KORELACIJA CITOGENETIKE I STEPENA FIBROZE KOSTNE SRŽI U PRIMARNOJ MIJELOFIBROZI

ORIGINALNI RAD

ORIGINAL ARTICLE

CORRELATION OF CYTOGENETICS AND GRADE OF BONE MARROW FIBROSIS IN PRIMARY MYELOFIBROSIS

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SAŽETAK

Uvod/Cilj: Primarna mijelofibroza je klonska mijeloproliferativna neoplazma koju karakterišu fibroza kostne srži i ekstramedularna hematopoeza. Fibroza često otežava aspiraciju i biopsiju kostne srži, što takođe otežava i dijagnozu bolesti. Uprkos tome, citogenetska analiza je moguća iz aspirata kostne srži ili iz periferne krvi. Kariotip je nezavisan prognostički parametar u primarnoj mijelofibrozi. Prognostički značaj stepena fibroze kostne srži u primarnoj mijelofibrozi je još uvek diskutabilan. Cilj studije bio je ispitati značaj fibroze kostne srži na ukupno preživljavanje pacijenata sa primarnom mijelofibrozom, kao i korelaciju kariotipa i stepena fibroze kostne srži ovih pacijenata.

Materijal i metode: Ispitivanje korelacije kariotipa i stepena fibroze kostne srži obuhvatilo je 120 od ukupno 136 pacijenata sa primarnom mijelofibrozom. Koristili smo stratifikaciju hromozomskih abnormalnosti prema preporukama prognostičkih skoring sistema: Lille, Mayo, Internacionalnog i Dinamičkog internacionalnog. Analiza kariotipa vršena je konvencionalnom citogenetskom metodom. Biopsije koštne srži pacijenata obojene su metodom Gordon Sweet.

Rezultati: Kod 136 pacijenata sa primarnom mijelofibrozom nije registrovana statistički značajna razlika ($p = 0,084$) u preživljavanju, bez obzira na stepen fibroze kostne srži. Takođe, nije registrovana korelacija između kariotipa ili hromozomskih aberacija različitog stepena rizika sa stepenom fibroze kostne srži (Lille $p = 0,293$; Mayo $p = 0,108$); Internacionalni prognostički skoring sistem $p = 0,086$; Dinamički internacionalni prognozni skoring sistem ($p = 0,613$) kod 120 pacijenata sa primarnom mijelofibrozom.

Zaključak: Iako nismo konstatovali korelaciju kariotipa i stepena fibroze kostne srži, smatramo da oba parametra treba da budu zastupljena u nekim od novih Prognoznih Skoring Sistema za primarnu mijelofibrozu.

Ključne reči: primarna mijelofibroza, kariotip, biopsija kostne srži

ABSTRACT

Introduction/Aim: Primary myelofibrosis is a clonal myeloproliferative neoplasm characterized by bone marrow fibrosis and extramedullary hematopoiesis. Fibrosis often complicates bone marrow aspiration and biopsy, further hindering disease diagnosis. Despite this, cytogenetic analysis is possible from bone marrow aspirate or peripheral blood. Karyotype is an independent prognostic parameter in primary myelofibrosis. The prognostic significance of bone marrow fibrosis grade in primary myelofibrosis remains debatable. This study aimed to investigate the impact of bone marrow fibrosis on overall survival in patients with primary myelofibrosis, as well as to examine the correlation between karyotype and bone marrow fibrosis grade in these patients.

Materials and Methods: The study of the correlation between karyotype and bone marrow fibrosis grade included 120 out of 136 patients with primary myelofibrosis. We used the stratification of chromosomal abnormalities according to the recommendations of prognostic scoring systems: Lille, Mayo, International, and Dynamic International. Karyotype analysis was performed using the conventional cytogenetic method. Bone marrow biopsies of patients were stained using the Gordon-Sweet method.

Results: In 136 patients with primary myelofibrosis, no statistically significant difference in survival was observed ($p = 0.084$) regardless of the bone marrow fibrosis grade. Additionally, no correlation was found between karyotype or chromosomal aberrations of varying risk levels and bone marrow fibrosis grade (Lille $p = 0.293$; Mayo $p = 0.108$; International Prognostic Scoring System $p = 0.086$; Dynamic International Prognostic Scoring System $p = 0.613$) in 120 patients with primary myelofibrosis.

Conclusion: Although we did not establish a correlation between karyotype and bone marrow fibrosis grade, we believe that both parameters should be incorporated into new prognostic scoring systems for primary myelofibrosis.

Keywords: primary myelofibrosis, karyotype, bone marrow biopsy

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UVOD

Primarna mijelofibroza (PMF) je klonska mijeloproliferativna neoplazma (MPN) koju karakterišu fibroza kostne srži (KS) i ekstramedularna hematopoeza [1,2]. Pacijenti sa PMF-om su heterogeni na prezentaciji. Poznato je da nepovoljni prognostički faktori uključuju stariju dob, prisustvo konstitucionih simptoma, anemiju, smanjen ili povišen broj leukocita, trombocitopeniju, cirkulišuće mijeloblaste i prisustvo klonskih hromozomskih aberacija [3,4].

Citogenetski podaci u PMF-i su često oskudni i neuverljivi. Njihov prognostički značaj ograničen je teškoćama u dobijanju metafaza dobrog kvaliteta iz aspirata KS [5]. Međutim, studije koje su izveštavale o uspešnoj citogenetici pokazale su da se rekurentne citogenetske abnormalnosti viđaju kod otprilike jedne trećine pacijenata pri postavljanju dijagnoze, i da se njihova učestalost povećava tokom bolesti. Najčešće aberacije u PMF-i (+1q, 13q-, 20q-, +9, +8) javljaju se kod dve trećine pacijenata sa patološkim kariotipom [6]. Druge abnormalnosti, kao što su balansirane translokacije, kompleksno aberantni kariotipovi i koegzistencija dva ili više nepovezana patološka klona, su retke. Ipak, citogenetika je jedan od osnovnih prognostičkih parametara u aktuelnim Prognoznim Skoring sistemima (PSS) u PMF-i.

Za razliku od citogenetike, fibroza je ključni histopatološki nalaz u biopsiji KS pacijenata sa PMF-om i predstavlja glavni dijagnostički kriterijum za PMF prema klasifikaciji Svetske zdravstvene organizacije (SZO) [7]. Fibroza je reaktivni fenomen koji nastaje zbog abnormalnosti sazrevanja megakariocita, koji oslobađaju povećane količine upalnih i fibrogenih citokina, a oni podstiču taloženje retikulina i kolagenih vlakana, stvaranje novih sudova i osteosklerozu [8].

Prema evropskom konsenzusu, fibroza KS procenjuje se u oblastima hematopoeze semikvantitativno, skalom od 4 nivoa: stepen 0 – odsutan; stepen 1 – slaba mreža retikulina sa mnogo ukrštanja, posebno u perivaskularnim područjima; stepen 2 – difuzno i gusto povećanje retikulina sa ekstenzivnim presecima, povremeno sa fokalnim snopovima kolagena i/ili fokalnom osteosklerozom; stepen 3 – difuzno i gusto povećanje retikulina sa ekstenzivnim presecima i grubim snopovima kolagena, često udruženim sa osteosklerozom [9]. Ovi kriterijumi su pojačani i revidirani novim kriterijumima SZO za dijagnozu PMF-a iz 2016. [10]. Još uvek ne postoji jasan stav o fibrozi KS kao prognostičkom parametru u PMF-u.

Cilj našeg istraživanja, čiji su rezultati predstavljeni u ovom radu, bio je ispitati postoji li korelacija između citogenetskih podataka i stepena fibroze KS u biopsiranim uzorcima pacijenata sa potvrđenom dijagnozom

INTRODUCTION

Primary myelofibrosis (PMF) is a clonal myeloproliferative neoplasm (MPN) characterized by bone marrow (BM) fibrosis and extramedullary hematopoiesis [1,2]. Patients with PMF present with heterogeneous clinical features. It is known that unfavorable prognostic factors include older age, the presence of constitutional symptoms, anemia, decreased or increased leukocyte count, thrombocytopenia, circulating myeloblasts, and the presence of clonal chromosomal aberrations [3,4].

Cytogenetic data in PMF are often scarce and inconclusive. Their prognostic significance is limited by the difficulty in obtaining high-quality metaphases from bone marrow aspirates [5]. However, studies reporting successful cytogenetic analysis have shown that recurrent cytogenetic abnormalities are observed in approximately one-third of patients at diagnosis, and their frequency increases throughout the disease. The most common aberrations in PMF (+1q, 13q-, 20q-, +9, +8) occur in two-thirds of patients with an abnormal karyotype [6]. Other abnormalities, such as balanced translocations, complex aberrant karyotypes, and the coexistence of two or more unrelated pathological clones, are rare. Nevertheless, cytogenetics remains one of the key prognostic parameters in current Prognostic Scoring Systems (PSS) for PMF.

Unlike cytogenetics, fibrosis is a crucial histopathological finding in BM biopsies of PMF patients and represents a primary diagnostic criterion for PMF according to the World Health Organization (WHO) classification [7]. Fibrosis is a reactive phenomenon caused by abnormal megakaryocyte maturation, leading to the release of increased amounts of inflammatory and fibrogenic cytokines. These cytokines stimulate the deposition of reticulin and collagen fibers, the formation of new blood vessels, and osteosclerosis [8].

According to the European consensus, BM fibrosis is assessed in hematopoietic areas using a semi-quantitative scale with four levels: grade 0 – absent; grade 1 – a loose network of reticulin with many intersections, especially in perivascular areas; grade 2 – diffuse and dense increase reticulin with extensive intersections, occasionally with focal bundles of collagen and/or focal osteosclerosis; grade 3 – diffuse and dense increase reticulin with extensive intersections and coarse collagen bundles, often associated with osteosclerosis [9]. These criteria were reinforced and revised by the new WHO diagnostic criteria for PMF introduced in 2016 [10]. There is still no clear consensus on BM fibrosis as a prognostic parameter in PMF.

The aim of our study, whose results are presented in this paper, was to investigate whether there is a correlation between cytogenetic data and the degree of bone marrow (BM) fibrosis in biopsied samples of pa-

PMF-a. Korelaciju smo testirali korišćenjem PSS-a (Lille, Mayo, IPSS i DIPS), koji imaju najčešću i najdužu primenu u kliničkoj praksi za prognozu PMF-a. Osim toga, ovi PSS-i jasno definišu hromozomske aberacije kao nezavisan prognozni parametar za bolesnike sa PMF-om. Svaki od primenjenih PSS-a daje stratifikaciju kariotipa, bilo da je normalan ili aberantan, kao i hromozomske aberacije u prognozne grupe različitog stepena rizika (povoljan, nepovoljan, srednje povoljan-1, srednje povoljan-2), što je olakšalo ispitivanje korelacije kariotipa, odnosno hromozomskih aberacija različitog stepena rizika, sa stepenom fibroze KS na skali od 0-3 prema evropskom konsenzusu (MF-0, MF-1, MF-2, MF-3) [9].

PACIJENTI I METODE

Pacijenti

U periodu od januara 2004. do decembra 2010. godine, na Klinici za hematologiju Univerzitetskog kliničkog centra Srbije u Beogradu, dijagnostikovana su 144 pacijenta sa PMF-om. Svi pacijenti ispunjavali su dijagnostičke kriterijume SZO za PMF [11,12]. Klinički, laboratorijski i patohistološki podaci, zajedno sa citogenetskim rezultatima, prikupljani su retrospektivno do 2013. godine. Pacijenti sa post-policitemijskom i post-esencijalnom trombocitemijskom mijelofibrozom (post-PV-MF i post-ET-MF) isključeni su iz studije.

Od 144 novodijagnostikovana pacijenata sa PMF-om, izveštaj o patohistološkim nalazima bio je dostupan za 136 pacijenata (136/144). Citogenetska analiza nije bila moguća kod 16 od 136 pacijenata (16/136). Centralni deo ispitivanja ove studije predstavljaju klinički i dijagnostički parametri 120 pacijenata kod kojih je bilo moguće ispitati korelaciju kariotipa sa stepenom fibroze KS. Njihovi podaci su bili dostupni u kliničkoj arhivi. Kod 24 pacijenta, od ukupno 144 (24/144) sa dijagnozom PMF-a, koji su lečeni na klinici u navedenom periodu, nisu mogli biti uključeni u studiju jer nisu imali jedan od dva nalaza: patohistološki ili citogenetski. U vreme prikupljanja podataka za ovu studiju, 78 pacijenata (65%) bilo je živo, dok 42 pacijenta (35%) nije preživelo.

Podaci o aspiratima i biopsijama KS korišćeni za istraživanje uzeti su iz arhive uz saglasnost pacijenata ili njihovih porodica. Studiju je odobrio Etički komitet Univerzitetskog kliničkog centra Srbije u Beogradu.

METODE

Citogenetska analiza

Konvencionalna citogenetska analiza vršena je na metafazama dobijenim iz nestimulisanih ćelija iz aspirata KS ili iz kultura periferne krvi, korišćenjem prethodno objavljene tehnike [13]. Kariotipovi su interpretirani

tients with a confirmed diagnosis of primary myelofibrosis (PMF). We tested this correlation using PSS (Lille, Mayo, IPSS, and DIPS), which are the most commonly and extensively used scoring systems in clinical practice for PMF prognosis. Additionally, these PSS clearly define chromosomal aberrations as an independent prognostic parameter for PMF patients. Each applied PSS stratifies the karyotype, whether regular or aberrant, as well as chromosomal aberrations, into prognostic groups of varying risk levels (favorable, unfavorable, intermediate-1, intermediate-2), which facilitated the examination of the correlation between karyotype, or chromosomal aberrations of different risk levels, with the degree of BM fibrosis on a scale of 0-3 according to the European consensus (MF-0, MF-1, MF-2, MF-3) [9].

PATIENTS AND METHODS

Patients

From January 2004 to December 2010, a total of 144 patients with PMF were diagnosed at the Clinic for Hematology of the University Clinical Center of Serbia in Belgrade. All patients met the WHO diagnostic criteria for PMF [11,12]. Clinical, laboratory, and pathohistological data, along with cytogenetic results, were collected retrospectively until 2013. Patients with post-polycythemia vera myelofibrosis (post-PV-MF) and post-essential thrombocythemia myelofibrosis (post-ET-MF) were excluded from the study.

Out of the 144 newly diagnosed PMF patients, pathohistological reports were available for 136 patients (136/144). Cytogenetic analysis was not possible in 16 of the 136 patients (16/136). The core focus of this study was the clinical and diagnostic parameters of 120 patients in whom the correlation between karyotype and BM fibrosis grade could be assessed. Their data were available in the clinical archives.

A total of 24 out of 144 PMF patients (24/144) diagnosed and treated at the clinic during the study period were excluded from the study because they lacked either a pathohistological or cytogenetic report. At the time of data collection for this study, 78 patients (65%) were alive, while 42 patients (35%) had not survived.

Data on BM aspirates and biopsies used for the research were obtained from the archive with the consent of the patients or their families. The Ethics Committee of the University Clinical Center of Serbia in Belgrade approved the study.

METHODS

Cytogenetic analysis

Conventional cytogenetic analysis was performed on metaphases obtained from unstimulated cells in bone

prema Međunarodnom sistemu za humanu citogenetsku nomenklaturu [14].

Patohistološka analiza

Biopsije KS obojene su metodom Gordon Sweet. Fibroza je ocenjena korišćenjem Bauermeister sistema iz 1971. godine [15] (skala 0–4) i konsenzusom revidovanog evropskog sistema (skala 0–3).

Statistika

Statistička analiza korelacije citogenetike i fibroze KS izvršena je korišćenjem nekoliko PSS-a. Preživljavanje se merilo od dijagnoze do poslednjeg kontakta ili smrti. Podaci su predstavljeni kao medijana (min-max) ili *n* (%). Razlika između Lile, IPSS, Mayo i DIPSS kategorija po BMF analizirana je Mann-Whitney U testom (dve grupe) i Kruskal-Wallis testom (tri ili četiri grupe). Preživljavanje je procenjeno korišćenjem Kaplan-Meier krive i Log-rank testa za grupna poređenja. Sve analize obavljene su korišćenjem SPSS 20.0 (IBM Corp.). Vrednosti verovatnoće (p) ≤ 0,05 smatrane su statistički značajnim.

REZULTATI

S obzirom na činjenicu da je fibroza KS jedan od vodećih parametara za dijagnozu PMF-a i jedan od dva parametra ispitivanja u našoj studiji, testirali smo i ukupno preživljavanje kod 136 pacijenata sa PMF-om u zavisnosti od stepena fibroze KS. Statističko ispitivanje ukupnog preživljavanja pokazalo je da se vreme preživljavanja ne razlikuje statistički značajno u odnosu na stepen fibroze KS (p = 0,084). Međutim, u grupi ispitanika bio je mali broj pacijenata sa fibrozom stepena 0 (MF-0), svega tri. Iz tog razloga smo iz statistike isključili grupu pacijenata sa MF-0, nakon čega smo utvrdili da postoji statistički značajna razlika (p = 0,043) u preživljavanju pacijenata sa fibrozom stepena MF-1, MF-2 i MF-3.

Iz ove grupe pacijenata sa PMF-om odabrali smo 120 pacijenata koji su imali kompletne kliničke, patohistološke i citogenetske podatke. U tabeli 1 prikazane su njihove karakteristike u vreme postavljanja dijagnoze. Srednja starost je bila 65,5 godina (raspon 28–80); 45 pacijenata (37,5%) bilo je mlađe od 60 godina. Odnos žena i muškaraca bio je 47/72. Medijana završetka praćenja u 2013. godini iznosila je 83 meseca, pri čemu je zabeleženo 42 (35,0%) smrtnih slučajeva. Prisustvo konstitucionih simptoma bilo je primećeno kod 23 pacijenta (19,3%), hemoglobin < 100g/L kod 16 (13,3%), broj leukocita < 4 ili > 30 x 10^9/L kod 15 (12,5%), broj trombocita < 100 x 10^9/L kod 9 (7,5%), cirkulišućih blasta ≥ 1% kod 21 (17,5%) i splenomegalija kod 68 (56,7%) pacijenata. Normalan kariotip je bio zastupljen

marrow aspirates or peripheral blood cultures using a previously published technique [13]. Karyotypes were interpreted according to the International System for Human Cytogenetic Nomenclature [14].

Pathohistological analysis

BM biopsies were stained using the Gordon-Sweet method. Fibrosis was assessed using the Bauermeister system from 1971 [15] (scale 0–4) and the consensus of the revised European system (scale 0–3).

Statistics

Statistical analysis of the correlation between cytogenetics and BM fibrosis was performed using several PSS. Survival was measured from diagnosis to the last contact or death. Data were presented as median (min-max) or n (%). Differences between Lille, IPSS, Mayo, and DIPSS categories by BMF were analyzed using the Mann-Whitney U test (for two groups) and the Kruskal-Wallis's test (for three or four groups). Survival was estimated using the Kaplan-Meier curve and the Log-rank test for group comparisons. All analyses were conducted using SPSS 20.0 (IBM Corp.). Probability values *(p)* ≤ 0.05 were considered statistically significant.

RESULTS

Given that BM fibrosis is one of the key parameters for diagnosing PMF and one of the two main parameters examined in our study, we also analyzed overall survival in 136 PMF patients based on the degree of BM fibrosis. The statistical analysis of overall survival revealed that survival time did not differ significantly according to BM fibrosis grade (p = 0.084). However, there were only three patients with grade 0 fibrosis (MF-0) in the study group. For this reason, we excluded the MF-0 group from the statistical analysis, after which we found a statistically significant difference (p = 0.043) in survival among patients with fibrosis grades MF-1, MF-2, and MF-3.

From this PMF patient group, we selected 120 patients with complete clinical, pathohistological, and cytogenetic data. Table 1 presents their characteristics at the time of diagnosis. The median age was 65.5 years (range 28–80), with 45 patients (37.5%) being younger than 60 years. The female-to-male ratio was 47/72. The median follow-up duration in 2013 was 83 months, with 42 deaths (35.0%) recorded. Constitutional symptoms were observed in 23 patients (19.3%), hemoglobin < 100 g/L in 16 (13.3%), leukocyte count < 4 or > 30 × 10^9/L in 15 (12.5%), platelet count < 100 × 10^9/L in 9 (7.5%), circulating blasts ≥ 1% in 21 (17.5%), and splenomegaly in 68 (56.7%) patients. A normal karyotype was present in 88 patients (88/120 or 73.3%), while 32

Tabela 1. Osnovne karakteristike 120 upisanih pacijenata

Table 1. Baseline characteristics of the 120 enrolled patients

Karakteristike / *Characteristics*	**Medijana (raspon) / *Median (range)***	**Broj pacijenata / *Number of patients***	**%**
Godine > 60 g / *Age > 60 years* Godine ≤ 6 0g / *Age ≤ 60 years*	65.5 (28–80)	75 45	62.5 37.5
Žene/Muškarci / *Female/Male*		47/72	39/61
Konstitucioni simptomi / *Constitutional symptoms*		23	19.3
Hb < 100g/L / *Hemoglobin < 100g/L*	129.5 (45–169)	16	13.3
Le < 4 ili >30x10^9/L / *Leukocyte count < 4 or >30x 10^9/L*	11.1 (1.6–83.2)	15	12.5
Tr < 100 x 10^9/L / *Platelets count < 100 x 10^9/L*	687.5 (28–684)	9	7.5
Blasti u perifernoj krvi ≥1% / *Circulating blasts ≥ 1%*	(0–9)	21	17.5
Palpabilna splenomegalija (cm) / *Palpable splenomegaly (cm)*	(0–24)	68	56.7
Kariotip / *Karyotype* Normalan / *Normal* Aberantan / *Aberrant*		120 88 32	 73.3 26.7
Smrtnost / *Deaths*		42	35.0

kod 88 pacijenata (88/120 ili 73,3%), a aberantan kod preostalih 32 bolesnika (32/120 ili 26,7%) (Tabela 1).

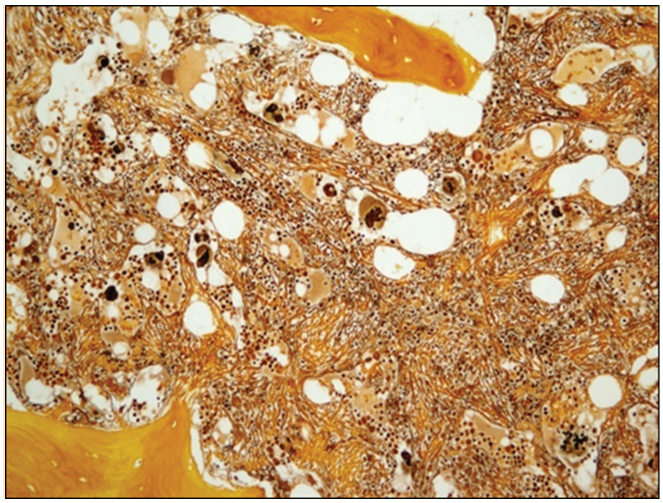
Svi pacijenti klasifikovani su prema Evropskom konsenzusu o stepenu fibroze KS [9]. Primeri morfoloških karakteristika PMF-a i stepena fibroze KS prikazani su na Slici 1. Stratifikacija pacijenata prema stepenu fibroze i citogenetskim podacima prikazani su u Tabeli 2. U redovima Tabele 2. dati su PSS-i i stratifikacija hromozomskih aberacija po prognoznim grupama različitog stepena rizika, dok su u kolonama tabele dati stratifikovani stepeni fibroze KS bolesnika sa skalom od 0-3. Distribucija broja pacijenata koji su imali adekvatan kariotip (normalan ili aberantan) ili hromozomske aberacije, određenog stepena rizika sa adekvatnim stepenom fibroze u KS prikazana je u tabeli. Pokazatelj da je distribucija pacijenata po broju i procentu tačna nalazi se u pretposlednjoj koloni, u kojoj je prikazan zbir ukupnog broja ispitanih bolesnika. Za svaki PSS ukupan broj bolesnika mora biti 120 (n = 120), što znači da i zbir procentualne zastupljenosti bolesnika sa odgovarajućim kariotipom ili hromozomskim aberacijama i sa određenim stepenom fibroze KS mora biti 100%.

Distrubucija fibroze KS naših pacijenata (n = 120) bila je takva da je najveći broj (n = 55 ili 45,8%) imao stepen fibroze MF-2, slede pacijenti sa stepenom fibroze MF-3 (n = 42 ili 35%), manje je pacijenata bilo sa fibrozom MF-1 (n = 20 ili 16,7%), i kao što smo pomenuli, najmanje je bilo pacijenata sa fibrozom MF-0 (n = 3 ili 2,5%) (Tabela 2).

Statistička analiza korelacije fibroze KS i kariotipa, korišćenjem Lille PSS-a [3] za citogenetiku, nije pokazala značajnu statističku razliku (p = 0,293) (Tabela 2). Ne

patients (32/120 or 26.7%) had an aberrant karyotype (Table 1).

All patients were classified according to the European consensus on BM fibrosis grading [9]. Examples of the morphological characteristics of PMF and BM fibrosis grades are shown in Figure 1. The stratification of patients based on fibrosis grade and cytogenetic data is presented in Table 2. The rows of Table 2 display the PSS classifications and the stratification of chromosomal aberrations into prognostic risk groups of different levels, while the columns present the stratified BM fibrosis grades of patients on a scale from 0 to 3. The distribution of patients with an adequate karyotype (normal or aberrant) or chromosomal aberrations of



Slika 1. MF-3, Gordon Sweet x200, Gusta retikulinska vlakna i snopovi kolagena

Picture 1. MF-3, Gordon Sweet x200, Dense reticulin fibers, and bundles of collagen

Tabela 2. Stratifikacija 120 pacijenata sa primarnom mijelofibrozom prema citogenetskim podacima iz aktuelnih prognostičkih sistema bodovanja i evropskog konsenzusa o stepenovanju fibroze koštane srži

Table 2. Stratification of 120 patients with primary myelofibrosis according to the cytogenetic data from current prognostic scoring systems and the European consensus on grading of bone marrow fibrosis

			Fibroza koštane srži (FKS) (*n* = 136)/ *Bone marrow fibrosis (BMF) (n = 136)* Korelacija kariotipa i FKS (*n* = 120) / *Correlation of karyotype and BMF (n = 120)*					
PSS	**Kariotip / *Karyotype***	**Kategorija rizika / *Risk category***	**Grade 0** (*n* = 3); n (%)	**Grade 1** (*n* = 20); n (%)	**Grade 2** (*n* = 55); n (%)	**Grade 3** (*n* = 42); n (%)	**Ukupno** (*n* = 120) n (%)	***p***
Lille [3]	1. normalan / *normal*	povoljan / *favorable*	0 (0)	17 (19.3)	44 (50.0)	27 (30.7)	88 (73.3)	0.293[a]
	2. aberantan / *aberrant*	nepovoljan / *unfavorable*	3 (9.4)	3 (9.4)	11 (34.4)	15 (46.9)	32 (26.7	
Mayo [16]	1. normalan / *normal*	povoljan / *favorable*	0 (0)	17 (19.3)	44 (50.0)	27 (30.7)	88 (73.3)	0.108[b]
	2. 13q- or 20q-	srednji / *intermediate*	0 (0)	0 (0)	1 (20.0)	4 (80.0)	5 (4.2)	
	3. ostale aberacije / *other aberrations*	nepovoljan / *unfavorable*	3 (9.4)	3 (9.4)	10 (37.0)	11 (40.7)	27 (22.5)	
IPSS [17]	1. +9 or 13q- or 20q-	povoljan / *favorable*	1 (12.5)	0 (0)	2 (25.0)	5 (62.5)	8 (6.7)	0.086[b]
	2. normalan / *normal*	srednji-1 / *intermediate-1*	0 (0)	17 (19.3)	44 (50.0)	27 (30.7)	88 (73.3)	
	3. ostale aberacije / *other aberrations*	srednji-2 / *intermediate-2*	2 (9.5)	3 (14.3)	9 (42.9)	7 (33.3)	21 (17.5)	
	4. +8 ili kompleks kariotip (≥ 3 aberacija) / *+8 or complex karyotype* (≥3 aberrations)	nepovoljan / *unfavorable*	0 (0)	0 (0)	0 (0)	3 (100.0)	3 (2.5)	
DIPSS [18]	1. sole 13q- t/dup(1q) sole 20q- sole +9 Ostale pojedinačne aberacije / *other sole aberrations* dve aberacije isključujući nepovoljne / *two aberrations excluding unfavorable* Normalni kariotip / *normal karyotype*	povoljan / *favorable*	2 (1.9)	19 (17.4)	51 (46.8)	37 (33.9)	109 (90.8)	0.613[a]
	2. kompleksni kariotip / *complex karyotype* (≥ 3 aberrations) sole +8 sole -5/5q- sole -7/7q- i(17q) inv(3) 12p- 11q23 Dve aberacije uključujući bar jednu nepovoljnu / *two aberrations including at least one unfavorable*	nepovoljan / *unfavorable*	1 (9.1)	1 (9.1)	4 (36.4)	5 (45.4)	11 (9.2)	

[a]Mann-Whitney U test [b]Kruskal-Wallis test

postoji korelacija između normalnog ili aberantnog kariotipa sa određenim stepenom fibroze u KS pacijenata. U grupi pacijenata sa normalnim kariotipom (povoljan) najviše je bilo pacijenata sa fibrozom MF-2 (50,0%), zatim MF-3 (30,7%) i MF-1 (19,3%). U grupi pacijenata sa aberantnim kariotipom (nepovoljan) najviše ih je bilo sa stepenom fibroze MF-3 (46,9%), zatim MF-2 (34,4%) i pacijenata sa fibrozom MF-1 i MF-0 (9,4%) (Tabela 2).

Korišćenjem citogenetske stratifikacije Mayo PSS-a [16] za ispitivanje povezanosti kariotipa i stepena fibroze KS nije potvrđena statistički značajna razlika u učestalosti stepena fibroze KS između posmatranih grupa pacijenata sa različitim kariotipom (p = 0,108) (Tabela 2). Najviše pacijenata sa stepenom fibroze MF-2 (50,0%) bilo je kod pacijenata sa normalnim kariotipom (*povoljan*). U grupi pacijenata sa aberacijama 13q- ili 20q- (*srednji*) najviše je bilo pacijenata sa MF-3 (80,0%) i jedan pacijent (20,0%) sa MF-2. Nije bilo pacijenata sa stepenom fibroze KS MF-1 i MF-0.

Kod pacijenata sa drugim hromozomskim aberacijama u kariotipu, najviše je bilo pacijenata sa stepenom fibroze KS MF-3 (40,7%), slede pacijenti sa MF-2 fibrozom (37,0%), pa pacijenti sa stepenom fibroze MF-1 i MF-0 sa istom učestalošću (9,4%).

Ispitivanjem povezanosti fibroze KS i kariotipa korišćenjem IPSS-a [17], takođe je utvrđeno da nema značajne statističke razlike u učestalosti stepena fibroze KS (p = 0,086) (Tabela 2). Ne postoji povezanost između kariotipa i hromozomskih aberacija različitog stepena značaja (povoljan, srednji-1, srednji-2, nepovoljan) sa stepenom fibroze KS. U grupi pacijenata sa povoljnim hromozomskim aberacijama (+9 ili 13q- ili 20q-) najviše je bilo pacijenata sa fibrozom MF-3 (62,5%), zatim pacijenata sa MF-2 (25,0%), jedan pacijent je bio sa MF-0 (12,5%). Nije bilo pacijenata sa fibrozom MF-1. Distribucija stepena fibroze KS kod pacijenata sa normalnim kariotipom (srednji-1) bila je ista kao kod primene Lille i Mayo PSS- a. U grupi pacijenata sa drugim hromozomskim abnormalnostima (srednji-2) najviše je bilo pacijenata sa MF-2 (42,9%), zatim sa fibrozom MF-3 (33,3%) i pacijenti sa fibrozom MF-1 (14,3%). Najmanje je bilo pacijenata sa fibrozom MF-0 (9,5%). U grupi pacijenata sa trizomijom hromozoma 8 i kompleksnim kariotipom (nepovoljan) bili su samo pacijenti sa fibrozom MF-3 (100,0%).

Primenom DIPSS-a [18] takođe nije registrovana statistički značajna povazanost (p = 0,613) između citogenetskih rezultata i dokumentovanog stepena fibroze KS. Prema preporukama DIPSS-a, pacijenti su svrstani u samo dve prognozne kategorije na osnovu nalaza kariotipa. Povoljnu kategoriju čine hromozomske aberacije koje predstavljaju dobar prognozni parametar, kao i u prethodno pomenutim PSS-ima, neke druge abe-

a specific risk level, along with the corresponding BM fibrosis grade, is illustrated in the table.

An indicator of the accuracy of the distribution of patients by number and percentage is provided in the penultimate column, which displays the total number of patients examined. For each PSS, the total number of patients must be 120 (n = 120), meaning that the sum of the percentage representation of patients with a corresponding karyotype or chromosomal aberrations and a specific bone marrow (BM) fibrosis grade must equal 100%.

The distribution of BM fibrosis among our patients (n = 120) revealed that the highest number (n = 55, 45.8%) had fibrosis grade MF-2, followed by patients with fibrosis grade MF-3 (n = 42, 35%). Fewer patients had fibrosis grade MF-1 (n = 20 or 16.7%), and as mentioned, the lowest number had fibrosis grade MF-0 (n = 3 or 2.5%) (Table 2).

Statistical analysis of the correlation between BM fibrosis and karyotype, using the Lille PSS [3] for cytogenetics, did not reveal a statistically significant difference (p = 0.293) (Table 2). There was no correlation between a regular or aberrant karyotype and the degree of bone marrow (BM) fibrosis in patients. Among patients with a normal karyotype (favorable), the majority had fibrosis MF-2 (50.0%), followed by MF-3 (30.7%) and MF-1 (19.3%). Among patients with an aberrant karyotype (unfavorable), the highest proportion had a fibrosis grade of MF-3 (46.9%), followed by MF-2 (34.4%), and those with fibrosis grades of MF-1 and MF-0 (9.4%) (Table 2).

Using the cytogenetic stratification of the Mayo PSS [16] to examine the association between karyotype and BM fibrosis grade, no statistically significant difference was confirmed in the frequency of BM fibrosis grades among the observed patient groups with different karyotypes (p = 0.108) (Table 2). The highest number of patients with fibrosis grade MF-2 (50.0%) was found in those with a normal karyotype (favorable). In the group of patients with 13q- or 20q- aberrations (intermediate), most had MF-3 (80.0%), while one patient (20.0%) had MF-2. There were no patients with BM fibrosis grades MF-1 or MF-0.

Among patients with other chromosomal aberrations in their karyotype, the majority had BM fibrosis grade MF-3 (40.7%), followed by MF-2 (37.0%), while MF-1 and MF-0 were present at the same frequency (9.4%).

Examining the association between BM fibrosis and karyotype using IPSS [17] also showed no significant statistical difference in the frequency of BM fibrosis grades (p = 0.086) (Table 2). There was no correlation between karyotype and chromosomal aberrations of different

racije (t/dup(1q), druge solo i dvostruke povoljne aberacije)), kao i normalan kariotip. Nepovoljnu kategoriju predstavljaju nepovoljne hromozomske aberacije kao i u IPSS-u (+8, kompleksan kariotip), kao i druge (-5/5q-, -7/7q-, i(17q), inv(3), 12p-, 11q23). U grupi pacijenata sa povoljnim kariotipskim karakteristikama najviše ih je bilo sa stepenom fibroze MF-2 (46,8%), zatim slede pacijenti sa MF-3 stepenom fibroze (33.9%), zatim pacijenti sa MF-1(17,4%), a najmanje je bilo pacijenata sa MF-0 stepenom fibroze KS (1,9%). Kod pacijenata sa nepovoljnim kariotipskim izmenama, stepen fibroze KS MF-3 bio je registrovan kod najvećeg broja pacijenata (45,4%), zatim slede pacijenti sa fibrozom gradusa MF-2 (36,4%). Pacijenti sa MF-1 i MF-0 stepenom fibroze bili su zastupljeni sa istom učestalošću (9,1%).

DISKUSIJA

Ukupno preživljavanje u PMF-i zavisi od kliničkih, laboratorijskih, citogenetskih i molekularnih karakteristika bolesti. Na njega utiču starost pacijenta, fizičko stanje i komorbiditeti. U to svrhu su kreirani mnogi PSS-i, koji kombinacijom svih ovih podataka omogućavaju svrstavanje pacijenata sa PMF-om u kategorije sa različitim nivoom rizika. Imperativ stratifikacije pacijenata sa PMF-om na osnovu stepena rizika je odabir što povoljnije, personalizovane terapije, koja će omogućiti pacijentu kvalitetniji život i duže preživljavanje.

U istoriji primene PSS-a u PMF-i, citogenetika je zauzela poziciju nezavisnog parametra u prognozi bolesti. U prvim ispitivanjima preživljavanja pacijenata u odnosu na citogenetiku, procenjeno je da je normalan kariotip povoljan, a aberantan kariotip nepovoljan prognozni parametar [3]. Kasnije je ustanovljeno da su neke hromozomske aberacije (+9, 13q-, 20q-) bile povezane sa dužim preživljavanjem u odnosu na normalan kariotip. Ove aberacije definisane su kao dobar prognozni parametar [16]. Neki drugi kariotipski rearanžmani (+8 ili kompleksani kariotip, -5/5q-, -7/7q-, 11q23, i(17q), inv(3), 12p-) dovođeni su u vezu sa kratkim preživljavanjem pacijenata, i po drugim PSS-a definisani su kao loš prognozni parametar [17,18]. Sve u svemu, citogenetska analiza i kariotip imaju značajno mesto u dijagnostici PMF-a, ali još značajnije mesto u prognozi PMF-a.

Suprotna je situacija sa stepenom fibroze KS kod PMF-a i uopšte kod MPN-i. Fibroza KS značajno različitog stepena može biti povezana sa tri glavna entiteta MPN-i (policitemijom verom – PV, esencijalnom trombocitemijom – ET i primarnom mijelofibrozom – PMF), bilo pri prezentaciji, kao kod PMF-e, ili nakon progresije bolesti PV-e i ET-e. Ažurirana klasifikacija SZO iz 2008. usvojila je Evropski konsenzus o ocenjivanju fibroze KS [9], četvorostepeni sistem sa ocenama 0–3 [7]. Razvoj

prognostic significance (favorable, intermediate-1, intermediate-2, unfavorable) with BM fibrosis grade.

Among patients with favorable chromosomal aberrations (+9 or 13q- or 20q-), the majority had a fibrosis grade of MF-3 (62.5%), followed by MF-2 (25.0%), while one patient had a grade of MF-0 (12.5%). There were no patients with MF-1 fibrosis. The distribution of BM fibrosis grades in patients with a normal karyotype (intermediate-1) was the same as in the Lille and Mayo PSS classifications.

In the group of patients with other chromosomal abnormalities (intermediate-2), most had MF-2 (42.9%), followed by MF-3 (33.3%), while MF-1 fibrosis was present in 14.3% of cases. The lowest number of patients had MF-0 fibrosis (9.5%). In the group of patients with on chromosome 8 and a complex karyotype (unfavorable), all patients had MF-3 fibrosis (100.0%).

The application of DIPSS [18] also did not reveal a statistically significant association ($p = 0.613$) between cytogenetic results and the documented degree of bone marrow fibrosis (KS). According to DIPSS recommendations, patients were classified into only two prognostic categories based on karyotype findings. The favorable category includes chromosomal aberrations that are considered suitable prognostic parameters, as in the previously mentioned PSSs, along with some other aberrations (t/dup(1q), other solo and double favorable aberrations), as well as a normal karyotype. The unfavorable category comprises poor prognostic chromosomal aberrations, such as those found in IPSS (+8, complex karyotype), along with others, including (-5/5q-, -7/7q-, i(17q), inv(3), 12p-, and 11q23).

Among patients with favorable karyotypic characteristics, the majority had fibrosis grade MF-2 (46.8%), followed by those with fibrosis grade MF-3 (33.9%), then those with MF-1 (17.4%), while the smallest number of patients had MF-0 fibrosis (1.9%). Among patients with unfavorable karyotypic alterations, fibrosis grade MF-3 was the most common (45.4%), followed by patients with MF-2 fibrosis (36.4%). Patients with MF-1 and MF-0 fibrosis grades were represented with the same frequency (9.1%).

DISCUSSION

Overall survival in PMF depends on the disease's clinical, laboratory, cytogenetic, and molecular characteristics. It is influenced by patient age, physical condition, and comorbidities. To address this, numerous PSSs have been developed, integrating these factors to categorize PMF patients into different risk levels. The imperative of risk stratification in PMF is to select the most favorable, personalized therapy that ensures a better quality of life and prolonged survival.

fibroze KS predstavlja postepenu evoluciju odsutne ili minimalne retikulinske fibroze, do izražene retikulinske i/ili kolagenske fibroze često povezane sa osteosklerozom, a precizno određivanje stepena fibroze je od suštinskog značaja, kako za početnu klasifikaciju MPN-i, tako i za identifikaciju progresije bolesti [19]. Iako je fibroza KS glavni dijagnostički kriterijum za PMF, stepen fibroze nije uključen u konvencionalne PSS-e. Nedavno je naglašeno da je tačna procena stepena fibroze KS ključna tačka za predviđanje prognoze PMF-e [8].

Analizom ukupnog preživljavanja svih 136 pacijenata sa PMF-om, u zavisnosti od stepena fibroze KS, dobili smo rezultat da ne postoji (p = 0,084) statistička značajnost u preživljavanju. U tom slučaju možemo doneti kontraverzni zaključak da stepen fibroze nije značajan činilac za preživljavanje kod pacijenata sa PMF-om, iako je jedan od osnovnih dijagnostičkih kriterijuma. Međutim, kada smo analizu ponovili bez 3 pacijenta sa stepenom fibroze MF-0, dobijena je statistička značajnost u preživljavanju (p = 0,043). Isključivanjem samo tri pacijenta sa stepenom fibroze MF-0 iz grupe od 136 pacijenata sa PMF-om, potvrđeno je da fibroza ipak utiče na ukupno preživljavanje kod PMF-e. To takođe sugeriše da bi trebalo dobro razmotriti sve dijagnostičke parametre pre donošenja definitivne dijagnoze da se radi o bolesti PMF-i, ili o eventualnoj transformaciji primarne bolesti PV-e i ET-e u sekundarnu bolest mijelofibrozu.

Većina naših pacijenata imala je fibrozu KS stepena MF-2 i MF-3 (46% i 35%). Skoro trećina pacijenata je imala MF-3, što je veoma interesantno s obzirom na činjenicu da se naša retrospektivna studija odnosi na podatke pacijenta prikupljenim tokom primarnih specijalističkih pregleda i prilikom postavljanja inicijalne dijagnoze.

Prema nekim literaturnim podacima [20] u pre fibrotičnoj fazi PMF-e (stepen fibroze MF-0), aberantan kariotip zastupljen je kod 18% pacijenata, a nepovoljno aberantan kariotip (≥ 3 aberacije, +8, -5/5q-, -7/7q-, i(17q), inv(3), 12p-, 11q23, kompleksan kariotip) kod 4–8% slučajeva. Iz tih podataka možemo zaključiti da niži stepen fibroze KS (MF-0 i MF-1) korelira sa povoljnim hromozomskim aberacijama i normalnim kariotipom i obrnuto, viši stepeni fibroze (MF-2 i MF-3) KS je u korelaciji sa nepovoljnom citogenetikom. Sličnu analizu smo uradili u našoj grupi bolesnika. Koristili smo jednostavne kategorizacije kariotipa (normalan, aberantan), po preporukama Lille PSS-a [3]. Primenili smo i naprednije kategorizacije kariotipa koje sadrže određene hromozomske aberacije (+9, 13q-,20q-, +8, kompleksan kariotip) sa različitim stepenom nepovoljnosti, kao što su Mayo PSS-em [16] i IPSS-em [17]. Takođe, primenjen je DIPSS-em [18] koji predlaže samo

In the history of PSS application in PMF, cytogenetics has been recognized as an independent prognostic parameter. Early survival studies based on cytogenetics indicated that a normal karyotype was a favorable prognostic factor, while an aberrant karyotype was considered unfavorable [3]. Later, it was found that certain chromosomal aberrations (+9, 13q-, 20q-) were associated with more prolonged survival compared to a normal karyotype. These aberrations were defined as good prognostic markers [16]. Conversely, other karyotypic rearrangements (+8 or complex karyotype, -5/5q-, -7/7q-, 11q23, i(17q), inv(3), 12p-) were linked to shorter survival and were classified as poor prognostic markers in other PSSs [17,18]. Overall, cytogenetic analysis and karyotyping play a significant role in the diagnosis of PMF but an even greater role in its prognosis.

The situation is different when it comes to BM fibrosis in PMF and MPN in general. BM fibrosis of varying degrees can be associated with the three main MPN entities-polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF)-either at presentation, as in PMF, or following disease progression in PV and ET. The updated 2008 WHO classification adopted the European consensus on BM fibrosis grading [9], which introduced a four-tiered system ranging from grade 0 to 3 [7]. The progression of BM fibrosis follows a gradual evolution from absent or minimal reticulin fibrosis to extensive reticulin and/or collagen fibrosis, often accompanied by osteosclerosis. Precise assessment of fibrosis grade is crucial for the initial classification of MPNs as well as for identifying disease progression [19]. Although BM fibrosis is a key diagnostic criterion for PMF, its grading has not been incorporated into conventional PSSs. Recently, it has been emphasized that an accurate assessment of BM fibrosis grade is a critical factor in predicting PMF prognosis [8].

By analyzing the overall survival of all 136 PMF patients based on the degree of BM fibrosis, we found no statistically significant difference in survival (p = 0.084). This could lead to the controversial conclusion that the degree of fibrosis is not an important factor in survival for PMF patients, despite being one of the key diagnostic criteria. However, when we repeated the analysis excluding the three patients with MF-0 fibrosis, statistical significance in survival emerged (p = 0.043). By removing only three MF-0 patients from the cohort of 136, it was confirmed that fibrosis indeed influences overall survival in PMF. This also suggests that all diagnostic parameters should be carefully considered before definitively diagnosing PMF or determining whether the disease represents a secondary transformation of PV or ET into myelofibrosis.

dve kategorije kariotipa sa definisanim povoljnim i nepovoljnim hromozomskim aberacijama. Ni u jednom od primenjenih PSS-a nije registrovana statistička značajnost u korelaciji kariotipa i stepena fibroze KS (Lille $p = 0,293$; Mayo $p = 0,108$; IPSS $p = 0,086$; DIPSS $p = 0,613$) (Tabela 2).

Naš osnovni cilj u radu je bio da testiramo povezanost, odnosno korelaciju citogenetskih rezultata i patohistoloških rezultata pacijenata sa dijagnozom PMF-e, sa pretpostavkom da postoji povezanost ova dva dijagnostička, prognostička parametra. Činjenica je da smo koristili PSS-e po kojima su normalan ili aberantan kariotip, odnosno hromozomske aberacije stratifikovane u prognozne kategorije na osnovu njihovag uticaja na ukupno preživljavanje pacijenata sa PMF-om. S druge strane, fibroza KS je nepovoljan dijagnostički nalaz za pacijente sa PMF-om i što je gradus fibroze veći, bolest je u višem stadijumu. Na osnovu ovih činjenica očekivali smo da će postojati pozitivna povezanost ili direktna korelacija citogenetskih i patohistoloških nalaza, odnosno da će aberantan kariotip ili „nepovoljne" hromozomske aberacije biti češće registrovane kod pacijenata sa stepenom fibroze MF-2 i MF-3, što bi se verifikovalo rezultatima statističkih analiza. Međutim, naši rezulati su suprotni, nije dokazana direkta korelacija prognozno „nepovoljnih" hromozomskih aberacija sa višim stepenima fibroze KS (MF-2 i MF-3), niti korelacija normalnog kariotipa i „povoljnih" hromozomskih aberacija sa stepenom fibroze KS MF-0 i MF-1u PMF-i.

ZAKLJUČAK

Koliko nam je poznato, naša studija je prvi pokušaj ispitivanja korelacije citogenetike i patohistologije, ova dva važna dijagnostička i prognostička parametra za PMF-u. Studija obuhvata 136 pacijenata sa PMF-om, što je relativno veliki broj pacijenata na dijagnozi za jedan kliničko, dijagnostički centar na Zapadnom Balkanu. Kod 120 pacijenata testirana je korelacija citogenetskih rezultata i patohistoloških podataka o stepenu fibroze KS, primenom korisnih PSS-a. Iako smo očekivali pozitivnu korelaciju između kariotipa (normalan, aberantan) i hromozomskih aberacija različitog stepena prognoze (povoljan, nepovoljan) sa stepenom fibroze KS po preporukama Evropskog konsenzusa, dobili smo suprotan rezultat. Ne postoji korelacija tipa kariotipa, odnosno hromozomskih aberacija različitog stepena prognoze sa stepenom fibroze KS kod bolesnika sa PMF-om. Ipak, naša je namera da se nastavi sa ispitivanjem korelacije stepena fibroze KS sa citogenetikom kao i "driver" mutacijama u PMF-u, kroz neku buduću, prospektivnu studiju.

The majority of our patients had BM fibrosis grades MF-2 and MF-3 (46% and 35%). Nearly one-third had MF-3, which is particularly interesting given that our retrospective study is based on patient data collected during initial specialist examinations and at the time of primary diagnosis.

According to some literature data [20], in the profibrotic phase of PMF (MF-0 fibrosis), an aberrant karyotype is present in 18% of patients, while an unfavorable aberrant karyotype (≥ 3 aberrations, +8, -5/5q-, -7/7q-, i(17q), inv(3), 12p-, 11q23, complex karyotype) is found in 4–8% of cases. Based on these data, we can conclude that lower degrees of BM fibrosis (MF-0 and MF-1) correlate with favorable chromosomal aberrations and a normal karyotype, whereas higher fibrosis grades (MF-2 and MF-3) are associated with unfavorable cytogenetics.

We conducted a similar analysis in our patient group. We employed simple karyotype categorizations (normal and aberrant) based on the Lille PSS recommendations [3]. Additionally, we applied advanced karyotype categorizations that included specific chromosomal aberrations (+9, 13q-, 20q-, +8, complex karyotype) with different levels of unfavorable prognosis, as classified by Mayo PSS [16] and IPSS [17]. The DIPSS model [18], which proposes only two karyotype categories (favorable and unfavorable chromosomal aberrations), was also applied. In none of these PSS models did we find a statistically significant correlation between karyotype and BM fibrosis grade (Lille $p = 0.293$; Mayo $p = 0.108$; IPSS $p = 0.086$; DIPSS $p = 0.613$) (Table 2).

Our primary aim was to test the association between cytogenetic and histopathological findings in PMF patients, assuming that a correlation exists between these two diagnostic and prognostic parameters. The fact is that we used PSS models in which normal or aberrant karyotypes and chromosomal aberrations were stratified into prognostic categories based on their impact on overall survival in PMF patients. On the other hand, BM fibrosis is considered an unfavorable diagnostic finding for PMF patients, with higher fibrosis grades indicating a more advanced disease stage. Based on these facts, we expected a positive association or direct correlation between cytogenetic and histopathological findings-that is, that aberrant karyotypes or "unfavorable" chromosomal aberrations would be more frequently observed in patients with MF-2 and MF-3 fibrosis, as confirmed by statistical analysis. However, our results were contrary to this expectation, as we found no direct correlation between prognostically "unfavorable" chromosomal aberrations and higher degrees of BM fibrosis (MF-2 and MF-3) or

SKRAĆENICE

PMF – primarna mijelofibroza
MPN – mijeloproliferativna neoplazma
KS – kosna srž
PSS – prognostički skoring sistem
SZO – svetska zdravstvena organizacija
PV – policitemija vera
ET – esencijalna trombocitemija

Dodatak: Ovaj rad predstavlja prikaz nekih od rezultata naučno istraživačkog ispitivanja dr sc. Vesne Đorđević radi izrade doktorske teze pod nazivom „Tip i učestalost hromozomskih aberacija i značaj molekularnog markera *JAK2*V617F u primarnoj mijelofibrozi".

Sukob interesa: Nije prijavljen.

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between a normal karyotype and "favorable" chromosomal aberrations with lower fibrosis grades (MF-0 and MF-1) in PMF.

CONCLUSION

To the best of our knowledge, our study is the first attempt to investigate the correlation between cytogenetics and histopathology-two crucial diagnostic and prognostic parameters for PMF. The study includes 136 PMF patients, which represents a relatively large number of cases at the time of diagnosis for a single clinical and diagnostic center in the Western Balkans. In 120 patients, we analyzed the correlation between cytogenetic findings and histopathological data on BM fibrosis grade using relevant PSS models.

Although we expected a positive correlation between karyotype (normal, aberrant) and chromosomal aberrations of different prognostic significance (favorable, unfavorable) with BM fibrosis grade, following the recommendations of the European consensus, our results showed the opposite. There was no correlation between karyotype type or chromosomal aberrations of different prognostic significance and BM fibrosis grade in patients with PMF.

However, we intend to continue investigating the correlation between BM fibrosis grade, cytogenetics, and "driver" mutations in PMF through a future prospective study.

ABBREVIATIONS AND ACRONYMS

PMF – primary myelofibrosis
MPN – myeloproliferative neoplasm
BM – bone marrow
PSS – prognostic Scoring System
WHO – world health organization
PV – polycythemia vera
ET – essential thrombocythemia

Appendix: This paper presents some of the results from the scientific research study conducted by Dr. Vesna Đorđević as part of her doctoral thesis entitled "Type and Frequency of Chromosomal Aberrations and the Significance of the JAK2V617F Molecular Marker in Primary Myelofibrosis."

Conflict of interest: None declared

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