Ultrapure water was purchased from A.S. Watson & Company (Guangzhou, China). Methanol and acetonitrile were purchased from Fisher Scientific (Fair Lawn, NJ, USA).

2.2 Instrumentation and Conditions

Analysis of imatinib in human plasma was achieved with the usage of ultra-high performance liquid chromatography-mass spectrometry tandem system (AB Sciex, USA) consisting of quaternary ultra-high pressure infusion pump (ExionLC AD), column oven (ExionLC AC), autosampler (ExionLC AD) and AB Sciex Triple Quad^{TM} 5500 mass spectrometer (Qtrap 5500). Imatinib and IS were separated on Waters BEH C_{18} column (2.1×50 mm, 1.7 μm; Waters, USA) at a temperature of 45°C. The mobile phase consisted of formic acid (0.1%) and acetonitrile with constant flow rate of 0.3 ml/min. Imatinib and IS were separated under gradient elution as follows: 0-6 min, 20%-80% B; 6-8 min, 80% B; 8-8.1min, 80%-20% B; 8.1-9 min, 20%.

The ionization of imatinib and IS was performed on an ESI^+ source and monitored in MRM mode with the m/z of 494.3-394.1 and 398.2-212.1, respectively. Product ion spectrum of the analytes was indicated in Figure 1. The ion source parameters comprised of ion source temperature of 500, capillary voltage of 5500

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V, Gas1 50 psi, Gas2 50 psi. The voltages of collision energy (CE) for imatinib and IS were optimized as 36 V and 41 V, respectively. The voltages of declustering potential (DP) were 100 V and 70 V.

2.3 Calibration Standards and Quality Control (QC) Samples

10 mg of imatinib was precisely weighed and then dissolved with methanol to prepare imatinib calibration standard and QC stock solution with a concentration of 1 mg/mL. The calibration standard stock solution was diluted with 50% methanol into a series of calibration standard solutions with concentrations of 2000, 1000, 500, 200, 100, 50, 20 and 10 ng/mL, and the QC stock solution was diluted into the solutions with concentrations of 1800, 300, 30 and 10 ng/mL in the same way. 10 mg of IS was precisely weighed and then dissolved with methanol to prepare IS stock solution with a concentration of 1 mg/mL. The IS solution of 10 ng/mL was obtained by dilution with acetonitrile used as precipitation agent.

2.4 Sample Preparation

50 μL of blank plasma samples were transferred to a 1.5 mL centrifuge tube, and then 50 μL of imatinib calibration standard solutions or QC solutions at different concentrations and 600 μL of precipitation agent were added successively. The mixture was vortex-mixed for 3 min, and centrifuged for 10 min at 15000$\times$g. 50 μL of the supernatant and 300 μL of 50% methanol were mixed and vortexed for 1 min. Subsequently, the mixture was centrifuged for 3 min under the same centrifugal condition and 3 μL were injected into UPLC system.

2.5 Method validation

The method validation was carried out for specificity, linearity, accuracy, precision, carryover, matrix effect, recovery, stability, dilution study following FDA guidelines and Chinese Pharmacopoeia.[21]

Six different lots of blank plasma samples were selected for the specificity assessment. Double blank plasma sample, blank plasma sample spiked with imatinib at LLOQ (10 ng/mL), plasma sample of the patient after 22.3 h of the last imatinib administration were processed to investigate possible endogenous interference. The response of the interference in blank plasma sample should be less than 20% of 10 ng/mL imatinib and 5% of IS.

Linearity was determined by plotting the peak area ratio (imatinib/IS) response versus the plasma concentrations of imatinib over the range from 10 to 2000 ng/mL. The weighting factor ($1/x^2$) of each calibration curve was used and should be >0.99. The accuracy (relative error, RE, %) of calibration standards should be 85%-115% and 80%-120% for LLOQ .

For inter- and intra- precision and accuracy studies, samples were prepared at 10, 30, 300, 1800 ng/mL with five replicates each analyzed in three consecutive runs. The deviation between the actual concentration and measured concentration was calculated to assess accuracy of imatinib, and it should be within $\pm$15% for 30, 300, 1800 ng/mL and $\pm$20% for LLOQ (10 ng/mL). The precision was expressed as relative standard deviation (RSD,%) and it should be <15% for 30, 300, 1800 ng/mL and <20% for LLOQ (10 ng/mL).

Carryover was assessed by injecting a blank plasma sample after the highest concentration calibrator (2000 ng/mL) in three independent runs. The response of the blank plasma sample should not >20% of the LLOQ and >5% of IS.

Recovery of imatinib were both determined at LQC, MQC and HQC concentration levels with five replicates each. The procedure was carried out according to the section of "sample preparation", and the peak area was recorded as A. In addition, blank plasma plasma was taken and prepared according to the section of "sample preparation". LQC, MQC and HQC solutions were added to the obtained supernatant, and the corresponding peak area was calculated as B. Recovery was assessed by the ratio of A to B. Matrix effect was calculated by the ratio of peak area obtained by adding LQC, MQC and HQC solutions to post-extracted blank plasma and peak area by added to water. Recovery and matrix effect of IS were also evaluated.

Three QC samples with 30, 300, 1800 ng/mL imatinib were taken, and five samples were measured in parallel

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for each concentration. The stability was investigated when untreated plasma samples were placed at room temperature for 4 h, repeated freeze-thaw cycles for 3 times, placed in -80 for 350 days and treated plasma samples at the autosampler for 24 h. The stability of the analyst in working solution was investigated for 14 days at -80 with the same concentration level.

Imatinib standard solution with the concentration greater than 2000 ng/mL was added to blank plasma. Then five replicates of this QC sample prepared in parallel were analyzed after being diluted 10 times with blank plasma to assess dilution stability.

2.6 Application of the method in Therapeutic Drug Monitoring

A total of 25 patients who were receiving adjuvant and neoadjuvant treatment with imatinib for GIST in the Fourth Hospital of Hebei Medical University, from October 2019 to October 2021, were enrolled in this study. The study was approved by the Ethics Committee of the Fourth Hospital of Hebei Medical University (No. 2019049) and and conducted in accordance with the Basic & Clinical Pharmacology &

Toxicology policy for experimental and clinical studies.[22] Written informed consents were obtained from themselves prior to their participation in the study. The exclusion criteria were age <18 years old; severe organ dysfunction; recurrence or distant metastasis at the initial diagnosis; poor compliance. Patients taking imatinib continuously at a daily dose for [?]7 days to reach imatinib steady-state concentration. Blood samples were collected into heparinized tubes before the next dosing. After centrifugation for 10 min at 3000xg, the supernatant was divided and stored in the refrigerator at -80 for testing.

Clinical data were recorded including demographic data (age, gender, height, weight), duration of medication, concomitant medication, time of blood sampling, primary tumor site, type of gene mutation, surgery, adverse reactions (nausea, vomiting, leukopenia, anemia, limb edema, periorbital edema, skin). Adverse reactions were graded according to the Common Terminology Criteria for Adverse Events, version 5.0.

2.7 Statistical Analysis

SPSS software version 21.0 was used for statistical analysis. Independent samples t-test was performed on the quantitative data which conform to the normal distribution.The data were expressed as mean+-standard deviation (SD). Otherwise, Manner-Whitney U test was used for analyzing the differences of clinical characteristics between two groups and the data were expressed as median (maximum-minimum). $p<0.05$ was considered statistically significant.

3 Results

3.1 Method evaluation

The separation of imatinib and IS was satisfactory and free of interfering peaks in the biological matrix. The retention time of imatinib and IS was 1.4 and 2.5 min, respectively (Figure 2). There was a good linear relationship between the concentration of imatinib (10-2000 ng/mL) in plasma and the ratio of peak area with R^2 greater than 0.99. The intra-batch and inter-batch precision, expressed as RSD%, about 10, 30, 300 and 1800 ng/mL QC samples were less than 4.72%. Accuracy for intra-batch and inter-batch assays ranged from 105.07-108.60% (Table 1). The matrix effect for 30, 300 and 1800 ng/mL imatinib and IS were 106.36+-3.98%、104.38±2.37%、102.22±3.39%、99.92±2.01%, along with RSD% less than 3.8%. The recovery were 100.76±3.08%, 104.21±3.30%, 102.68±3.65% and 99.01±4.39%, respectively. The RSD values were less than 3.6%. The stability of imatinib at different conditions conformed to the verification guidelines of biological sample quantitative analysis methods, and the RSD values were less than 5.83%. The accuracy ranged from 92.17 to 105.96%. In addition, work-solution stability of imatinib for 14 days was also assessed and the accuracy was within ±15% (Table 2). The average accuracy was 107.36% for 10-fold dilutions with RSD values less than 3.2%. The imatinib C_{ssmin} would not be affected as plasma samples processed with dilution pre-treatment.

3.2 Patient characteristics

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Twenty-five samples from 25 patients included in the study were analysed. According to the evaluation of relevant examination results, 9 patients received preoperative imatinib adjuvant treatment (neoadjuvant treatment group) because of large volume of tumor, special location or invasion of surrounding organs, and the difficulty of complete resection, while 16 patients directly underwent surgical resection followed by imatinib chemotherapy (adjuvant treatment group).

In the neoadjuvant treatment group, there were 6 males (66.7%) and 3 females (33.3%). The patients were between 34 and 67 years old, with an median age of 57 years. There were 7 patients (77.8%) with primary site of tumor at stomach and 2 patients (22.2%) at small intestine. 7 patients were tested as c-kit 11 gene mutation, and 2 patients without genetic testing. In the adjuvant treatment group, there were 6 males (37.5%) and 10 females (62.5%). The patients were between 42 and 72 years old, with an median age of 58 years. There were 11 patients (68.8%) with primary site of tumor at stomach and 5 patients (31.2%) at small intestine. 10 patients were tested as c-kit 11 gene mutation, 1 patient as c-kit 9 gene mutation and 5 patients without genetic testing. Patient characteristics are summarized in Table 3.

3.3 C_{ssmin} plasma concentration

In the neoadjuvant treatment group, all patients initially received the imatinib doses of 400 mg daily. The mean±SD (ranges) of the imatinib C_{ssmin} were 1858.7±1118.9 ng/mL (range 1083-4722 ng/mL).

In the adjuvant treatment group, 1 patient received 200 mg/d imatinib and the imatinib C_{ssmin} was 823 ng/mL. 4 patients received 300 mg/d imatinib and the mean±SD (ranges) of the imatinib C_{ssmin} were 1216.6±705.9 ng/mL (range 584-2052 ng/mL). 9 patients received 400 mg/d imatinib and the mean±SD (ranges) of the imatinib C_{ssmin} were 1741.0±435.5 ng/mL (range 977.4-2252 ng/mL). 2 patients received 600 mg/d imatinib and the imatinib C_{ssmin} were 2692 ng/mL and 1446 ng/mL, respectively. The distribution of imatinib in 25 GIST patients received neoadjuvant imatinib therapy and adjuvant imatinib therapy was showed in Figure 3.

3.4 Clinical characteristics and Imatinib C_{ssmin}

Currently, the recommended dosage of imatinib targeted therapy is 400 mg/d. The difference between the neoadjuvant treatment group (male=6; female=3) and the adjuvant treatment group (male=4; female=5) was evaluated at the dosage of 400 mg/d. The C_{ssmin} of two groups was similar (p=0.402, figure 4).

Considering no significant difference in C_{ssmin} of imatinib between above two group, correlation between C_{ssmin} and gender, age, Body Mass Index (BMI), medication duration, primary tumor site of 18 patients receiving 400 mg/d imatinib were compared, respectively. As in figure 5, significant difference was observed in C_{ssmin} of imatinib between female and male patients. The median value of women was significantly higher than that of men (1998 ng/mL vs 1468 ng/mL, p=0.016). There was no correlation between C_{ssmin} and age (r=-0.028, p=0.911), BMI (r=0.098, p=0.700), medication duration (r=-0.069, p=0.787).

3.5 Adverse reactions

In the neoadjuvant treatment group, leukopenia appeared in 3 patients (20%), anemia in 1 patients (6.7%), periorbital edema in 7 patients (46.7%), and skin in 4 patients (26.6%). One patient developed grade 3 skin, and one patient developed grade 4 leukopenia. Two patients experienced dose adjustment due to adverse reactions.

In the adjuvant treatment group, nausea and vomiting appeared in 4 patients (10.5%), leukopenia in 6 patients (15.8%), anemia in 3 patients (7.9%), limb edema in 6 patients (15.8%), periorbital edema in 12 patients (31.6%), and skin in 7 patients (18.4%). One patient developed grade 3 leukopenia, and no adverse reaction of grade 4-5 occurred in all patients. Two patients experienced dose adjustment due to adverse reactions.

4 Discussion

Correlation between imatinib C_{ssmin} and clinical efficacy has been confirmed in many studies. Demetri et

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al. considered 1100 ng/mL as the threshold for clinical effect in patients with unresectable or metastatic GIST.[18] Rapid disease progression and low survival benefit have been observed without C_{ssmin} reaching the concentration threshold. Another European multicenter study showed that imatinib C_{ssmin}greater than 760 ng/mL can prolong the progression free survival (PFS) of patients diagnosed with GIST.[23] Therefore, explicit threshold value is a prerequisite for TDM to enhance clinical response, efficacy and safety of imatinib. However, the relationship between imatinib C_{ssmin} and prognosis of patients receiving adjuvant imatinib therapy and preoperative imatinib neoadjuvant therapy is unclear. In our research, the C_{ssmin} of 16 patients with imatinib adjuvant therapy and 9 patients with neoadjuvant therapy were determined by this developed UPLC-MS/MS method, and the characteristics of the C_{ssmin} of the above patients were preliminarily explored.

The recommended dosage of imatinib for adjuvant treatment or neoadjuvant treatment is 400 mg/d. In this research, the average C_{ssmin} of imatinib with neoadjuvant treatment at the dosage of 400 mg/d was 1858.7 ng/mL, and that of adjuvant treatment was 1741.0 ng/mL. Statistical analysis indicated that the C_{ssmin} of imatinib with neoadjuvant treatment was not obvious different from that with adjuvant treatment, which revealed that the C_{ssmin} may not be affected by tumor load. For 18 patients given 400 mg/d imatinib, the median of C_{ssmin}tend to be higher in female than male patients (1998 ng/mL vs 1468 ng/mL, $p<0.05$). Wu et al. also found a significant decrease of approximately 19% in the mean C_{ssmin} of male patients compared to the female. It can be suspected that the C_{ssmin} of imatinib may be related to gender.[24] Meanwhile, imatinib $C_{ssmin}$was found to be obviously related with gender in a Korean study ($p=0.010$).[25] For efficacy, Lv et al. has indicated that male gender proved to be a negative indicator of survival with the 5-year overall survival (OS) for male and female was 83.37% and 87.68%, respectively.[26] Since imatinib is a substrate of P-glycoprotein (P-gp) efflux transporter, the reason for this result may be the difference of this efflux transporter activity in different gender groups.[27,28] Due to the higher activity of P-gp in male patients than the female, more imatinib could be transported from the intracellular to the extracellular, thereby decreasing imatinib concentration in intracellular and reducing clinical efficacy.

Of the 9 patients who received imatinib neoadjuvant treatment, 7 patients mainly had large tumor diameter initially showed in CT, and the other 2 patients were treated before surgery because of the special tumor location and the difficulty of complete resection. The duration of preoperative imatinib neoadjuvant treatment was 5-13 months. The primary tumor of 6 patients were shrinking, that of 2 patients had no significant change, but that of 1 patient was growing. This may be related to unclear initial genotype or secondary mutation during preoperative neoadjuvant treatment. Several studies on imatinib dosage and clinical efficacy show that imatinib is most sensitive to GIST with c-kit exon 11 mutation and also has good efficacy for those with PDGFRA exon 18 non-D842V mutation, while the dosage of imatinib is required to increase for GIST with c-kit exon 9 mutation to achieve considerable clinical efficacy.[29-32] In neoadjuvant treatment group, 1 patient with tumor growth and 1 patient without tumor changing were not tested for gene. The other 7 patients were tested as c-kit 11 gene mutation. Similarly, 1 GIST patients with adjuvant treatment was tested as c-kit 9 gene mutation. After surgical operation given at a dosage of 400 mg/d for 15 months, the dosage increased to 600 mg/d as a result of tumor progression. Therefore, for GIST with indications for neoadjuvant and adjuvant treatment, identification of genotype is important for adjusting drug dosage to improve clinical efficacy.

Imatinib is generally well tolerated, with a low risk of adverse reactions and the occurrence of adverse reactions and severity are often related to the dosage of imatinib.[33] The most common adverse effects include mild to moderate edema, nausea and vomiting, diarrhea, muscle cramps, and rash, while the incidence of myelosuppression and elevated transaminase levels is low.[17] In the imatinib neoadjuvant treatment group, the adverse reactions after imatinib treatment were mainly leukopenia, anemia, periorbital edema, and skin. Only one patient had grade 4 leukopenia requiring drug control. In the imatinib adjuvant treatment group, the adverse reactions were mainly nausea and vomiting, anemia, limb edema, limb edema and skin. No obvious grade 4 and 5 adverse reactions were found. Previous study has showed that the incidence of adverse reactions is positively correlated with the level of imatinib C_{ssmin}.[34,18] There was no obvious relationship between adverse reactions and imatinib C_{ssmin} in this study due to limited sample size of GIST patients.

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However, the determination of C_{ssmin} in Chinese patients using imatinib can effectively reduce the risk of adverse reactions. Fever and neutropenia was caused by high levels of imatinib C_{ssmin} (4722 ng/mL) and not be tolerated in one patient with neoadjuvant treatment. The drug needs to be discontinued, and the dosage will be reduced to 200 mg/d after appropriate treatment. As a result, the C_{ssmin} dropped to 1640 ng/mL without related adverse reactions. After 11 months of targeted therapy, relevant imaging examination showed tumor shrinking.

Among 25 patients in this study, the C_{ssmin} of 20 patients was higher than 1100 ng/mL. For neoadjuvant treatment group, the C_{ssmin} was from 1083 to 4722 ng/mL, and that of 78% patients was within 1100 ng/mL and 2000 ng/mL after receiving 400mg/d imatinib. Meanwhile, tumor shrinking was found in 67% patients to complete resection. For adjuvant treatment group, the C_{ssmin} was in the range of 584 ng/mL and 2692 ng/mL, and that of 75% patients ranged from 1100ng/mL to 2692 ng/mL with the dosage of 200, 300, 400 and 600 ng/mL. During the follow-up, 44% of the patients did not adjust the dosage of imatinib between 4 months and 14 months. It is assumed that the C_{ssmin} threshold of 1100ng/mL is applicable to most GIST patients in our study. It is impossible to determine the explicit C_{ssmin} threshold of imatinib with adjuvant treatment and neoadjuvant treatment by reason of the limited small number of patients. The sample size can be expanded to further to explore the characteristics of imatinib C_{ssmin} in special populations.

5 Conclusion

In our study, a UPLC-MS/MS method for analysis of imatinib was established. Under the optimized experimental conditions, this method showed good selectivity and reliability and has been used for routine imatinib monitoring for patients receiving adjuvant and neoadjuvant therapy for GIST. We found that gender may be the affecting factor of imatinib C_{ssmin}. Whether the imatinib C_{ssmin} threshold of 1100 ng/mL could be contributed to oncotherapy for patients with adjuvant and neoadjuvant therapy for GIST should be further studied.

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Conflict of interest

The author reports no conflicts of interest in this work.

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Preparation concentration (ng/mL)	Intra-day precision	Intra-day precision	Intra-day precision	Inter-day precision	Inter-day precision	Inter-day precision
	Measured concentration (ng/mL)	RSD (%)	Accuracy (%)	Measured concentration (ng/mL)	RSD (%)	Accuracy (%)
10	10.51±0.50	4.72	105.07	10.72±0.43	4.05	107.22
30	31.76±1.01	3.18	105.87	31.89±0.82	2.58	106.28
300	320.00±6.04	1.89	106.66	316.56±6.51	2.06	105.52
1800	1935.40±38.82	2.01	107.52	1954.80±45.16	2.31	108.60

Table 1 Evaluation of the inter- and intra-batch precision by the proposed UPLC-MS/MS method for determination of imatinib in human plasma (mean ± SD, n=5)

Table 2 Evaluation of stability of imatinib in human plasma and work-solution (Mean ± SD, n=5)

Condition Test	LQC(30 ng/mL) Mean conc. (ng/ml)	LQC(30 ng/mL) RSD (%)	LQC(30 ng/mL) Accuracy (%)	MQC(300 ng/mL) Mean conc. (ng/ml)	M R
Room temperature for 4h	30.25±1.17	3.87	100.81	302.94±7.78	2.

Autosampler for 24h	27.65±0.92	3.32	92.17	286.20±12.11	4.
3-freeze thaw cycles at -80	31.54±1.68	5.34	105.12	301.02±17.54	5.
-80 for 350 days	30.50±0.78	2.57	101.67	305.80±8.22	2.
Work-solution stability for 14 days	29.37±1.23	4.17	97.91	313.28±13.82	4.

Table 3 Clinical Characteristics of the Enrolled Patients

Clinical Characteristics	No. of Patients (%) neoadjuvant treatment group (n=9)	No. of Patients (%) adjuvant treatment group (n=16)
Gender		
Male	6 (66.7)	6 (37.5)
Female	3 (33.3)	10(62.5)
Age:Median [range], y	57[34-67]	58[42-72]
Weight: Median [range], kg	67[52-90]	61.5[45-85]
Height:Median[range],cm	168[152-177]	165[150-178]
Primary site		
Stomach	7(77.8)	11(68.8)
Small intestine	2(22.2)	5(31.2)
Kinase mutation		
c-kit 11 mutation	7(77.8)	10(62.5)
c-kit 9 mutation	-	1(6.3)
unknown	2(22.2)	5(31.2)
Dosage		
200 mg/d	-	1(6.3)
300 mg/d	-	4(25.0)
400 mg/d	9(100)	9(56.2)
600 mg/d	-	2(12.5)
Medication duration:Median[range],d	110[30-300]	39[8-420]

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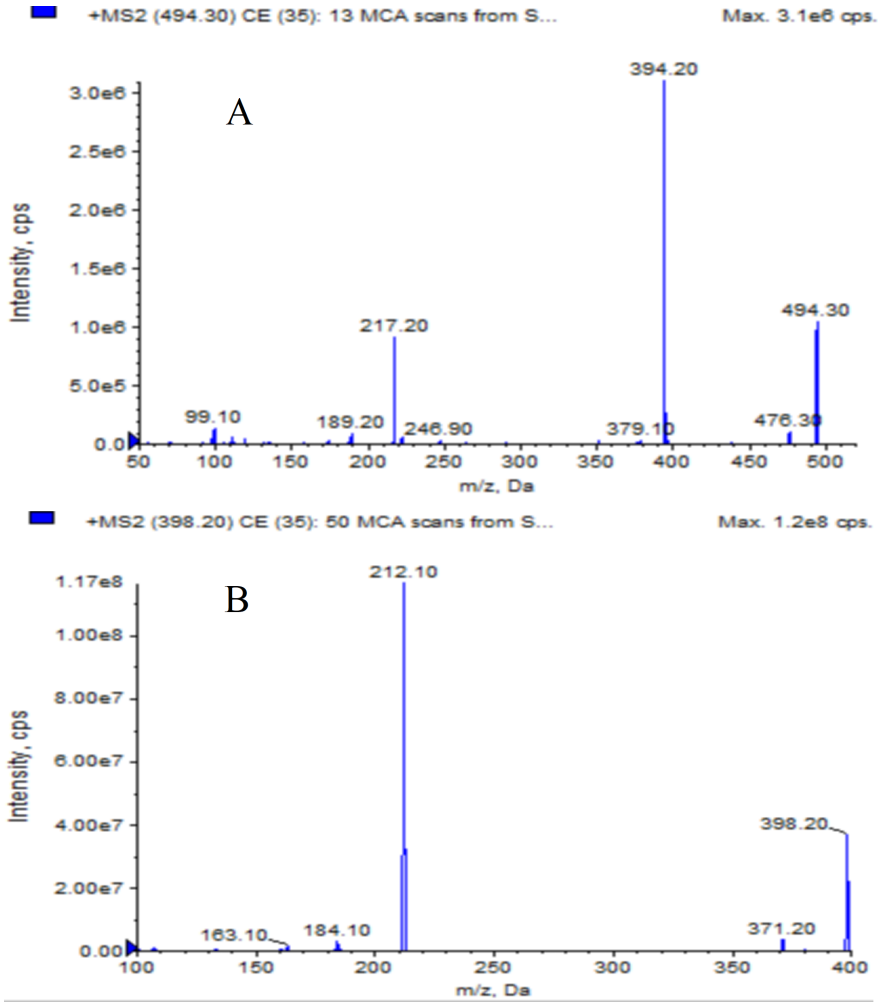

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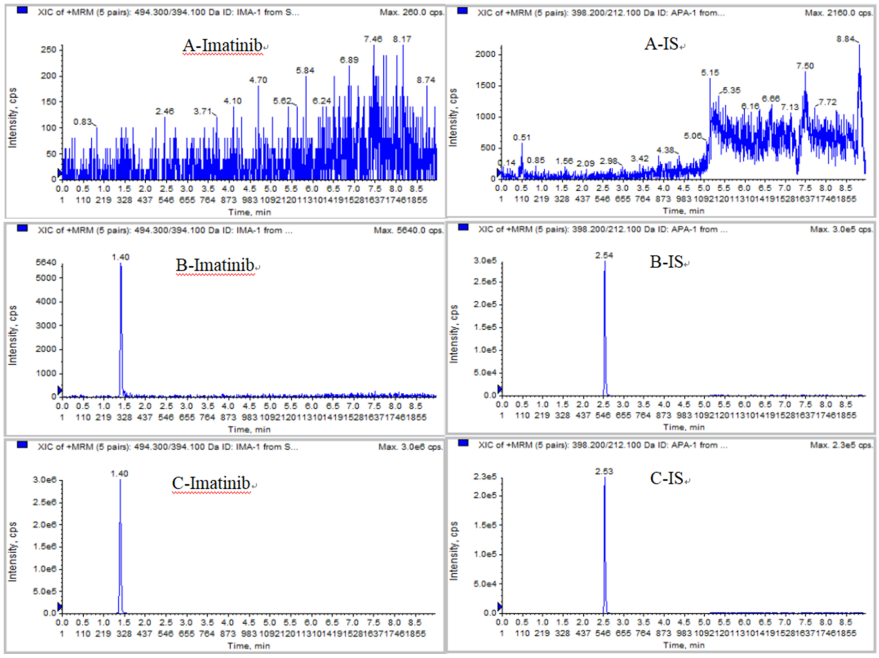

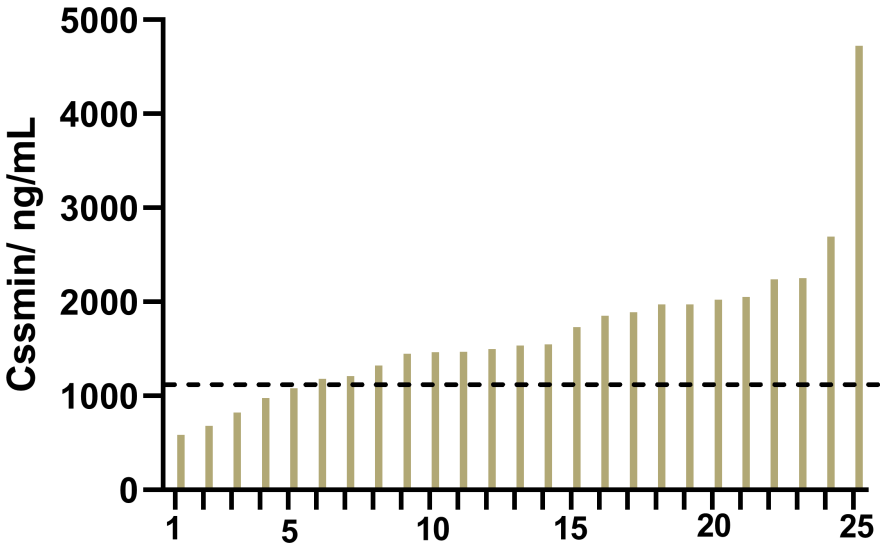

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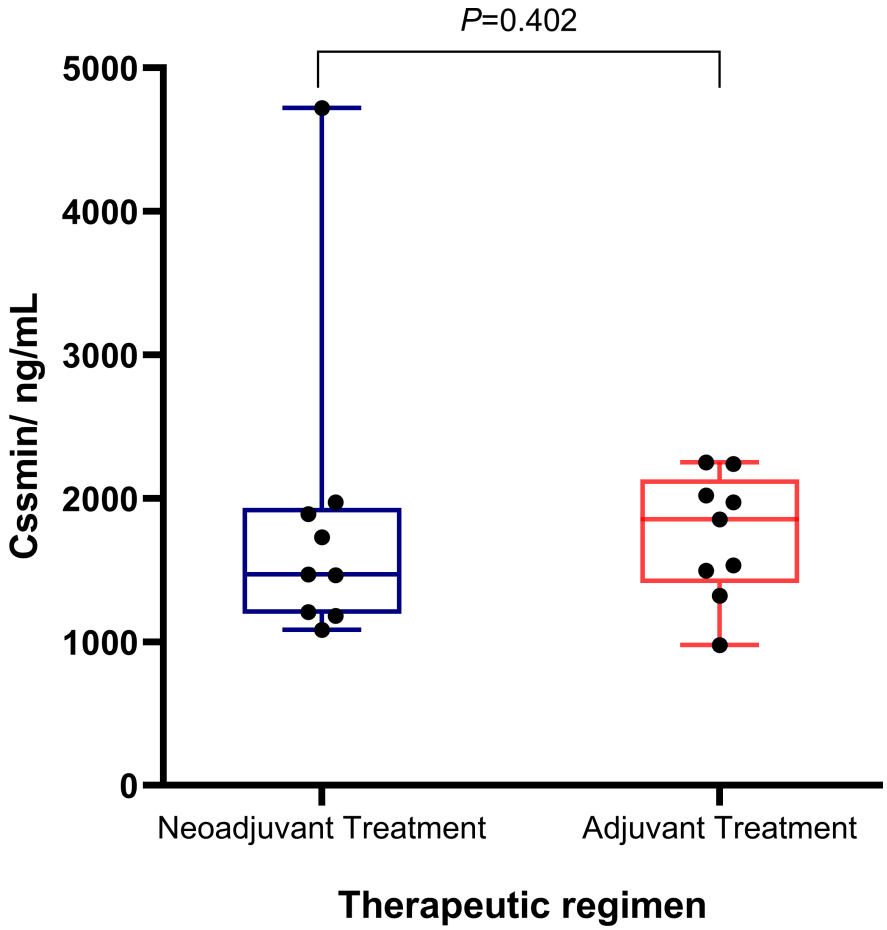

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