PROGRESIJA BOLESTI KOD PACIJENATA SA PRIMARNOM MIJELOFIBROZOM NISKOG RIZIKA – PRIKAZ SLUČAJA

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CASE REPORT

DISEASE PROGRESSION IN PATIENTS WITH LOW-RISK PRIMARY MYELOFIBROSIS – CASE REPORT

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SAŽETAK

Uvod: Medijana preživljavanja (OS) pacijenata sa primarnom mijelofibrozom (PMF) niskog rizika je preko 15 godina, ali prema „MOST" prospektivnoj studiji, progresiju bolesti ima skoro 60% ovih pacijenata.

Cilj: Predstaviti tok bolesti i lečenje pacijenata sa PMF-om niskog rizika.

Prikaz slučajeva: Svi pacijenti su imali utvrđenu PMF, međunarodni prognostički skoring sistem (IPSS) niskog rizika i normalnu početnu citogenetiku.

Slučaj 1: Pacijent, muškarac starosti 61 godina, primljen je u maju 2016. godine sa Tr 772x10^9/L, LDH 566U/L, bez splenomegalije, JAK2V617F+. Prvobitno je lečen hidroksiureom (HU) od 2016. do 2020. godine. Od 2021. godine uveden je ruksolitinib zbog razvoja splenomegalije i leukocitoze, uz progresiju bolesti 18 meseci kasnije (slezina 26cm, Plt 20x10^9/L, 10% blasta u srži, složen kariotip: -5, del 7q, mar+). Uveden je azacitidin, ali zbog sepse dolazi do smrtnog ishoda u oktobru 2022. godine. OS je 66 meseci.

Slučaj 2: Pacijentkinja, žena starosti 47 godina, primljena je u julu 2011. godine sa Tr 899x10^9/L, LDH 899U/L, slezinom 15x7cm, JAk2V617F+, ASXL+. Inicijalno je lečena sa HU, a od oktobra 2013. godine ruxolitinibom, zbog progresije splenomegalije (slezina 19,3cm, LDH 1881U/L), uz potonju normalizaciju veličine slezine, broja Tr i LDH. Pacijentkinja je u remisiji. OS je 126 meseci.

Slučaj 3: Pacijent, muškarac starosti 64 godine, primljen je u maju 2012. godine sa Tr 1457x10^9/L, LDH 631U/L, graničnom splenomegalijom, JAk2V617F-, MPL+, SRSF2+, U2AF1+, ASXL1+. Inicijalno je lečen sa HU (maj 2012. godine), a od aprila 2019. godine danazolom zbog pojave transfuzione zavisnosti. Zbog srčane insuficijencije, smrtni ishod je nastupio u februaru 2020. godine. OS je 96 meseci.

Zaključak: Neki pacijenti sa PMF-om niskog rizika imaju progresiju bolesti, a buduća istraživanja će pokazati da li rana sekvencioniranja sledeće generacije (NGS) analiza non-driver mutacija i rano uvođenje terapije doprinose promeni toka bolesti.

Ključne reči: mijeloproliferativna neoplazma, primarna mijelofibroza, prognoza

ABSTRACT

Introduction: The median survival (OS) of patients with low-risk primary myelofibrosis (PMF) is over 15 years, but according to the "MOST" prospective study, disease progression occurs in almost 60% of these patients.

Aim: to present the disease course and treatment outcome of patients with low-risk PMF.

Case report: All patients were diagnosed with PMF, low-risk International Prognostic Scoring System (IPSS), and normal initial cytogenetics.

Case 1: A 61-year-old male patient was admitted in May 2016 with Tr 772x10^9/L, LDH 566U/L, no splenomegaly, JAK2V617F+. He was initially treated with hydroxyurea (HU) from 2016 to 2020. In 2021, he was introduced to ruxolitinib due to the development of splenomegaly and leukocytosis, with disease progression 18 months later (spleen 26cm, Plt 20x10^9/L, 10% blasts in the marrow, complex karyotype: -5, del 7q, mar+). Azacitidine was introduced, but death occurred due to sepsis in October 2022. OS is 66 months.

Case 2: A 47-year-old female patient was admitted in July 2011 with Tr 899x10^9/L, LDH 899U/L, spleen 15x7cm, JAk2V617F+, and ASXL+. She was initially treated with HU and has been treated with ruxolitinib since October 2013 due to the progression of splenomegaly (spleen 19.3cm, LDH 1881U/L), with subsequent normalization of spleen size, number of Tr, and LDH. The patient is in remission. OS is 126 months.

Case 3: A 64-year-old male patient was admitted in May 2012 with Tr 1457x10^9/L, LDH 631U/L, borderline splenomegaly, JAk2V617F-, MPL+, SRSF2+, U2AF1+, ASXL1+. He was initially treated with HU (May 2012) and since April 2019 with danazol due to the emergence of transfusion dependence. Due to heart failure, death occurred in February 2020. OS is 96 months.

Conclusion: Some patients with low-risk PMF have disease progression and future studies will show whether early Next Generation Sequencing (NGS) analysis of non-driver mutations and early initiation of therapy contribute to changing the course of the disease.

Keywords: myeloproliferative neoplasm, primary myelofibrosis, prognosis

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UVOD

Primarna mijelofibroza (PMF) predstavlja stečeni klonalni poremećaj matične ćelije hematopoeze uz abnormalnu proliferaciju mijeloidnih ćelija. PMF je Bcr-Abl (Breakpoint cluster region protein-Abelson tyrosine-protein kinase, eng.) negativna mijeloproliferativna neoplazma (MPN) [1,2]. Karakteriše se prisustvom konsitucionalnih simptoma, splenomegalijom usred ekstramedularne hematopoeze i mogućnošću razvoja leukemijske transformacije [1]. Većina pacijenata ima prisutnu mutaciju gena za Janus kinazu (JAK)2/V617F) [3]. Dijagnoza PMF se određuje prema ICC kriterijumima (2022.). Prisustvo nukleiranih eritrocita, nezrelih granulocita i dakriocita je tipično, ali prefibrotični PMF ne mora da pokaže jasnu leukoeritroblastozu. Fibroza koštane srži je obično povezana sa mutacijama JAK2, CALR ili MPL mutacijama. Kariotip najčešće pokazuje abnormalnosti po tipu +9, del(13k), del(20k) i hromozoma br 1 [4-6]. Prognostički model baziran je na razvoju međunarodnog prognostičkog skoring sistema (IPSS) iz 2009. godine, koji se koristi u inicijalnoj dijagnostici i uključuje starost >65god., hemoglobin <100g/l, Le >25x10^9/l, prisustvo > 1% cirkulišućih blasta i razvoj konstitucionih simptoma. U zavisnosti od broja prisustnih štetnih faktora, pacijenti su statifikovani u grupe niskog, srednjeg-1, srednjeg-2 i visokog rizika bolesti [7]. DIPSS (dinamički IPSS skor) koristi iste prognostičke varijable, ali je primenljiv bilo kad u toku bolesti [8]. Konvencionalni tretman je ograničenog uticaja na preživljavanje pacijenata i uključuje "watch and wait" za asimptomatske pacijente, stimulatore eritropoeze, androgene ili imunomodulatore za anemiju, primenu citoreduktivnih lekova (hidroksiurea kod razvoja splenomegalije i konstitucionalnih simptoma), u pojedinačnim slučajevima i splenektomiju ili radioterapiju [9]. Ciljana molekularna terapija (JAK inhibitori) je efikasna i kod JAK2+ i JAK2- pacijenata. Ruksolitinib je terapija izbora za PMF sa splenomegalijom i/ili razvojem konstitucionalnih simptoma. Alogena transplantacija matičnih ćelija ostaje jedini način izlečenja PMF, ali je ograničena na pacijente sa visokim i srednjim 2 rizikom. U toku su mnoge studije kombinacije JAK inhibitora sa drugim agensima [9]. Više studija je pokazalo da lečenje mijelofibroze u ranijem toku bolesti, dok je pacijent niskog, ili srednjeg-1 rizika ili što je pre moguće ukoliko ima srednji i visoki rizik, rezultira većom relativnom efikasnošću i manjom toksičnošću u poređenju sa kasnijim započinjanjem lečenja [10]. Cilj ovog rada je bio da prikažemo bolesnike sa PMF niskog stepena rizika, kod kojih je došlo do biološke progresije bolesti, i da istaknemo njihove klinčke i biološke karakteristike.

INTRODUCTION

Primary myelofibrosis (PMF) is an acquired clonal disorder of hematopoietic stem cells with abnormal proliferation of myeloid cells. PMF is a Bcr-Abl (Breakpoint cluster region protein-Abelson tyrosine-protein kinase, Eng.) negative myeloproliferative neoplasm (MPN) [1,2]. It is characterized by the presence of constitutional symptoms, splenomegaly in the midst of extramedullary hematopoiesis, and the possibility of leukemic transformation [1]. Most patients have a Janus kinase gene mutation (JAK)2/V617F) [3]. The diagnosis of PMF is determined according to the ICC criteria (2022). The presence of nucleated erythrocytes, immature granulocytes, and dacryocytes is typical, but prefibrotic PMF does not necessarily show clear leukoerythroblastosis. Bone marrow fibrosis is usually associated with JAK2, CALR, or MPL mutations. The karyotype most often shows abnormalities of type +9, del(13k), del(20k), and chromosome number 1 [4-6]. The prognostic model is based on the development of the International Prognostic Scoring System (IPSS) from 2009, which is used in the initial diagnosis and includes age >65 years, hemoglobin <100g/l, Le >25x10^9/l, presence > 1% of circulating blasts and development of constitutional symptoms. Depending on the number of harmful factors present, patients were stratified into groups of low, medium-1, medium-2, and high risk of the disease [7]. DIPSS (dynamic IPSS score) uses the same prognostic variables but is applicable at any time during the disease [8]. Conventional treatment has a limited impact on patient survival and includes "watch and wait" for asymptomatic patients, erythropoiesis stimulators, androgens or immunomodulators for anemia, the use of cytoreductive drugs (hydroxyurea in the development of splenomegaly and constitutional symptoms), and in individual cases, splenectomy or radiotherapy [9]. Targeted molecular therapy (JAK inhibitors) is effective in both JAK2+ and JAK2- patients. Ruxolitinib is the therapy of choice for PMF with splenomegaly and/or the development of constitutional symptoms. Allogeneic stem cell transplantation remains the only cure for PMF but is limited to high- and intermediate-2-risk patients. Many studies of the combination of JAK inhibitors with other agents are underway [9]. Several studies have shown that treatment of myelofibrosis earlier in the course of the disease, while the patient is at low or intermediate-1 risk, or as early as possible if he has intermediate and high risk, results in greater relative efficacy and less toxicity compared to later initiation of treatment [10]. This work aimed to present patients with low-risk PMF, in whom biological progression of the disease occurred, and to highlight their clinical and biological characteristics.

PRIKAZI SLUČAJEVA

Prvi slučaj

Kod pacijenta, muškog pola, starosti 61 godinu, u maju 2016. godine u krvnoj slici konstatovana je leukocitoza i trombocitoza (Hb 158 g/L, MCV 90fl, Le 12,9x10^9/L, Tr 792x10^9/L, u diferencijalnoj formuli neutrofili 66%, limfociti 26%, bez leukoeritroblastoze), a u biohemijskim analizama povišen LDH (556U/L). Ultrazvuk abdomena nije pokazao prisustvo splenomegalije. Pacijent nije imao nikakve tegobe na prikazu. U sprovedenoj hematološkoj dijagnostici, nalaz bioptata koštane srži pokazao je fibrozu gr I, uočen je spontani razvoj formacija eritroidnih kolonija, konvencionalna citogenetika je bila uredna (20 metafaza). Konstatovana je JAK2V617F mutacija (PCR). Nivo serumskog eritropetina bio je uredan. Postavljena je dijagnoza prefibrotičnog PMF-a, niskog stepena rizika prema IPSS skoru, uz MPN 10 skor 10/100.

Uz primenu acetilsalicilne kiseline od 100 mg *per os* dnevno, po sprovednoj dijagnostici i daljoj progresiji trombocitoze i leukocitoze, započeto je (jun 2016. godine) lečenje hidroksiureom (HU), 500 mg *per os* dnevno, u periodu 2016. do 2020. godine. Decembra 2019. godine, kontrolni ultrazvuk abdomena i dalje je bio uredan. Međutim, u avgustu 2020. godine (53 meseca od dijagnoze PMF) konstatovana je progresija leukocitoze (30x10^9/l, uz Hb 129g/L i Tr 161x10^9/L), te je doza HU eskalirana na 1000 mg *per os* dnevno. Oktobra 2020. godine (55 meseci od dijagnoze PMF) pacijent prijavljuje mučninu, gubitak od 5 kg telesne težine za mesec dana, noćno znojenje i preznojavanje. Konstantovana je dalja progresija leukocitoze (50x10^9/L, 3% blasta u perifernom razmazu) i porast LDH (1627 U/L). Doza HU je eskalirana na 1500 mg *per os* dnevno, uz potonju pojavu trombocitopenije.

U oktobru 2020. godine sprovedena je hematološka reevaluacija. Aspiraciona punkcija nije pokazala akutnu transformaciju MPN-a (2% blasta). U bioptatu koštane srži uočena je fibroza gr II, 4% CD34+ ćelija. Nalaz konvencionalne citogenetike u 20 mitoza bio je uredan. Konstatovano je prisustvo PMF-a, intermedijarnog-2 rizika prema DIPSS skoru, MPN skor 19/100. U februaru 2021. godine radiografski je konstatovana dalja progresija splenomegalije (22,2 cm), bez splahnične tromboze. Uveden je Ruxolitinib® u dozi 2x20 mg *per os* dnevno, a mesec dana kasnije, zbog leukocitoze 146x10^9/l (uz Hb 120 g/L i Tr 101x10^9/l), ponovo je dodata HU (500 mg *per os* dnevno). Nakon 18 meseci od početka primene ruksolitiniba (avgust 2022. godine), konstatovana je dalja progresija bolesti (produbljivanje trombocitopenije, progresija splenomegalije do 26 cm), uz pojavu aberantnog kariotipa sa 2 nezavisna

CASE REPORTS

First case

In a patient, male, age 61, in May 2016, leukocytosis and thrombocytosis were found in the blood count (Hb 158 g/L, MCV 90fl, Le 12.9x10^9/L, Tr 792x10^9/L, in the differential formula neutrophils 66%, lymphocytes 26%, without leukoerythroblastosis), and in biochemical analyzes elevated LDH (556U/L). Abdominal ultrasound did not show the presence of splenomegaly. The patient did not have any symtoms during the examination. In the performed hematological diagnosis, the findings of the bone marrow biopsy showed fibrosis gr I, spontaneous development of erythroid colony formations was observed, and conventional cytogenetics was normal (20 metaphases). JAK2V617F mutation was detected (PCR). The serum erythropoietin level was average. A diagnosis of prefibrotic PMF, low risk according to the IPSS score, with an MPN 10 score of 10/100, was made.

Along with the use of acetylsalicylic acid of 100 mg per body per day, after the implementation diagnosis and further progression of thrombocytosis and leukocytosis, treatment with hydroxyurea (HU), 500 mg per body per day, was started (June 2016), in the period from 2016 to 2020. In December 2019, the follow-up abdominal ultrasound was still normal. However, in August 2020 (53 months after the diagnosis of PMF), a progression of leukocytosis was noted (30x10^9/L, with Hb 129 g/L and Tr 161x10^9/l), and the dose of HU was escalated to 1000 mg per person per day. In October 2020 (55 months after the diagnosis of PMF), the patient reported nausea, weight loss of 5 kg in one month, night sweats, and sweating. Further progression of leukocytosis (50x10^9/L, 3% blasts in peripheral smear) and LDH increase (1627 U/L) were constant. The dose of HU was escalated to 1500 mg *per os* per day, with the subsequent occurrence of thrombocytopenia.

In October 2020, a hematological reevaluation was carried out. Aspiration puncture showed no acute transformation of MPN (2% blasts). In the bone marrow biopsy, fibrosis grade II was observed in 4% CD34+ cells. The findings of conventional cytogenetics in 20 mitoses were normal. The presence of PMF, intermediate-2 risk, according to the DIPSS score, and the MPN score of 19/100 was established. In February 2021, further progression of splenomegaly (22.2 cm) was detected radiographically, without splenic thrombosis. Ruxolitinib® was introduced at a dose of 2x20 mg per body per day, and a month later, due to leukocytosis 146x10^9/l (with Hb 120 g/L and Tr 101x10^9/l), HU was added again (500 mg *per os* per day). After 18 months from the start of ruxolitinib administration (August

klona (-5, del 7q, +mar). Ponovljena aspiracija srži pokazala je 10% blasta *u srži. Kod pacijen*ta je započeto lečenje primenom azacitidina, ali usled pojave septičnog stanja sa razvojem septičnog šoka dolazi do letalnog ishoda u oktobru 2022. godine. Period ukupnog preživljavanja (OS, Overall Survival, eng.) je iznosio 66 meseci.

Drugi slučaj

Pacijentkinja starosti 47 godina javila se hematologu u julu 2011. godine zbog pojave priritusa i abdominalnog bola, kao i nalaza krvne slike (Hb 128 g/L, MCV 89fL, Le 8.7x10^9/L, Tr 772x10^9/l, u diferencijalnoj formuli neutrofili 66%, limfociti 25%, monociti 5%, bez leukoeritroblastoze). U laboratorijskim analizama LDH je iznosio 899 U/L. Inicijalni ultrazvuk abdomena pokazao je slezinu promera 15x7cm. Sprovedena je hematološka dijagnostika. U bioptatu koštane srži uočena je fibroza Gr I, bio je pozitivan spontani razvoj formacija eritroidnih kolonija, a konvencionalna citogenetika bila je uredna (u 4 analizirane metafaze- nizak mitotski indeks). Konstatovana je JAK2V617F mutacija-homozigot (PCR). NGS (Next Generation Sequencing- mutacioni panel Prof. Skode) analiza utvrdila je prisustvo ASXL1 mutacije. Nivo serumskog eritropoetina je bio uredan. Konstatovana je dijagnoza prefibrotičnog PMF, niskog stepena rizika prema IPSS skoru, MPN 10 skor 25/100.

Inicijalno je uvedena acetilsalicilna kiselina (100 mg *per os* dnevno), a od jula 2012. godine je uvedena HU (500mg *per os* dnevno), s obzirom na progresivnu splenomegaliju (18x7cm) i porast LDH (1221U/L). Tada je utvrđeno prisustvo intermedijarnog-1 rizika prema DIPSS skoru. U oktobru 2013. godine pacijentkinja prijavljuje malaksalost, noćno znojenje, preznojavanje i ponovnu pojavu abdominalnog bola. Slezina se palpirala do nivoa umbilikusa. U krvnoj slici Hb 130x10^9/L, Le 13x10^9/l, Tr 563x10^9/L, postoji tendencija daljeg rasta LDH (1881U/L), uz uredne druge zapaljenske parametre. Kontrolni ultrazvuk abdomena pokazao je dalju progresiju splenomegalije (19,3x7,5cm). Pacijentkinja nije bila motivisana za rebiopsiju koštane srži. Konstatovano je i dalje prisustvo intermedijarnog-1 rizika (DIPSS), uz porast MPN 10 skora na 36/100.

Istog meseca je uveden ruxolitinib u dozi od 2x20 mg *per os* dnevno (u okviru "compassionate" programa). U daljem toku lečenja ruxolitinibom dolazi do potpune regresije splenomegalije (13x5 cm februara 2015. godine), uz potpunu laboratorijskih parametara. Na poslednjoj kontroli, u januaru 2024. godine (i dalje ruxolitinib 2x20 mg *per os* dnevno), u krvnoj slici Hb 124 g/L, Le 4.2x10^9/l, Tr 413x10^9/l. LDH 478U/L. Poslednji ultrazvuk abdomena je pokazao promer slezine 10x3 cm. Pacijentkinja nije prijavljivala tegobe na poslednjoj

2022), further progression of the disease was noted (deepening thrombocytopenia, progression of splenomegaly up to 26 cm), with the appearance of an aberrant karyotype with 2 independent clones (-5, del 7q, +mar). A repeat marrow aspiration showed 10% blasts in the marrow. The patient started treatment with azacitidine, but due to the appearance of a septic condition with the development of septic shock, the patient died in October 2022. The overall survival (OS) period was 66 months.

Second case

A 47-year-old patient consulted a hematologist in July 2011 due to the appearance of pruritus and abdominal pain, as well as blood count findings (Hb 128 g/L, MCV 89fL, Le 8.7x10^9/L, Tr 772x10^9/l, in the differential formula neutrophils 66%, lymphocytes 25%, monocytes 5%, without leukoerythroblastosis). In laboratory analyses, LDH was 899 U/L. The initial abdomen ultrasound showed a spleen with a diameter of 15x7cm. A hematological diagnosis was carried out. In the bone marrow biopsy, Gr I fibrosis was observed, there was a positive, spontaneous development of erythroid colony formations, and conventional cytogenetics were normal (metaphase in 4 analyzed - low mitotic index). JAK2V617F mutation-homozygote was established (PCR). NGS (Next Generation Sequencing - Prof. Skoda mutation panel) analysis determined the presence of ASXL1 mutation. The serum erythropoietin level was average. The diagnosis of prefibrotic PMF, low risk, according to the IPSS score, and MPN 10 score 25/100 was established.

Acetylsalicylic acid (100 mg *per os* per day) was initially introduced, and from July 2012, HU (500 mg *per os* per day) was introduced, considering progressive splenomegaly (18x7cm) and an increase in LDH (1221U/L). Then, the presence of intermediate-1 risk was determined according to the DIPSS score. In October 2013, the patient reported malaise, night sweats, sweating, and the recurrence of abdominal pain. The spleen was palpated up to the level of the umbilicus. In the blood count, Hb 130x10^9/L, Le 13x10^9/l, Tr 563x10^9/L, there is a tendency for further growth of LDH (1881U/L), along with normal other inflammatory parameters. Control ultrasound of the abdomen showed further progression of splenomegaly (19.3x7.5 cm). The patient was not motivated to undergo a bone marrow rebiopsy. The presence of intermediate-1 risk (DIPSS) was still established, with an increase in the MPN 10 score to 36/100.

In the same month, ruxolitinib was introduced at a dose of 2x20 mg *per os* per day (within the "compassionate" program). In further treatment with ruxolitinib, there is a complete regression of splenomegaly (13x5 cm in February 2015), with complete laboratory pa-

kontroli. Pacijentkinja je u stabilnoj remisiji osnovne bolesti. Period bez progresije bolesti iznosi 126 meseci. Dalje praćenje uz nastavak ruxolitiniba je u toku.

Treći slučaj

Pacijent muškog pola, starosti 64 godine, inicijalno se javio hematologu u maju 2012. godine zbog nalaza trombocitoze u krvnoj slici, uz podatak da je za povišene trombocite znao od novembra 2010. godine (tada Tr 989x10^9/L), ali se nije ispitivao. Prilikom pregleda, u krvnoj slici su registrovani Hb 107g/L, MCV 82fl, Le 11,2x10^9/L, Tr 1457x10^9/l, u diferencijalnoj formuli neutrofili 62%, limfociti 20%, monociti 5%. U laboratorijskim nalazima LDH je iznosio 631U/L, a feritin 9 ugL, uprkos prethodnoj primeni oralnih preparata gvožđa. Ultrazvuk abdomena iz maja 2012. godine pokazao je slezinu veličine 12x5 cm. Pacijent, izuzev blagog svraba kože, nije imao druge tegobe.

U sprovedenoj hematološkoj dijagnostici, nalaz bioptata koštane srži pokazao je fibrozu gr I, uočen je spontani razvoj formacija eritroidnih kolonija. Konvencionalna citogenetika bila je uredna (4 metafazenizak mitotski indeks). PCR metodom nije potvrđena JAK2V617F mutacija. NGS metodom (mutacioni panel Profesora Skode) utvrđeno je prisustvo MPL, SRSF2, U2AF1 i ASXL1 mutacije. Nivo serumskog eritropoetina bio je uredan. Postavljena je dijagnoza prefibrotičnog PMF, niskog stepena rizika prema IPSS skoru, uz MPN 10 skor 5/100.

Inicijalno je uvedena acetilsalicilna kiselina (100 mg *per os* dnevno), ali je obustavljena u maju 2012. godine zbog pojave izražene epistakse (utvrđena aktivnost Von Willebrand-ovog faktora 34%). Istog meseca je uvedena HU (500 mg *per os* dnevno), čije je doziranje u daljem toku lečenja bilo korigovano u zavisnosti od parametara krvne slike. HU je primenjivana sve do aprila 2019. godine, kada je konstatovano prisustvo transfuziono zavisnog anemijskog sindroma, te je lečenje nastavljeno Danazolom (2x200 mg *per os* dnevno).

U oktobru 2019. godine urađena je hematološka reevaluacija. Rebiopsija koštane srži pokazala je prisustvo fibroze, sada gr III, dok aspiraciona punkcija nije pokazala akutnu transformaciju (8% blasta). Uočeno je prisustvo kompleksnog kariotipa, 46 XY, del (20)q11/(5), 46 XY/15/. PCR za *JAK2V617F* mutaciju je i dalje bio negativan. U krvnoj slici su zabeleženi Hb 72g/L, Le 19.1x10^9/l, Tr 487x10^9/L, u perifernom razmazu krvi 6% blasta sa leukoeritroblastozom, retikulociti 5.2%. LDH je iznosio 2498 U/L, a feritin 247,8 mg/L. Ultrazvučni nalaz iz oktobara 2019. godine pokazao je slezinu veličine 14 cm.

U daljem toku lečenja pacijent je prijavljivao izraženu malaksalost i zamor, te je nastavljena transfuzi-

rameters. At the last control, in January 2024 (still ruxolitinib 2x20 mg *per os* per day), in the blood count Hb 124g/L, Le 4.2x10^9/l, Tr 413x10^9/l. LDH 478U/L. The last abdomen ultrasound showed a diameter of the spleen of 10x3 cm. The patient did not report any complaints at the last check-up. The patient is in stable remission of the underlying disease. The period without disease progression is 126 months. Further follow-up with the continuation of ruxolitinib is ongoing.

Third case

A male patient, aged 64, initially consulted a hematologist in May 2012 due to the findings of thrombocytosis in the blood count, with the information that he had known about elevated platelets since November 2010 (then Tr 989x10^9/L), but he did not examine it. During the initial examination, the blood count showed Hb 107 g/L, MCV 82fl, Le 11.2x10^9/L, Tr 1457x10^9/l, in the differential formula neutrophils 62%, lymphocytes 20%, monocytes 5%. In the laboratory findings, LDH was 631U/L, and ferritin was 9 ugL, despite previous administration of peroral iron medications. An abdominal ultrasound from May 2012 showed a spleen measuring 12x5 cm. The patient, except for mild skin itching, had no other complaints.

In the performed hematological diagnosis, the findings of the bone marrow biopsy showed fibrosis grade I, and spontaneous development of erythroid colony formations was observed. Conventional cytogenetics was normal (metaphase 4 - low mitotic index). The PCR method did not confirm the JAK2V617F mutation. The presence of MPL, SRSF2, U2AF1, and ASXL1 mutations was determined using the NGS method (Professor Skoda's mutation panel). The serum erythropoietin level was average. A diagnosis of prefibrotic PMF, low risk according to IPSS score, with an MPN 10 score of 5/100, was made.

Acetylsalicylic acid (100 mg *per os* per day) was initially introduced, but it was discontinued in May 2012 due to severe epistaxis (Von Willebrand factor activity was determined to be 34%). In the same month, HU (500 mg *per os* per day) was introduced, the dosage of which was corrected in the further course of treatment depending on the blood count parameters. HU was applied until April 2019, when the presence of transfusion-dependent anemia syndrome was established, and the treatment was continued with Danazol (2x200 mg *per os* per day).

In October 2019, a hematological reevaluation was performed. Bone marrow rebiopsy showed the presence of fibrosis, now gr III, while aspiration puncture showed no acute transformation (8% blasts). The presence of a complex karyotype, 46 XY, part (20)q11/(5), 46 XY/15/, was observed. PCR for the JAK2V617F mutation was still negative. The blood count showed

ona subsitutcija koncentratima eritrocitma, dva puta mesečno. I pored navedene terapije, konstitucionalni simptomi su se pogoršavali, uz održavanje transfuzione zavisnosti. Nastavljeno je sa primenom Danazola, međutim, usred razvoja srčane insuficijencije, došlo je do smrtnog ishoda u februaru 2020 godine. OS je iznosilo 96 meseci.

DISKUSIJA

Mnogi pacijenti kod kojih je postavljena dijagnoza PMF niskog/ nižeg stepena rizika manifestuju jasne kliničke znake i simptome osnovne bolesti i stoga bi imali koristi od primene aktivnog lečenja [10]. Na prikazu ovi pacijenti najčešće imaju malaksalost (30%), LDH je povećana kod ¼ pacijenata, slezina je palpabilna kod 19%, a leukocitoza je prisutna u 15% slučajeva [10]. Prema nalazima „MOST" observacione studije (The Myelobrosis and Essential Thrombocythemia Observational Study; NCT02953704), u trenutku uključivanja u studiju 41% pacijenata (od 232 ispitanika) pripadalo je grupi niskog rizika prema DIPSS skoru, a 59% grupi intermedijarnog-1 rizika ali je splenomegalija konstatovana kod 41% pacijenta sa niskim rizikom, a manje (31%) kod pacijenata sa intermedijarnim-1 rizikom [11]. Samo jedan pacijent u našoj miniseriji je imao jasne simptome na prikazu, drugi samo blagi pruritus. Interesantno, svi naši pacijenti imali su značajnu trombocitozu na prikazu i samo blagu leukocitozu u dva slučaja. Dva naša pacijenta prikazala su se splenomegalijom, u jednom slučaju graničnom.

Prema aktuelnim preporukama NCCN-a (National Comprehensive Cancer Network, eng.), ruxolitinib predstavlja dobru terapijsku opciju za rano lečenje pacijenata sa PMF nižeg stepena rizika, što dokazuju i podaci JUMP I ROBUST studija pacijenata sa intermedijarnim-1 rizikom, i koje potvrđuju benefit rane primene ruxolitiniba, uz 50% ili veću redukciju splenomegalije kod čak 61% pacijenata (nakon 48 nedelja terapije), uz značajnu redukciju inicijalne simptomatologije [12,13]. Rezultati COMBI studije ukazali su na to da je u podgrupi pacijenata sa PMF (dominantno niskog ili intermedijarnog-1 stepena rizika), kombinovana primena ruxolitiniba i malih doza pegilovanog interferona alfa 2a, dovela do remisije u 44% slučajeva, uz prihvatljivu toksičnost [14]. Svi naši pacijenti imali su inicijalnu dijagnozu prefibrotičnog PMF niskog stepena rizika, svi su inicijalno lečeni primenom antiagregacione terapije, a potom sa HU zbog progresije splenomegalije ili leukocitoze ili trombocitoze. Kod dva pacijenta je uveden ruxolitinib, uz kompletni odgovor u jednom slučaju i aktuelno održavanje remisije bolesti nakon više od 10 godina primene ruxolitiniba, ali i dalje progresije bolesti do smrtnog ishoda u drugom slučaju. Treba

Hb 72g/L, Le 19.1x10^9/l, Tr 487x10^9/L, in the peripheral blood smear 6% blasts with leukoerythroblastosis, reticulocytes 5.2%. LDH was 2498 U/L, and ferritin was 247.8 mg/L. The ultrasound findings from October 2019 showed a spleen measuring 14 cm.

In the further course of treatment, the patient reported severe weakness and fatigue, and transfusion substitution with erythrocyte concentrates was continued twice a month. Despite the mentioned therapy, the constitutional symptoms worsened, while the transfusion dependence was maintained. The use of Danazol was continued; however, amid the development of heart failure, there was a fatal outcome in February 2020. OS was 96 months.

DISCUSSION

Many patients diagnosed with low/low-risk PMF manifest clear clinical signs and symptoms of the underlying disease and would, therefore, benefit from active treatment [10]. At presentation, these patients most often have weakness (30%), LDH is increased in ¼ of patients, the spleen is palpable in 19%, and leukocytosis is present in 15% of cases [10]. According to the findings of the "MOST" observational study (The Myelobrosis and Essential Thrombocythemia Observational Study; NCT02953704), at the time of inclusion in the study, 41% of patients (out of 232 subjects) belonged to the low-risk group according to the DIPSS score, and 59% to the intermediate-1 risk group, but splenomegaly was noted in 41% of patients with low risk, and less (31%) in patients with intermediate-1 risk [11]. Only one patient in our miniseries had apparent symptoms at presentation; the other had only mild pruritus. Interestingly, all our patients had significant thrombocytosis at presentation and only mild leukocytosis in two cases. Two of our patients presented with splenomegaly, in one case borderline.

According to the current NCCN (National Comprehensive Cancer Network) recommendations, ruxolitinib represents an excellent therapeutic option for the early treatment of patients with PMF of lower risk, as evidenced by the data of the JUMP and ROBUST studies of patients with intermediate-1 risk, which confirm the benefit early administration of ruxolitinib, with a 50% or more significant reduction of splenomegaly in as many as 61% of patients (after 48 weeks of therapy), with a substantial reduction of initial symptoms [12,13]. The results of the COMBI study indicated that in a subgroup of patients with PMF (predominantly low or intermediate-1 risk), the combined use of ruxolitinib and low-dose pegylated interferon alfa 2a led to remission in 44% of cases, with acceptable toxicity [14]. All our patients had an initial diagnosis of low-risk

napomenuti da je kod drugog pacijenta bila prisutna progresivna leukocitoza koja značajno smanjuje ukupno preživljavanje sa medijanom OS od 13,3 meseca. U ovakvim slučajevima, prema rezultatima nekih retrospektivnih analiza, primena ruxolitiniba pokazala je najveći benefit, sa produženjem medijane OS na 21,2 meseci [15], što ugrubo odgovara i medijani OS kod našeg pacijenta, a po uočenoj progresiji prethodne blage leukocitoze.

Non-driver mutacije imaju značajan uticaj na klinički tok bolesti, ali i ishod lečenja. ASXL1 non-driver mutacije imaju značajnu ulogu u progresiji pre-PMF u pravi PMF, kao i u stepenu fibroze, ali i leukemijskoj transformaciji PMF [15]. SRSF2 mutacije pokazale su tendenciju redukcije klonske proliferacije u toku uočene progresije bolesti. ASXL1 mutacije nisu direktno odgovorne za leukemogenezu, ali dovode do akceleracije akumulacije direktnih leukemogenih faktora, npr. mutiranih produkata RAS-signalnog puta. Stoga, aktuelni literaturni podaci idu u prilog tome da ASXL1 mutacija ima kritičnu ulogu u kliničkom toku PMF, uz uticaj na pogoršanje fibroze i povećanje mogućnosti leukemijske transformacije. Obzirom na svoju značajnu frekventnost u PMF, ASXL1 mutacije bi u budućnosti mogle biti značajne za target-terapiju [16]. Kod dva naša pacijenta je rađen NGS, uz ASXL1 prisutvo mutacije u oba slučaja sa potpuno drugačijim kliničkim ishodom. Naime, jedna pacijentkinja je u dugogodišnjoj remisji uz primenu ruxolitiniba, dok je drugi pacijent imao smrtni ishod, ali nije lečen sa JAK inhibitorom, već antigregacionom terapijom, HU i danazolom po postizanju transfuzione zavisnosti. Ni kod jednog našeg pacijenta nije dokazana leukemijska transformacija.

Klinički tok bolesti kod pacijenata sa PMF nižeg rizika pokazuje nepredvidljivost, što potvrđuju prethodni, ali i najnoviji rezultati „MOST" observacione studije prema kojima 60% pacijenata (medijana praćenja grupe od 232 pacijenta sa MF niskog/intermedijernog-1 rizika je bila 52,9 meseci) ima znakova progresije bolesti, dok je 8.3% pacijenta imalo leukemijsku transformaciju ili umrlo [11,17]. Treba napomenuti da je 84 pacijenta imalo PMF niskog rizika, a kriterijumi progresije bolesti su bili Hb <100 g/L, Tr <100×10^9/L, konstitucionalni simptomi, pojava ili progresija splenomegalije, prisutvo blasta >1% u perifernoj krvi, Le >25×10^9/L, smrtni ishod usled progresije bolesti, leukemijska transformacija i više od jedne transfuzije Er. U našoj miniseriji se uočava jasna progresija bolesti u sva tri slučaja, ali uz različite terapijske odgovore i klinički ishod bolesti. Dva pacijenta su imala smrtni ishod, OS su iznosili 66 i 96 meseci, dok je treća pacijentkinja u stabilnoj remisiji sa OS od 156 meseci. Ona je takođe nosilac ASXL1 mutacije (Tabela 1).

prefibrotic PMF; all were initially treated with antiplatelet therapy and then with HU due to the progression of splenomegaly or leukocytosis or thrombocytosis. In two patients, ruxolitinib was introduced, with a complete response in one case and current maintenance of disease remission after more than 10 years of ruxolitinib administration, but still disease progression to death in the other case. Of note, another patient had progressive leukocytosis, which significantly reduced overall survival with a median OS of 13.3 months. In such cases, according to the results of some retrospective analyses, the use of ruxolitinib showed the most significant benefit, with an extension of the median OS to 21.2 months [15], which roughly corresponds to the median OS in our patient, and according to the observed progression of the previous mild leukocytosis.

Non-driver mutations have a significant impact on the clinical course of the disease as well as the outcome of treatment. ASXL1 non-driver mutations play an essential role in the progression of pre-PMF to true PMF, as well as in the degree of fibrosis and the leukemic transformation of PMF [15]. SRSF2 mutations tended to reduce clonal proliferation during the observed disease progression. ASXL1 mutations are not directly responsible for leukemogenesis but lead to an acceleration of the accumulation of direct leukemogenic factors, e.g., mutated products of the RAS signaling pathway. Therefore, current literature data supports that ASXL1 mutation plays a critical role in the clinical course of PMF, affecting worsening fibrosis and increasing the possibility of leukemic transformation. Considering its significant frequency in PMF, ASXL1 mutations could be important for target therapy in the future [16]. In two of our patients, NGS was performed, with ASXL1 mutation in both cases, resulting in a completely different clinical outcome. Namely, one patient is in long-term remission with the use of ruxolitinib, while the other patient had a fatal outcome but was not treated with a JAK inhibitor but with antiaggregation therapy, HU, and danazol after achieving transfusion dependence. No leukemic transformation was proven in any of our patients.

The clinical course of the disease in patients with lower-risk PMF shows unpredictability, which is confirmed by the previous and the most recent results of the "MOST" observational study, according to which 60% of patients (the median follow-up of a group of 232 patients with low/intermediate-1 risk MF was 52.9 months) have signs of disease progression, while 8.3% of patients had leukemic transformation or died [11,17]. It should be noted that 84 patients had low-risk PMF, and the criteria for disease progression were Hb <100 g/L, Tr <100×10^9/L, constitutional symptoms, the appearance or progression of splenomegaly, the pres-

Tabela 1. Dijagnoza, lečenje i ishod bolesti u našoj grupi pacijenata

Table 1. Diagnose, treatment, and disease outcomes in our group of our pts

Pacijent / *Patient*	Dijagnoza / *Diagnose*	JAK2V617F mutacioni status / *JAK2V617F mutation*	NGS mutacije / *NGS mutations*	Lečenje / *Treatment*	Neželjeni događaji / *Adverse events*	Ukupno preživljavanje / *Overall survival* (meseci / *months*)	Ishod / *Outcomes*
Br. 1 / *No1*	prePMF IPSS „nizak rizik / *IPSS low risk*" MPN 10- 10/100	+	Nije urađen / *Not done*	ASA HU Rukoslitinib Azacitidin	Progresija 18m nakon uvođenja rukolitiniba / *Disease progression 18mts after ruxo introduction*	66	Smrtni ishod / *Death*
Br. 2/ *No2*	prePMF IPSS „nizak rizik/ *IPSS low risk*" MPN 10- 25/100	+	ASXL1+	ASA, HU, Ruksolitinib/ Ruxolitinib	Ne/ *None*	126	Kompletna remisija / *Complete remission*
Br. 3/ *No3*	prePMF IPSS „nizak rizik/ *IPSS low risk*" MPN 10- 5/100	-	MPL+, SRSF2+ U2AF1+ ASXL1+	ASA HU Danazol	Epistaksa / *Epistaxis* Transfuziona zavisnost / *Transfusion dependence* Srčana insuficijencija / *Cardiac insufficiency*	96	Smrtni ishod / *Death*

Legenda: PMF– Primarna mijelofibroza; IPSS – Internacionalni Prognostički Skor sistem; MPN 10 – skor procene simptoma mijeloproliferativne neoplazme 10; JAK2V617F – Janus kinaza2 V617F; NGS – Sekvencioniranje sledeće generacije; ASA – Acetilsalicilna kiselina; HU- Hidrokisurea; m- meseci

Legend: *PMF– Primary myelofibrosis; IPSS – International Prognostic Score System; MPN 10 – Myeloproliferative neoplasm symptom assessment 10; JAK2V617F – Janus kinase2 V617F; NGS – Next Generation Sequencing; ASA – Acetilsalicylic acid; HU-Hydroxyurea; m- months*

ZAKLJUČAK

Aktuelni podaci idu u prilog značajnom prisustvu progresije bolesti i kod pacijenata sa incijalnim PMF niskog rizika, što zahteva regularno praćenje ovih pacijenata uz blagovremeno uključivanje/izmenu aktuelne terapije u slučaju progresije. Naši podaci takođe potvrđuju da je PMF niskog rizika jedan entitet sa mnogo različitih lica. Buduća istraživanja će pokazati da li rana NGS analiza non-driver mutacija, kao i rano uvođenje terapije doprinose promeni toka bolesti, kao i da li će non-driver mutacije biti nova meta u ciljanoj terapiji za PMF.

Sukob interesa: Nije prijavljen.

ence of blasts >1% in peripheral blood, Le >25×10^9/L, death due to disease progression, leukemic transformation and more than one transfusion Er. In our miniseries, a clear progression of the disease can be observed in all three cases, but with different therapeutic responses and clinical outcomes of the disease. Two patients had a fatal outcome: OS was 66 and 96 months, while the third was a patient in stable remission with an OS of 156 months. She is also a carrier of the ASXL1 mutation (Table 1).

CONCLUSION

Current data supports the significant presence of disease progression in patients with low-risk initial PMF, which requires regular monitoring of these patients with timely inclusion/change of current therapy in case of progression. Our data confirm that low-risk PMF is one entity with many different faces. Future research will show whether early NGS analysis of non-driver mutations and early therapy introduction contribute to changing the course of the disease and whether non-driver mutations will be a new target in targeted therapy for PMF.

Conflict of interest: None declared

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