Klinička značajnost otkrivanja mutacije T315I u domeni ABL kinaze kod bolesnika rezistentnih na liječenje imatinib mesilatom

Clinical significance of T315I ABL kinase domain mutation detection in patients resistant to imatinib mesylate therapy

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Sažetak

Uvod: Kronična mijeloična leukemija (engl. *chronic myeloid leukemia*, CML) je mijeloproliferativna bolest koju karakterizira prisutnost fuzijskog gena bcr-abl i posljedično fuzijskog proteina bcr-abl. Iako je otkriće inhibitora tirozin-kinaze (engl. *tyrosine kinase inhibitor*, TKI), imatinib mesilata (IM) poboljšalo liječenje bolesnika oboljelih od CML, dio bolesnika razvija rezistenciju na lijek, što dovodi do povišene razine bcr-abl prijepisa. Jedan od mogućih razloga te rezistencije su mutacije u domeni ABL kinaze. Neke se mutacije mogu prevladati povećanjem doze lijeka ili primjenom nove generacije TKI. Jedina mutacija rezistentna na trenutno dostupne TKI jest T315I. Cilj ovog istraživanja bio je otkriti da li je prisutnost T315I kod bolesnika rezistentnih na liječenje imatinib mesilatom povezana s povećanom ili neprekidno visokom razinom bcr-abl prijepisa. Također, cilj je bio procijeniti moguću razliku u razini bcr-abl prijepisa kod bolesnika rezistentnih na liječenje imatinib mesilatom sa i bez T315I mutacije.

Ispitanici i metode: U ispitivanje su bila uključena 24 bolesnika oboljela od CML s neodgovarajućim odgovorom na liječenje imatinib mesilatom. Provedena je kvantitativna lančana reakcija polimerazom u stvarnom vremenu (engl. *real time quantitative polymesare chain reaction, RQ-PCR*) prema protokolu udruženja *Europa protiv raka* (engl. *Europe Against Cancer*), a za dokazivanje mutacije T315I rabljena je metoda alel specifične oligonukleotidne PCR (engl. *allele specific oligonucleotide PCR*, ASO PCR).

Rezultati: Kod 4 od 24 bolesnika dokazana je mutacija T315I (17%). Izračunat je i medijan omjera bcr-abl/abl koji je iznosio 19% za bolesnike s T315I mutacijom i 13% za bolesnike bez mutacije, no razlika između tih dviju skupina nije bila statistički značajna (P = 0,394)

Zaključak: Dokazivanje prisutnosti mutacije T315I ključno je u terapijskom pristupu bolesnicima s CML, budući da je liječenje izbora za nositelje te mutacije transplantacija matičnih stanica.

Ključne riječi: kronična mijeloična leukemija; rezistencija na imatinib mesilat; mutacija T315I

Abstract

Background: Chronic myeloid leukemia (CML) is a myeloproliferative disease characterized by the presence of bcr-abl fusion gene and consequently bcr-abl fusion protein. Although the discovery of tyrosine kinase inhibitor (TKI), imatinib mesylate (IM), improved the treatment of CML patients, a proportion of patients develop resistance to the drug resulting in increased bcr-abl level. One of possible reasons for resistance are the mutations in ABL kinase domain. Some mutations can be overcome by increasing the drug dose or by using the new generation of TKI. The only mutation resistant to currently available TKI is T315I. The aim of this study was to detect if the presence of T315I in patients resistant to imatinib mesylate therapy is associated with the increase or constantly high bcr-abl level. We also aimed to assess the possible difference in bcr-abl level in imatinib-resistant patients with and without T315I mutation.

Materials and methods: The study included 24 CML patients with inadequate response to IM therapy. Real time quantitative PCR was performed according to Europe Against Cancer protocol and allele specific oligonucleotide PCR was used for T315I mutation detection.

Results: T315I was detected in 4 out of 24 patients (17%). Calculated median bcr-abl/abl levels were 19% for T315I positive and 13% for T315I negative patients, but the difference was not statistically significant (P = 0.394).

Conclusions: T315I detection is essential in therapy approach for CML patients as the treatment of choice for T315I carriers is stem-cell transplantation.

K**eywords:** chronic myeloid leukemia; imatinib mesylate resistance; T315I mutation

Pristiglo: 16. studenog 2009. *Received: November 16, 2009*
Prihvaćeno: 16. prosinca 2009. *Accepted: December 16, 2009*

Uvod

Kronična mijeloična leukemija (engl. *chronic myeloid leukemia*, CML) je mijeloproliferatvna bolest karakterizirana kromosomskom translokacijom t(9;22) (q34;q11), poznatom kao filadelfijski kromosom (engl. *Philadelphia chromosome*). Posljedica te translokacije je onkogenetički fuzijski gen bcr-abl koji proizvodi protein bcr-abl s pojačanom aktivnošću tirozin-kinaze. Nova saznanja o CML i otkriće molekularnih putova tirozin-kinaze, doveli su do otkrića „pametnog lijeka", imatinib mesilata (engl. *imatinib mesylate*, IM), koji se kompetitivno veže na vezna mjesta za ATP i specifično inaktivira bcr-abl kinazu (1,2). Uvođenje IM u liječenje CML omogućilo je visoki postotak citogenetičkih i molekularnih remisija kod većine bolesnika (1). Standardno liječenje kronične faze CML uključuje 400 mg IM na dan s citogenetičkim praćenjem postotka stanica pozitivnih na filadelfijski kromosom i molekularnim praćenjem razine *bcr-abl* prijepisa metodom kvantitativnog PCR-a (RQ-PCR) (1,3,4).

Međutim, kod određenog broja bolesnika ili uopće nema odgovora ne terapiju imatinib mesilatom, ili se on s vremenom izgubi. Jedan od mogućih razloga za to su mutacije gena za ABL kinazu koje su otkrivene kod više od 50% bolesnika s CML otpornih na liječenje IM (1).

Postoji nekoliko tipova mutacija koje se mogu podijeliti u četiri skupine temeljem kristalografske strukture cABL: mutacije koje se vežu na katalitičku domenu proteina bcr-abl i tako izravno onemogućuju IM, one koje se nalaze unutar ATP veznog mjesta, one koje se nalaze unutar aktivacijske petlje koja sprečava kinazu kako bi postigla neaktivnu konformaciju potrebnu za vezanje IM i one koje se nalaze unutar katalitičke domene (1).

Do sada su opisane barem 73 različite mutacije kod bolesnika s CML rezistentnih na IM (5). Sve mutacije nemaju istu važnost, neke su čak funkcionalno nevažne (3). Prema odgovoru na liječenje IM, mutacije se dijele na visoko rezistentne mutacije u domeni kinaze (T315I, Y253H/K, E255K/V, H396P/R) i umjereno rezistentne mutacije (M244V, M351T, F359V). Umjereno rezistentne mutacije u domeni kinaze mogu se prevladati povišenom dozom lijeka IM od 600 ili 800 mg na dan (1,3).

Razvoj druge generacije inhibitora tirozin-kinaze (engl. *tyrosine kinase inhibitor*, TKI) nilotiniba i dasatiniba, koji se strukturno razlikuju od IM, koristi bolesnicima nositeljima visoko rezistentnih mutacija, budući da oba lijeka suzbijaju učinak svih poznatih mutacija koje dovode do rezistencije na imatinib, osim mutacije T315I (2,5). To je točkasta mutacija u kojoj dolazi do zamjene C→T, što dovodi do promjene aminokiseline treonina u izoleucin na položaju 315 (Thr315→Ile) u cABL. Thr315 se nalazi na ključnom položaju odgovornom za stvaranje vodikovih veza s IM ili drugim TKI, čineći time lijek djelotvornim. Izoleucin sadrži jednu ugljikovodikovu skupinu više u postraničnom lancu, koja stvara steričke smetnje za vezivanje TKI, ali ne

Introduction

Chronic myeloid leukemia (CML) is a myeloproliferative disease characterized by chromosomal translocation t(9;22) (q34;q11), also known as Philadelphia chromosome. The consequence of this translocation is a bcr-abl oncogenic fusion gene which produces bcr-abl protein with enhanced tyrosine kinase activity. Increased knowledge about CML and the discovery of the molecular pathways of tyrosine kinase has led to the creation of a "smart drug", imatinib mesylate (IM), which competitively binds at the ATP binding site and specifically inactivates bcr-abl kinase (1,2). The introduction of IM in the treatment of CML has enabled high rate of cytogenetic and molecular remission in most patients (1). Standard treatment of chronic phase CML is 400 mg IM per day with cytogenetic monitoring of percentage of Philadelphia chromosome positive cells and molecular monitoring of bcr-abl transcript level by real time quantitative PCR (RQ-PCR) (1,3,4).

However, a proportion of patients fail to respond to IM therapy or lose the response. One of the possible reasons for that are ABL kinase mutations that are found in more than 50% IM resistant CML patients (1).

There are several types of mutations that can be divided in four groups based on crystallographic structure of cABL: mutations that bind to the catalytic domain of the bcr-abl protein and so directly impair IM, those that are within the ATP binding site, those within activation loop that prevent the kinase from achieving the inactive conformation required for IM binding, and those within the catalytic domain (1).

So far, at least 73 different mutations have been described in IM resistant CML patients (5). Not all mutations have the same relevance; some are even functionally irrelevant (3). According to response to IM they are divided into highly resistant kinase domain mutations (T315I, Y253H/K, E255K/V, H396P/R) and moderately resistant mutations (M244V, M351T, F359V). Moderately resistant kinase domain mutations can be overcome by an increase in IM dose to 600 or 800 mg per day (1,3).

The development of second generation tyrosine kinase inhibitors (TKI), nilotinib and dasatinib, that are structurally different from IM brought benefits for patients bearing highly resistant mutations because both drugs are active against all known imatinib resistant mutations except the T315I (2,5). In this mutation, single nucleotide C→T change results in threonine to isoleucine substitution at position 315 (Thr315→Ile) of cABL. Thr315 forms critical hydrogen bond with IM or other TKI, making the drug effective. Isoleucine contains an extra hydrocarbon group in the side chain, which results in steric clash with TKI but do not interfere with ATP binding (6). Therefore, T315I represents kind of a "gatekeeper" that controls ac-

utječe na vezivanje ATP (6). Stoga, T315I predstavlja vrstu *čuvara* (engl. *gatekeeper*) koji kontrolira ulaz malih molekula inhibitora i zbog toga je tu mutaciju teško nadvladati, ne samo imatinib mesilatom, već i ostalim molekulama koje kompetitiraju s ATP (7). Zbog svih problema povezanih s T315I, liječenje izbora za nositelje T315I mutacije je alogena transplatacija matičnih stanica (1).

Cilj ovog istraživanja bio je otkriti je li prisutnost T315I mutacije kod bolesnika rezistentnih na liječenje imatinib mesilatom povezana s povišenom ili neprekidno visokom razinom bcr-abl prijepisa. Cilj je također bio utvrditi postoji li razlika u razinama bcr-abl prijepisa kod bolesnika rezistentnih na liječenje imatinib mesilatom sa i bez T315I mutacije.

Materijali i metode

Ispitanici

U istraživanje su bila uključena 24 bolesnika (10 žena i 14 muškaraca) kojima je dijagnosticirana CML i čiji odgovor na liječenje IM nije bio odgovarajući. Svi bolesnici liječeni su u Zavodu za hematologiju Klinike za unutarnje bolesti Medicinskog fakulteta Sveučilišta u Zagrebu i Kliničkog bolničkog centra Zagreb.

Prema Baccaraniju i suradnicima, neodgovarajući se odgovor može podijeliti u tri skupine (3):

1. Suboptimalni odgovor – manji nego značajni molekularni odgovor (engl. *major molecular response*, MMoR) (MMoR se definira kao omjer bcr-abl/abl ≤ 0,1 prema Međunarodnoj ljestvici (engl. *International Scale*, IS) nakon 18 mjeseci praćenja) ili gubitak MMoR.
2. Bez odgovora na liječenje IM – manji nego potpuni citogenetički odgovor (engl. *complete cytogenetic response*, CCyR) (CCyR se definira kao izostanak Ph pozitivnih metafaza nakon 18 mjeseci praćenja) ili gubitak CCyR ili kompletnog hematološkog odgovora.
3. Povećanje veće od 1 log omjera bcr-abl/abl između dva mjerenja tijekom praćenja.

Tijekom istraživanja kod svih je bolesnika bolest bila u kroničnoj fazi.

Metode

RNA i DNA izolirane su iz koštane srži ili perifernih krvnih stanica. Genomska DNA ekstrahirana je metodom isoljavanja (8). Za dokazivanje mutacije T315I primijenjena je metoda alel specifičnog oligonukleotidnog PCR-a (ASO-PCR) prema Kangu i sur. (9). Uzorak DNA bolesnika s potvrđenom mutacijom T315I korišten je kao pozitivni kontrolni uzorak. RNA je izolirana protokolom izdvajanja s trizolom (engl. *Trizol RNA extraction protocol*) (10) te obrnuto prepisana u cDNA (High capacity cDNA reverse Transcription Kit with RNAse inhibitor, Applied Biosystems,

cess of small molecule inhibitors and this is why it is such a difficult mutation to inhibit, not only by IM, but also by other ATP competitors (7). Because of all the problems related to T315I, the treatment of choice for T315I carriers is allogenic stem-cell transplantation (1).

The aim of this study was to detect if the presence of T315I in patients resistant to imatinib mesylate therapy is associated with the increase or constantly high bcr-abl level. We also aimed to assess the possible difference in bcr-abl level in imatinib-resistant patients with and without T315I mutation.

Materials and methods

Subjects

The study included a total of 24 patients (10 women and 14 men) diagnosed with CML whose response to therapy with IM was inadequate. Patients were diagnosed and treated at Hematology Division, Department of Medicine, Zagreb Clinical Hospital Center. Inadequate response can be divided in three groups according to Baccarani et al. (3):

1. Suboptimal response - less than major molecular response (MMoR is defined as bcr-abl/abl level ≤ 0.1 to International Scale (IS) at 18 months of follow-up) or loss of MMoR.
2. IM failure - less than complete cytogenetic response (CCyR is defined as no Ph positive metaphase found at 18 months of follow-up), loss of CCyR or complete hematological response.
3. More than 1 log increase in bcr-abl/abl level between 2 measurements during follow-up.

At the time of investigation all patients were in chronic phase of the disease.

Methods

RNA and DNA were isolated from bone marrow or peripheral blood cells. Genomic DNA was extracted using the salting out method (8). Allele specific oligonucleotide (ASO-PCR) for T315I detection was performed according to Kang et al. (9). DNA sample from patient with confirmed T315I mutation was used as positive control.

RNA was isolated using Trizol RNA extraction protocol (10) and reversely transcribed using High capacity cDNA reverse Transcription Kit with RNAse Inhibitor (Applied Biosystems, Foster City, California, USA) according to manufacturer's instructions. RQ-PCR was performed according to Europe Against Cancer protocol (11) using TaqMan technology (LightCycler, Roche, Mannheim, Germany). Amplification of fusion bcr-abl and abl as a control gene was performed using Fusion*Quant Kit for RQ-PCR analysis* of bcr-abl Mbcr (Ipsogen, Marseille, France) according to

Foster City, Kalifornija, SAD) prema uputama proizvođača. RQ-PCR je provedena prema protokolu udruženja *Europa protiv raka* (engl. *Europe Against Cancer*) (11) tehnologijom TaqMan (LichtCycler, Roche, Mannheim, Njemačka). Umnažanje fuzijskog gena bcr-abl i abl kao kontrolnog gena izvedeno je metodom RQ-PCR (FusionQuant Kit for RQ-PCR analysis of bcr-abl Mbcr, Ipsogen, Marseille, France) prema uputama proizvođača. Omjeri bcr-abl/abl izračunati su prema IS.

Statistička analiza

Podaci su prikazani kao medijan i raspon te su procijenjeni neparametrijskim statističkim testom. Razlike između dviju skupina (s i bez mutacije) ispitane su Mann-Whitneyevim testom, dok je razlika u omjerima bcr-abl/abl između tri ispitivane skupine s neodgovarajućim odgovorom na liječenje IM ispitana Kruskal-Wallisovim testom. Razina statističke značajnosti postavljena je na 0,05. Statistička analiza provedena je programskim paketom MedCalc verzija 9.3.2.0 (Frank Schoonjans, Mariakerke, Belgium).

Rezultati

Kod 24 bolesnika s CML bez odgovarajućeg odgovora na liječenje IM provedeno je dokazivanje mutacije T315I metodom ASO-PCR. Na slici 1. prikazano je elektroforetsko razdvajanje umnoženih produkata ASO-PCR-om.

Rezultati RQ-PCR i status mutacije T315I u skupinama s neodgovarajućim odgovorom na liječenje IM prikazani su u tablici 1. Od 24 ispitana bolesnika, 12 bolesnika nije odgovorilo na liječenje IM (citogenetički podaci nisu prikazani), 6 bolesnika je imalo suboptimalni odgovor,a kod 6 bolesnika je dokazano povećanje omjera bcr-abl/abl više od 1 log između dva mjerenja u praćenju. Kod bolesnika kod kojih nije dokazana mutacija T315I raspon omjera bcr-abl/abl bio je 0,14-46,25%, za razliku od skupine s dokazanom mutacijom T315I čiji je raspon omjera bcr-abl/abl bio 10,95-30,45%.

manufacturer's instructions. Bcr-abl/abl levels were calculated according to IS.

Statistical analysis

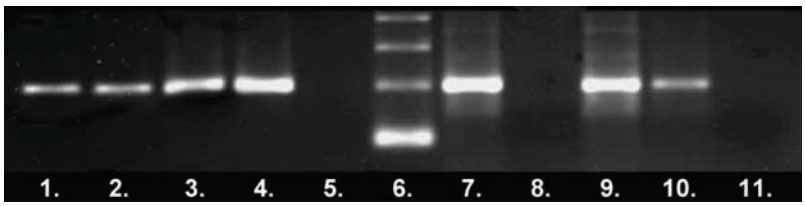
Data are presented as a median and ranges and assessed by non-parametric statistics. Difference between two groups (with and without mutation) was tested with Mann-Whitney test whereas difference in bcr-abl/abl levels between three studied groups with inadequate IM response was tested with Kruskal-Wallis test. The level of significance was set at 0.05. Statistical analysis was performed using the MedCalc software package version 9.3.2.0 (Frank Schoonjans, Mariakerke, Belgium).

Results

In twenty-four CML patients who were monitored by RQ-PCR and who had inadequate response to IM therapy T315I mutation detection was performed. Electrophoretic separation of representative ASO-PCR amplification products is shown in Figure 1.

RQ-PCR results and T315I mutation status in inadequate IM therapy response groups are shown in Table 1. Among 24 CML patients studied, 12 patients had IM therapy failure (cytogenetic data not shown), 6 patients had suboptimal response, and 6 patients had more than 1 log increase of bcr-abl/abl level between two measurements during follow-up. In patients without T315I the range of bcr-abl/abl was 0.14-46.25%, unlike in those with T315I whose bcr-abl/abl range was 10.95-30.45%.

In order to establish the possible difference in bcr-abl/abl level between CML patients with and without T315I mutation, median bcr-abl/abl levels were calculated. Results are presented in Table 2 and in Figure 2. In T315I positive patients the median bcr-abl/abl level was 66% higher than in T315I negative patients.



1. Patient 1
2. Patient 2
3. Patient 7
4. Positive control
5. Blank probe

PCR products with control primers

6. Molecular Weight Marker

7. Patient 1
8. Patient 2
9. Patient 7
10. Positive control
11. Blank probe

PCR products with T315I mutation primers

Slika 1. Elektroforetsko razdvajanje reprezentativnih ASO-PCR produkata.

Figure 1. Electrophoretic separation of representative ASO-PCR amplification products.

Tablica 1. Omjeri bcr-abl/abl (medijan i raspon) i status mutacije T315I kod tri ispitivane skupine s neodgovarajućim odgovorom na liječenje IM

Table 1. Bcr-abl/abl levels (median and range) and T315I mutation status in three studied groups with inadequate IM responses

IM therapy response	Bcr-abl/abl (% IS) median (range)	T315I positive (N)	P (Kruskal-Wallis test)
IM therapy failure (N = 12)	15.38 (0.27-45.43)	3	0.526
Suboptimal (N = 6)	6.80 (0.14-46.25)	0	
> 1 log increase (N = 6)	14.38 (7.45-30.45)	1	

IM – imatinib mesilate; IS - International Scale

Kako bi se odredila razlika u omjerima bcr-abl/abl između skupina bolesnika s i bez mutacije T315I, izračunat je medijan omjera bcr-abl/abl. Rezultati su prikazani u tablici 2. i na slici 2. Kod bolesnika s dokazanom mutacijom T315I, medijan omjera bcr-abl/abl bio je 66% viši nego kod bolesnika bez mutacije T315I.

Rasprava

Nakon što je prije više od 150 godina CML prvi puta opisana kao bolest, veći je napredak u liječenju učinjen u proteklom desetljeću nego u prvih sto godina od otkrića bolesti. Otkriće tirozin-kinazne aktivnosti proteina bcr-abl, dovelo je do sinteze nove serije spojeva, inhibitora tirozin-kinaze. Jedan od njih, imatinib mesilat, pokazuje vrlo visoku i relativno specifičnu biokemijsku aktivnost te posjeduje prihvatljiv farmakokinetički i toksični profil, pa je vrlo brzo uveden u kliničku praksu (6). To je dovelo do revolucionarnog koraka u liječenju CML, odgovoru na liječenje i naposljetku procjeni liječenja osjetljivijim tehnikama. Stoga je Europska mreža za leukemiju (engl. *European Leukemia Net*) izradila protokol za obradu CML koji uključuje kliničku procjenu, krvnu sliku, citogenetički pregled koštane srži i molekularno praćenje razine bcr-abl prijepi-

Discussion

After the initial description of CML more than 150 years ago, little progress has been made in the treatment of this disease for more than a century compared to the past decade. Recognition of tyrosine kinase activity of the bcr-abl proteins has led to the discovery of a new series of compounds, namely tyrosine kinase inhibitors. One of them, imatinib mesylate, was found to have high and relatively specific biochemical activity and an acceptable pharmacokinetic and toxic profile, and was rapidly introduced into clinical practice (6). This resulted in revolutionary step in CML therapy, response to therapy and consequently estimation of response by more sensitive techniques. Therefore European Leukemia Net established a protocol for CML management which includes clinical assessment, blood count, cytogenetic revision of bone marrow, and molecular monitoring of bcr-abl level every 3 months from the beginning of the therapy (3).

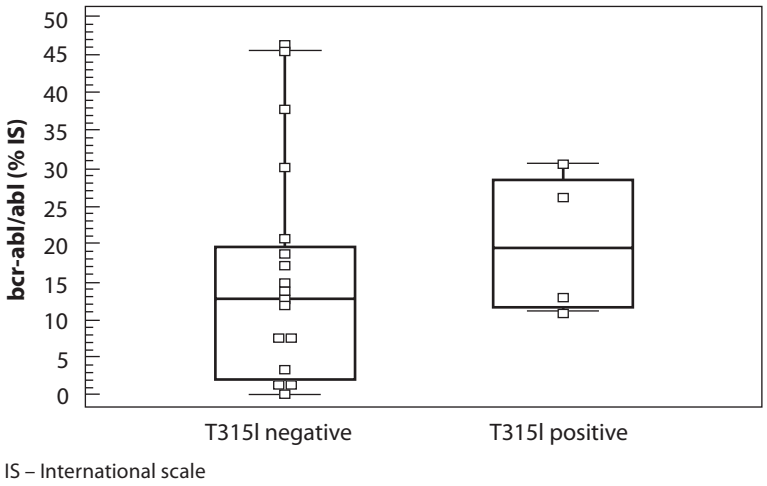
Major cause of resistance to IM or relapse during IM therapy are ABL kinase point mutations (7,12,13). The only mutation resistant to currently available TKI is T315I, and T315I carriers are eligible for allogeneic stem-cell transplantation only. This is why the detection of ABL kinase

Tablica 2. Omjeri bcr-abl/abl (medijan i raspon) kod T315I pozitivnih i negativnih bolesnika

Table 2. Bcr-abl/abl levels (median and range) in T315I postive and negative patients

Patients	Bcr-abl/abl level (% IS) median (range)	P
T315I positive (N = 4)	19.36 (10.95-30.45)	0.394
T315I negative (N = 20)	12.80 (0.14-46.25)	

IS – International Scale



Slika 2. Grafički prikaz omjera bcr-abl/abl za T315I negativne i pozitivne bolesnike.

Figure 2. Box-and-Whisker plots of bcr-abl/abl levels for T315I negative and positive patients.

sa svaka 3 mjeseca od početka liječenja (3). Glavni razlog rezistencije na liječenje IM ili recidiva tijekom liječenja IM su točkaste mutacije ABL kinaze (7,12,13). Jedina mutacija rezistentna na trenutno dostupan TKI jest T315I. Stoga je nosiocima mutacije T315I jedino rješenje alogenična transplatacija matičnih stanica. To je razlog zašto je otkrivanje mutacija ABL kinaze vrlo korisno u racionalnoj terapijskoj obradi bolesnika s CML (12).

Postoji nekoliko metoda za otkrivanje mutacija u domeni kinaze. Jedna od najčešće primjenjivanih metoda probira u rutinskom praćenju bolesnika je direktno sekvenciranje. Nedostatak te metode je njena niska osjetljivost (20%). Metoda s višom osjetljivošću od direktnog sekvenciranja je denaturirajuća tekućinska kromatografija visoke djelotvornosti (engl. *Denaturing high performance liquid chromatography*) (1-5%). Za razliku od tih metoda, alel specifična oligonukleotidna lančana reakcija polimeraze (*engl. Allele-Specific Oligonucleotide PCR*, ASO-PCR) ima najvišu osjetljivost koja može doseći čak 0,1%, no probir kroz cijelu domenu gena ABL nije moguć, budući da ta metoda zahtjeva različite početnice za svaku mutaciju (1,2,12,13).

U ovo istraživanje su bila uključena 24 bolesnika s neodgovarajućim odgovorom na liječenje IM i stalnim povećanjem ili visokim omjerom bcr-abl/abl kod praćenja. Sukladno ranije objavljenim podacima, pretpostavili smo da bi mutacija T315I mogla biti razlogom neodgovarajućeg odgovora na terapiju (1). Među svim bolesnicima, u 4 je slučaja otkrivena mutacija T315I (17%). Tri bolesnika s dokazanom mutacijom T315I su iz skupine bez odgovora na liječenje IM, što je bilo i očekivano s obzirom da je taj mutirani podklon rezistentan na liječenje IM (4). Kod jednog bolesnika s dokazanom mutacijom T315I i povećanjem omjera bcr-abl/abl više od 1 log, mutacija se proširila vjerojatno kao rezultat selektivne inhibicije IM (4).

mutations is very useful for rational therapeutic management of CML patients (12).

Several methods have been established for detection of kinase domain mutations. Direct sequencing is one of the most used screening methods in routine monitoring of patients, but its disadvantage is low sensitivity (20%). Denaturing high performance liquid chromatography is a more sensitive method (1-5%) compared to direct sequencing. Unlike those methods, ASO-PCR has the highest sensitivity that can even reach 0.1% but the screening through the whole ABL gene domain is not possible as this method requires different primers for each mutation (1,2,12,13).

Twenty-four patients with inadequate response to IM therapy and constant increase or high bcr-abl/abl levels during follow-up were included in this study. It was assumed that, according to literature, T315I could be the possible cause (1). Among all patients tested, T315I was detected in four cases (17%). Three patients bearing T315I mutation were in IM failure group, which was expected since this mutant subclone is uniformely resistant to treatment with IM (4). In one T315I positive patient with more than 1 log increase of bcr-abl/abl level, the mutation probably expanded as a result of selective pressure exerted by IM (4).

Calculated median bcr-abl/abl levels were 19.36% for T315I positive and 12.80% for T315I negative patients, but the difference was not statistically significant (P = 0.394). These high bcr-abl levels are related to poor prognosis including progression to blast crisis and therefore indication for T315I mutation detection (3). If T315I is detected, therapy approach and treatment drastically change: patient is no more on expensive and inadequate therapy and there is more time for finding a donor for allogenetic stem-cell transplantation. Therefore monitoring is not

Izračunat medijan omjera bcr-abl/abl iznosio je 19,36% za skupinu pozitivnu na T315I i 12,80% za skupinu negativnu na T315I, no razlika nije bila statistički značajna (P = 0,394). Visoke razine bcr-abl prijepisa povezuju se s lošom prognozom koja uključuje progresiju do blastične krize i upućuje na dokazivanje prisutnosti mutacije T315I (3). Ukoliko se dokaže mutacija T315I, drastično se mijenja terapijski pristup: bolesnik više neće primati skupo i neodgovarajuće liječenje, a dobiva se više vremena za pronalazak darivatelja koštane srži. Stoga praćenje nije samo važno da se osigura najbolje moguće liječenje bolesnika, već je poželjno i s farmakoekonomskog gledišta (13).

U zaključku, otkrivanje mutacije T315I treba biti uključeno u obradu bolesnika oboljelih od CML zajedno s molekularnim, citogenetičkim i kliničkim praćenjem. Rezultat ove analize izravno utječe na terapijski pristup, budući da je liječenje izbora za nosioce mutacije T315I alogenična transplantacija matičnih stanica.

only important for ensuring that a patient receives the best treatment, but that is also convenient from a pharmacoeconomic point of view (13).

In conclusion, T315I detection should be included in management of CML patients together with molecular, cytogenetic and clinical monitoring. The result of this analysis directly affects the therapy approach, since the treatment of choice for T315I carriers is allogeneic stem-cell transplantation.

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