Hematological Diseases and Therapies

Which Factor Is Responsible for The Prognosis in Myelodysplastic Syndrome Patients with an Isolated Del(5q) or A Cytogenetic Abnormality in Addition to Del(5q): Blast Counts or Cytogenetics?

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Abstract

Background/Aim: In MDS patients carrying a del(5q) mutation, number of additional chromosomal abnormalities is of prognostic value. In this study, we aimed to determine the clinical and laboratory characteristics, OS, and prognostic factors with an effect on OS in MDS patients who have isolated del(5q) or additional chromosomal abnormalities along with del(5q).

Materials and Methods: We have included 34 patients diagnosed according to the WHO 2008 diagnostic criteria during the time period of 2008-2017 who have either del(5q) or one or more other cytogenetic abnormalities in addition to del(5q). Any patients who are positive for del(5q) but not diagnosed with MDS were not included to the study.

Results: Bone marrow (BM) blast ratio was <5% in 14 (41.2%) patients, whereas BM blast ratio of 20 patients (58.8%) was ≥5%. 5q deletion was in isolated form in 16 (47.1%) patients, whereas additional cytogenetic abnormality was detected in 18 (52.9%) patients. Among the accompanying abnormalities, del(7q) was the most common. In 7 (20.5%) patients, MDS progressed into AML. According to MDS IPSS risk scoring, patients were classified as follows: 9 in low-risk group (26.4%), 4 in intermediate-1 risk group (11.7%), 9 in intermediate-2 risk group (26.4%), 12 in high-risk group (%35.2). Based on the results of multivariate analysis, in MDS patients with 5q deletion, presence of additional cytogenetic abnormality, a BM blast ratio of ≥5%, IPSS risk score, presence of cytopenia and MDS subtype as per WHO definition were identified as the prognostic factors with a negative effect on OS (p=0.02, p=0.007, p=0.001, p=0.007, and p=0.003, respectively).

Conclusion: In del(5q) positive MDS patients, most important prognostic factors affecting OS are: BM blast ratio of ≥5%, presence of cytogenetic abnormality in addition to del(5q), a high IPSS risk score, WHO MDS sub-type presenting with a gradually increasing blast number, and number of cytopenia.

Introduction

Myelodysplastic Syndrome (MDS) is a heterogeneous clonal stem cell disorder characterized with insufficient hematopoiesis leading to cytopenia, and is associated with a variable Overall Survival (OS) and a relatively high risk of progression into Acute Myeloid Leukemia (AML) [1,2]. In approximately 50% of MDS patients, abnormal karyotype could be detected at the time of di-

agnosis [3,4]. Most frequent karyotype abnormality in MDS patients is the interstitial deletion at the long arm of chromosome 5 (del[5q]) which is detected in about 10-15% of the cases [5]. The diagnosis of traditional '5q- syndrome' as per WHO diagnostic criteria requires a blast percentage of <5% in bone marrow and <1% in peripheral blood, absence of Auer rods, and presence of an isolated del(5q) [6]. The current treatment for patients with 5q syndrome is lenalidomide and is an effective treatment [7]. Patients with 5q- syndrome are of advanced age with a female/male ratio of 7/3 [8]. At the time of diagnosis, patients usually have macrocytic anemia, normal to elevated platelet count, hypolobulated megakaryocytes in Bone Marrow (BM), and a BM blast ratio of <5% [6,9]. Treatment of MDS patients with 5q deletions also includes supportive treatments such as transfusion and iron chelation. In addition, alternative options, depending on the risk group, include EPO, G-CSF, thalidomide, lenalidomide ATRA, low-dose cytarabine, 5-azacitidine, decitabine, and allogeneic stem cell transplantation [10]. MDS patients with isolated del(5q) and BM blast ratio of <5% are predisposed to a longer lifespan than those bearing other chromosome abnormalities along with 5q del and a BM blast ratio of ≥5% [10,11]. Additional chromosomal abnormalities or increased number of blasts for 5q deletion also pose a risk of higher AML transformation [12,13]. In this study, we aimed to determine the clinical and laboratory characteristics, OS, and prognostic factors with an effect on OS in MDS patients who have isolated del(5q) or additional chromosomal abnormalities along with del(5q).

Materials and Methods

Patients and Diagnostic Criteria

We have included 34 patients diagnosed according to the WHO 2008 diagnostic criteria and treated in Eskisehir Osmangazi University, Medical Faculty, Department of Internal Medicine, Division of Hematology during the time period of 2008-2017 who have either del(5q) or one or more other cytogenetic abnormalities in addition to del(5q). Any patients who are positive for del(5q) but not diagnosed with MDS were not included to the study. Approval of local ethic committee was obtained for the study.

Cytogenetic Studies: Conventional Cytogenetic Analysis and Fluorescence in Situ Hybridization Analysis

Sample Preparation

Bone marrow samples from newly diagnosed MDS patients by the Hematology Department were referred to the Cancer Cytogenetics Section of the Medical Genetics Department. The bone marrow samples were cultured for 24-48 hours in RPMI-1640 medium with and without mitogen stimulation and with 10µg/ml colcemid concentration. Then chromosomal slides were prepared according to standard procedures (0.075 M KCl treatment and fixation with Carnoy's fixative).

Slide Pretreatment and Denaturation

Slides were prepared by dropping cell suspensions onto frozen microscopic slides and then left to dry overnight at room temperature before use. Slides were dehydrated by the treatment of 100 %-70 %-50 % -30 % ethanol series and 0.1xSSC (Standard Saline Citrate) solution. Then the slides were treated with 2xSSC solution at 70°C and denaturated in 0.07 M NaOH solution. The slides were transferred into cold 1xSSC and then into 2xSSC solutions for 1 min. each. Then the slides were transferred into ethanol series and air-dried before hybridization.

FISH Analysis

In the MDS FISH analysis, probes for the locus specific LSI EGR1(5q31)/D5S23 DC (locus specific D7S486 (7q31)/CEP7 DC, CEP8, locus specific 11q23 (LSI MLL), 17p13.1 (LSI TP53) and LSI D20S108(20q12) (Vysis, Downers Grave, IL, USA) were used. FISH was performed according to manufacturer's specifications. Probes were denatured at 73°C ±1°C for 5 minutes and then they were applied immediately to the previously determined regions of the slides. Following overnight hybridization at 37°C, post hybridization washes were performed and air-dried in darkness. The slides were counterstained by using DAPI (4'-6'-diamidine-2-phenylindole) and were stored at -20°C in the dark.

Microscopy

Slides were analyzed with Olympus BX61 fluorescence microscope and images captured with a CCD camera using image analysis system (Applied Imaging). At least 200 nuclei using areas of the slides on which the cells were spread were analyzed for each probe. The cut-off points for positive values was determined for each probe from five bone-marrow samples collected from individuals with iron deficiency anemia. The cut-off values for each probe are as following: EGR1(5q31)/D5S23 deletion %5, D7S486 (7q31)/CEP7 deletion %5, centromere 8; 3%, 11q23 (LSI MLL) deletion 7.5 %, 17p13.1 (LSI TP53) deletion 9%, D20S108(20q12) deletion 8%.

Prognostic Factors

Patient characteristics were evaluated in order to identify any possible prognostic factors and demographic features with an effect on OS and progression into AML. For the patients following were assessed: age, gender, hematological parameters (ie. hemoglobin level, Absolute Neutrophil Count (ANC), Absolute Lymphocyte Count (ALC), Absolute Monocyte Count (AMC), platelet count, Mean Corpuscular Volume (MCV), erythrocyte sedimentation rate (ESR), serum ferritin level, serum albumin level, Lactate Dehydrogenase Level (LDH)), MDS classification of patients according to WHO 2008 classification, number of cytopenia(s), percentage of BM blasts, overall survival as of diagnosis, mortality rate, reasons of mortality, transformation from MDS into AML and duration of the transformation, risk classification of patients according

to IPSS (low, intermediate-1, intermediate-2, and high), treatments received, and post-treatment transfusion independence.

Statistical Analysis

Continuous data was presented as Mean ± Standard Deviation. Categorical data was presented in percentage (%). Shapiro Wilk's test was used to figure out whether the data is normally distributed. In order to compare the groups incompliant with normal distribution, Mann-Whitney U test and Kruskal-Wallis H test were used in cases with two groups and in cases with three or more groups, respectively. Pearson's Exact Chi Square analysis was adopted to analyze the generated cross tables. Analysis of independent markers affecting on the survival time was carried out by survival analysis (Kaplan Meier). Analyses were carried out with IBM SPSS Statistics 21.0 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.) program. A p-value of < 0.05 was considered statistically significant.

Results

Characteristic of the Patients

At the time of diagnosis patients were 63.3. ± 14.3 (30-85) years old, on average, and out of 34 patients, 20 (58.8%) were female and 14 (41.2%) were male. F/M ratio was 1.4. Among the patients who are positive for 5q deletion, 13 (38.2%) had 'isolated 5q syndrome', 4 (11.8%) had 'MDS-RAEB Type-1', 16 (47.1%) had 'MDS-RAEB Type-2', and 1 (2.9%) had 'MDS-unclassified'. Bone marrow (BM) blast ratio was <5% in 14 (41.2%) patients, whereas BM blast ratio of 20 patients (58.8%) was ≥5%. 5q deletion was in isolated form in 16 (47.1%) patients, whereas additional cytogenetic abnormality was detected in 18 (52.9%) patients. Among the

accompanying abnormalities, del(7q) was the most common. In 7 (20.6%) patients MDS progressed into AML. According to MDS IPSS risk scoring, patients were classified as follows: 9 in low-risk group (26.4%), 4 in intermediate-1 risk group (11.7%), 9 in intermediate-2 risk group (26.4%), 12 in high-risk group (%35.2). In addition to MDS, 23 (67.6%) patients were inflicted by a comorbid disease. Due to the positive history of malignancies, 4 (11.7%) patients had received treatment with cytotoxic agents. Out of these malignancies, 2 (50%) were endometrial carcinoma and the other 2 (50%) were breast carcinoma. Allogeneic stem cell transplantation was performed on 4 (11.7%) patients, BM blast ratio of those patients was ≥5%. Transfusion independence was achieved in 11 (33.3%) out of 33 (97%) patients in need of transfusion. Macrocytosis was detected in 6 (17.6%) of patients. Demographic, clinical and laboratory differences among the patients once the patients were divided into 3 groups based on whether they have isolated del(5q) or del(5q) +1 cytogenetic abnormality or ≥ +2 cytogenetic abnormalities are given in (Tables 1 and 2), any differences identified when patient are divided into 2 based on whether their BM blast count is <5% or ≥5% are shown in (Table 3). Kaplan-Meier curve and Overall survival time plots of patients in (Figure 1) depicts 3 different cytogenetic categories (isolated 5q deletion, 5q deletion+1 and 5q deletion +≥2 cytogenetic abnormalities); BM blast ratio of <5% and ≥5%, and BM blast ratio of <5%, 5-10%, and ≥10%. Kaplan-Meier curves in order to evaluate the effect of cytogenetics and BM blast number jointly on OS time is given in (Figure 2), with patient characteristics given in (Table 4). Other than the transfusion, patients had received the following treatments: 6 (17.6%) patients EPO, 2 (0.05%) patient's methylprednisolone, 4 (11.7%) patients danazol, 2 (0.05%) patients G-CSF, 12 (35.2%) patients lenalidomide, 1 (0.03%) patient thalidomide, 14 (41.2%) patients 5-azacytidine, and 4 (11.7%) patients decitabine.

	Isolated del(5q) group n=16	del(5q) and 1 additional abnormality group n=12	del (5q) and ≥2 additional abnormalities group n=6	p
Age (median,Q1-Q3)/years	68,5 (57,5-75)	62,5 (44-68)	69 (46-80,5)	p>0.05
Gender (Male/Female) (n)	Mar-13	06-Jun	05-Jan	**p=0,016**
AML transformation (n,%)	1 (%6,3)	6 (%50)	0	**p=0.012**
Overall survival time (mean±SD) /months	66,59±11,3	24,35±7,69	6,66±2,53	**p=0.011 (1 vs 3 p=0.012)**
Mortality (n, %)	9 (%56)	10 (%83,3)	5 (%83,3)	p>0.05
Hb (median, Q1-Q3)/g/dl	9 (7,12-10,3)	8 (7,3-9,9)	10 (8,4-10,9)	p>0.05
WBC (median, Q1-Q3)/mm3	3500 (2155-5770)	2730 (2300-4025)	1550 (722-4350)	p>0.05

ANC (median, Q1-Q3)/mm3	1730 (1025-3657)	1100 (685-2250)	950 (52,5-2050)	p>0.05
AMC (median, Q1-Q3)/mm3	250 (162,5-437,5)	300 (60-550)	110 (0-425)	p>0.05
ALC (median, Q1-Q3)/mm3	1100 (755-1675)	1200 (500-1625)	800 (167,5-1750)	p>0.05
Platelet (median, Q1-Q3)/mm3	223000 (78500-363500)	49000 (36000-64500)	25000 (10500-49750)	**p=0.001 (1 vs 2 p=0.038 and 1 vs 3 p=0.002)**
MCV (median, Q1-Q3)/fL	96,4 (94,5-109,2)	87,5 (85,4-98,7)	90,8 (86,9-97,6)	**p=0.025 (1 vs 2 p=0.033)**
ESR (median, Q1-Q3) mm/h	52 (19-95)	84 (54,7-106,2)	83 (39,5-99,5)	p>0.05
Serum ferritin (median, Q1-Q3)/ ng/ml	658,7 (118,7-1262)	983,7 (545,2-1145)	2334 (170,3-3574)	p>0.05
LDH (median, Q1-Q3)/ IU/L	341 (307-586)	396 (204-455)	320 (214-576)	p>0.05
Albumin (median, Q1-Q3)/ mg/dl	4,25 (4,12-4,67)	4,1 (3,5-4,8)	3,2 (2,87-3,85)	**p=0.011 (1 vs 3 p=0.008)**
BM blast count <%5 (n,%)	11 (%68,8)	2 (%16,7)	1 (%16,7)	**p=0.008**
BM blast count ≥%5 (n,%)	5 (%31,2)	10 (%83,3)	5 (%83,3)	**p=0.008**
BM blast count % (median, Q1-Q3)	3 (5-12,5)	11,5 (7,25-16,5)	12 (4,5-15,5)	p>0.05
Transfusion independence (n,%)	7 (%53,8)	3 (%27,3)	1 (%16,7)	p>0.05

AML: Acute Myeloid Leukemia; Anc: Absolute Neutrophil Count; Amc: Absolute Monocyte Count; Alc: Absolute Lymphocyte Count; Bm: Bone Marrow; Esr : Erythrocyte Sedimentation Rate; Hb: Haemoglobin; Ldh: Lactate Dehydrogenase; Mcv: Mean Corpuscular Volume; Sd: Standard Deviation; Wbc: White Blood Cell Count.

Table 1: Demographic, clinical and laboratory characteristics of MDS patients with isolated del(5q) or del(5q) plus additional cytogenetic abnormality/abnormalities.

	Isolated del(5q) group	del(5q) and 1 additional abnormality group	del (5q) and ≥2 additional abnormalities group	p
	n(%)	**n(%)**	**n(%)**	
Age < 60 years ≥ 60 years	4 (%25) 12 (%75)	6 (%50) 6 (%50)	2 (%33,3) 4 (%66,7)	p>0.05
Hemoglobin < 10 g/dl ≥ 10 g/dl	11 (%68,8) 5 (%31,3)	9 (%75) 3 (%25)	3 (%50) 3 (%50)	p>0.05
Absolute neutrophil count < 1500/mm3 ≥ 1500/mm3	6 (%50) 6 (%50)	6 (%66,7) 3 (%33,3)	4 (%66,7) 2 (%33,3)	p>0.05
Platelet count < 100000/mm3 ≥ 100000/ mm3	3 (%23,1) 10 (%76,9)	10 (%83,3) 2 (%16,7)	6 (%100) 0	**P<0.0001**

Mean corpuscular volume <100 fL≥100 fL	8 (%66,7) 4 (%33,3)	7 (%77,8) 2 (%22,2)	6 (%100) 0	p>0.05
Bone marrow blast count <%5% 5-10%11-20	11 (%68,8) 1 (%6,3) 4 (%25)	2 (%16,7) 3 (%25) 7 (%58,3)	1 (%16,7) 1 (%16,7) 4 (%66,7)	**P=0.045**
Cytopenia None One Two Three	0 9 (%56,3) 2 (%12,5) 5 (%31,3)	0 1 (%8,3) 4 (%33,3) 7 (%58,3)	0 0 1 (%16,7) 5 (%83,3)	**p=0.015**
IPSS risk group Low Intermediate-1 Intermediate-2 High	9 (%56,3) 3 (%18,8) 3 (%18,8) 1 (%6,3)	0 1 (%8,3) 4 (%33,3) 7 (%58,3)	**0** **0** 2 (%33,3) 4 (%66,7)	**p=0.002**
WHO subtype 5q syndrome MDS-RAEB Type 1 MDS-RAEB Type 2 MDS-U	11 (%68,8) 1 (%6,3) 4 (%25) 0	1 (%8,3) 3 (%25) 7 (%58,3) 1 (%8,3)	1 (%16,7) 0 5 (%83,3) 0	**p=0,007**

Ipss: International Prognostic Scoring System; Mds: Myelodysplastic Syndrome; Mds-U: Mds Unclassifiable; Raeb: Refractory Anemia with Excess of Blasts; Who: World Health Organization.

Table 2: Laboratory characteristics, MDS risk groups and WHO-classified sub-types of MDS patients with isolated del(5q) or del(5q) plus additional cytogenetic abnormality/abnormalities.

	BM blast count <%5 n=14	BM blast count ≥%5 n=20	p
Age (median, Q1-Q3)/ years	69 (58-76)	66 (47,7-72,7)	p>0.05
Gender (Male/Female)	4/10	10/10	p>0.05
AML transformation (n, %)	1 (%7)	6 (%30)	**p=0.004**
IPSS risk group (n, %) Low Intermediate-1 Intermediate-2 High	9 (%64,3) 3 (%21,4) 2 (%14,3) 0	0 1 (%5) 7 (%35) 12 (%60)	**p<0.0001**
OS time (median, Q1-Q3)/ months	52,5 (16,5-85,2)	9,5 (3,25-18,7)	**p=0.002**
Mortality (n,%)	8 (%57)	16 (%80)	**p=0.004**
Hb (median, Q1-Q3) gr/dl	9 (6,8-10,4)	8,9 (7,6-10,1)	p>0.05
WBC (median, Q1-Q3) /mm3	4700 (1800-5900)	2630 (1650-3550)	p>0.05

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ANC (median, Q1-Q3) /mm3	2900 (1100-3700)	1100 (677,5-1840)	**p=0.008**
AMC (median, Q1-Q3) /mm3	200 (200-300)	155 (20-625)	p>0.05
ALC (median, Q1-Q3) /mm3	1100 (740-1700)	1100 (450-1550)	p>0.05
Platelet (median, Q1-Q3) /mm3	223000 (51000-356000)	50000 (23250-95000)	**p=0.016**
MCV (median, Q1-Q3) /fL	95,1 (89,3-110,4)	92,3 (87,1-97,1)	p>0.05
ESR (median, Q1-Q3)/ mm/h	46,5 (18,5-80,7)	84 (52-104)	p>0.05
Serum ferritin (median, Q1-Q3)/ ng/ml	658 (118,7-1262)	1034 (542-2077)	p>0.05
LDH (median, Q1-Q3) IU/L	312,5 (233,7-508,7)	349,5 (208,5-475,2)	p>0.05
Albumin (median, Q1-Q3)/ mg/dl	4,3 (4,2-4,8)	4,0 (3,7-4,4)	p>0.05
ALC: Absolute Lymphocyte Count; AML: Acute Myeloid Leukemia; ANC: Absolute Neutrophil Count; AMC: Absolute Monocyte Count; BM: Bone Marrow; ESR: Erythrocyte Sedimentation Rate; Hb: Hemoglobin; LDH: Lactate Dehyrdogenase; MCV: Mean Corpuscular Volume; OS: Overall Survival; WBC: White Blood Cell Count.			

Table 3: Demographic, clinical and laboratory characteristics of MDS patients with BM blast count of <5% or ≥5%.

Categories	HR (%95 CI)	p-value
Karyotype	2.736 (1.172-6.387)	0.02
Bone marrow blast count	3.62 (1.416-9.267)	0.007
IPSS risk group	9.557 (2.441-37.423)	0.001
Cytopenia	4.158 (1.483-11.664)	0.007
WHO MDS subtype	8.684 (2.126-35.472)	0.003
IPSS: International Prognostic Scoring SystemMDS: Myelodysplastic syndrome; WHO: World Health Organization.		

Table 4: Results of multivariate analysis of prognostic factors affecting OS.
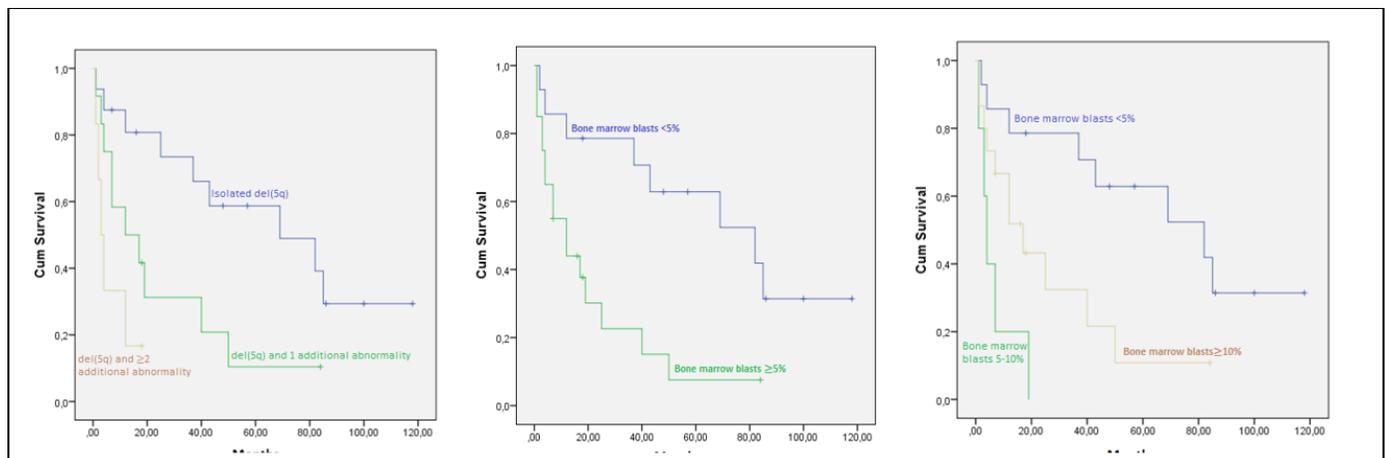
Figure 1: OS time according to Kaplan-Meier curves in MDS patients 1A. In isolated del(5q) group, del(5q) and 1 additional abnormality group and del(5q) and ≥2 additional abnormalities group 1B. By BM blast ratio of <5% or ≥5% 1C. OS time by BM blast ratio of <5%, 5-10% or ≥10%.
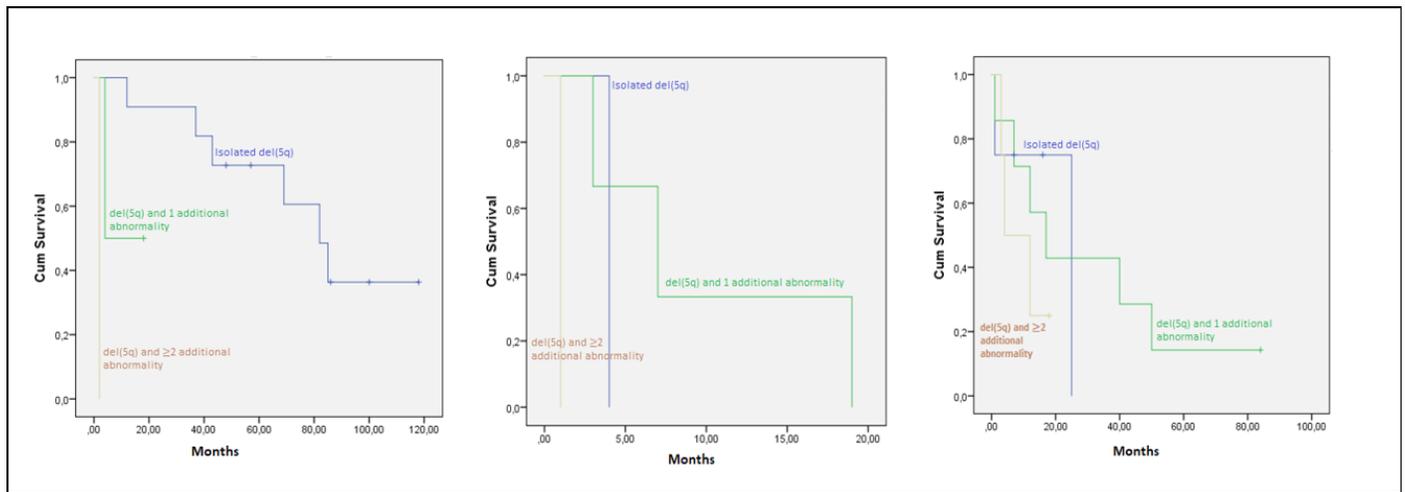
Figure 2: Overall survival time of isolated del(5q), del(5q) +1 additional abnormality and del(5q) + ≥2 additional abnormalities by different bone marrow blast ratios 2A. BM blast ratio <5%, 2B.BM blast ratio 5-10%, 2C. BM blast ratio ≥10%.

Outcome and prognostic factors in patients

AML transformation developed in 7 (20.5%) of our patients. Average duration from diagnosis until transformation into AML was 16.7 (3-48) months, on average. Overall mean survival time of the patients was 32.1 (1-118) months. 24 (70.6%) of our patients died. Most common cause of mortality was sepsis in 10 (41.6%) patients. In the patient group with blast percentage ≥5%, a significantly higher incidence of additional cytogenetic abnormalities was found (p=0.045). In MDS patient groups with del(5q) and additional cytogenetic abnormality/abnormalities, cytopenia number was higher, IPSS risk score was greater, and WHO-defined MDS subtypes presenting with increased blast counts were more common (p=0.015, p=0.002, and p=0.007, respectively). Kaplan-Meier curves indicate a significantly shortened OS time in patients groups with BM blast percentage ≥5%, additional cytogenetic abnormality/abnormalities, higher IPSS risk score, WHO-defined MDS subtypes presenting with increased blast counts, or higher number of cytopenia (p=0.004, p=0.003, p<0.0001, p=0.007, and p=0.008, respectively) Once the patients were divided into 3 groups as <5%, 5-10% and ≥10%, based on the BM blast ratios, the effect of isolated del(5q) and additional cytogenetic abnormalities on survival time was separately evaluated by blast ratios. Accordingly, in the patient group with blast percentage <5%, isolated del(5q) has significantly longer OS compared to the del(5q) +1 cytogenetic abnormality and ≥ +2 cytogenetic abnormalities (mean OS 79.8, 11, and 2 months, respectively, p=0.001). On the other hand, in the patient group with 5-10% blasts, no difference was revealed whether the patients have isolated del(5q) or del(5q) +1 cytogenetic abnormality or ≥ +2 cytogenetic abnormalities (mean OS 4, 9, and 1 month, respectively p=0.116). Similar to the latter, when the blast ratio was ≥10%, patients did not pursue any different OS

depending on having isolated del(5q) or del(5q) +1 cytogenetic abnormality or ≥ +2 cytogenetic abnormalities (mean OS 19, 30, and 9 months, respectively p=0.622). Based on the results of multivariate analysis, in MDS patients, presence of additional cytogenetic abnormality/abnormalities [HR (%95 CI) 2.736 (1.172- 6.387)], a BM blast ratio of ≥5% [HR (%95 CI) 3.62 (1.416-9.267)], IPSS risk score [HR (%95 CI) 9.557 (2.441-37.423)], presence of cytopenia [HR (%95 CI) 4.158 (1.483-11.664)] and MDS subtype as per WHO definition [HR (%95 CI) 8.684 (2.126-35.472)] were identified as the prognostic factors with a negative effect on OS (p=0.02, p=0.007, p=0.001, p=0.007, and p=0.003, respectively). Age, gender, hemoglobin level, ANC, platelet count and MCV were found to have no effect on OS (p>0.05). AML transformation was most frequently encountered in MDS group with del(5q) +1 abnormality and in MDS patient group with a BM blast ratio of ≥5% (p=0.012, p=0.004, respectively). No prognostic factor was detected to have an impact on the AML transformation. However, small sample size of our AML transformation patients has to be taken into account (p>0.05).

Discussion

Karyotype abnormality is a well-established prognostic factor for MDS [2-13]. In MDS patients carrying a del(5q) mutation, number of additional chromosomal abnormalities holds a prognostic value. Isolated del (5q) and a BM blast percentage of <5% have been associated with a reduced relapse rate and an improved relapse-free survival, on contrary to the existence of additional abnormality which has been associated to poor prognosis [14-17]. Have mentioned the number of chromosomal abnormalities, platelet count, and BM blast percentage as the most substantial predictors in their study on factors affecting OS and AML transformation

of del(5q) positive MDS patients [2]. In the study by. no difference was detected between the isolated del(5q) group and del(5q) group with an additional abnormality regarding OS and leukemia transformation [18]. In our study, we have 34 del(5q) positive MDS patients, among whom, in the patient group with one or more additional cytogenetic abnormalities, we have identified a higher number of cytopenia, a greater IPSS risk score, and more frequent occurrence of WHO-defined MDS subtypes manifesting with increased blast counts. Furthermore, we have identified most important prognostic factors affecting OS as: BM blast ratio of $\geq 5\%$, presence of cytogenetic abnormality in addition to del(5q), a high IPSS risk score, WHO MDS sub-type presenting with a gradually increasing blast number, and a high number of cytopenias. In concert with some studies available in the literature, and unlike some other studies, we did not find any effect of age, gender, hemoglobin level, ANC, platelet count, or MCV on OS ($p > 0.05$). Albeit only a small number of our patients experienced AML transformation, in compliance with some previous studies, we did not identify any prognostic factors acting on AML transformation. 5q syndrome typically manifest with macrocytic anemia and normal to elevated platelet count [6]. MDS patients with isolated del(5q) are prone to have a greater MCV and platelet number compared to the patient group with additional one or more abnormalities [19]. Similar to the literature data, both MCV and platelet number was significantly higher in our study, in the MDS patients with isolated del(5q) compared to the patient group having del(5q) plus 1 or ≥ 2 abnormality. A low MCV value and poor platelet count might predict the likelihood of additional abnormalities in del(5q) positive MDS patients. Studies evaluating the effect of blast ratio on OS as indicated by Kaplan-Meier curves plotted in del(5q) positive MDS patients found out a longer survival time in isolated del(5q) patients compared to the patient groups with additional abnormalities in both BM blast ratio sub-divisions of $<5\%$ and $<10\%$. Therefore, occurrence of isolated del(5q) has been concluded as a better predictor than BM blast ratio [2]. In our study, we have divided the patients into three groups based on their BM blast ratio as $<5\%$, 5-10%, and $\geq 10\%$. In our patient group with a blast ratio of $<5\%$, isolated del(5q) patients survived significantly longer than those with del(5q) +1 or $\geq +2$ cytogenetic abnormalities. On the other hand, no difference was detected in the survival time among the individuals with isolated del(5q), del(5q) +1 or $\geq +2$ cytogenetic abnormalities neither in 5-10% nor in $\geq 10\%$ blast ratio sub-groups. While isolated form of del(5q) prolongs the survival provided that the BM blast ratio is $<5\%$, once the BM blast ratio exceeds $\geq 5\%$, whether del(5q) is isolated or additional abnormalities are present makes no difference on survival. Consequently, in del(5q) positive MDS patients, prognostic effect of BM blast percentage on OS is as important as the cytogenetic abnormalities concomitant to del(5q).

Conclusion

Among the del(5q) positive MDS patients, longest OS is achieved by the group with isolated del(5q) and BM blast ratio of $<5\%$, whereas shortest survival is experienced by those with del(5q) +1 or $\geq +2$ cytogenetic abnormalities and BM blast ratio of $\geq 5\%$. In del(5q) positive MDS patients, most important prognostic factors affecting OS are: BM blast ratio of $\geq 5\%$, presence of cytogenetic abnormality in addition to del(5q), a high IPSS risk score, WHO MDS sub-type presenting with a gradually increasing blast number, and high number of cytopenia. Not only the abnormalities in addition to del(5q) but also the BM blast ratio determines the prognosis in MDS del(5q) positive patients.

References

1. Rollison DE, Howlader N, Smith MT, Strom SS, Merritt WD, et al. (2008) Epidemiology of myelodisplastic syndrome and chronic myeloproliferative disorders in the United States, 2001-2004, using data from the NAACCR and SEER programs. Blood 112: 45-52.

2. Mallo M, Cervera J, Schanz J, Such E, Manero-Garcia G, et al. (2011) Impact of adjunct cytogenetic abnormalities for prognostic stratification in patients with myelodysplastic syndrome and deletion 5q. Leukemia 25: 110-120.

3. Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, et al. (2009) The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood 114: 937-951.

4. Mahmood R, Altaf C, Ahmed P, Khan SA, Malik HS, et al. (2018) Myelodysplastic Syndrome in Pakistan: Clinicohematological Characteristics, Cytogenetic Profile, and Risk Stratification. Turk J Hematol 35:109-115.

5. Sole F, Espinet B, Sanz GF, Cervera J, Calasanz MJ, et al. (2000) Incidence, characterization and prognostic significance of chromosomal abnormalities in 640 patients with primary myelodysplastic syndromes. Grupo Cooperativo Espanol de Citogenetica Hematologica. Br J Haematol 108: 346-356.

6. Tefferi A, Vardiman JW (2009) Myelodysplastic syndromes. N Engl J Med 361: 1872-1885.

7. List A, Kurtin S, Roe DJ, Buresh A, Mahadevan D, et al. (2005) Efficacy of lenalidomide in myelodysplastic syndromes. N Engl J Med 352: 549-557.

8. Giagounidis AA, Germing U, Aul C (2006) Biological and prognostic significance of chromosome 5q deletions in myeloid malignancies. Clin Cancer Res 12: 5-10.

9. Boultwood J, Pellagatti A, McKenzie AN, Wainscoat JS (2010) Advances in the 5q- syndrome. Blood. 116: 5803-5811.

10. Fenaux P, Kelaidi C (2006) Treatment of the 5q- syndrome. Hematology Am Soc Hematol Educ Program. 2006:192-198.

11. Hosono N, Makishima H, Mahfouz R, Przychodzen B, Yoshida K, et al. (2017) Recurrent genetic defects on chromosome 5q in myeloid neoplasms. Oncotarget 8: 6483-6495.

12. Haase D, Germing U, Schanz J, Pfeilstocker M, Nosslinger T, et al. (2007) New insights into the prognostic impact of the karyotype in MDS and correlatio with subtypes: evidence from core dataset of 2124 patients. Blood 110: 4385-4395.

13. Solé F, Luño E, Sanzo C, Espinet B, Sanz GF, et al. (2005) Identification of novel cytogenetic markers with prognostic significance in a series of 968 patients with primary myelodysplastic syndromes. Haematologica 90: 1168-1178.

14. Giagounidis AA, Germing U, Haase S, Hildebrandt B, Schlegelberger B, et al. (2004) Clinical, morphological, cytogenetic, and prognostic features of patients with myelodysplastic syndromes and del(5q) including band q31. Leukemia 18: 113-119.

15. Stewart B, Verdugo M, Guthrie KA, Appelbaum F, Deeg HJ et al. (2003) Outcome following haematopoietic cell transplantation in patients with myelodysplasia and del (5q) karyotypes. Br J Haematol 123: 879-885.

16. Lee HR, Oh B, Hong DS, Zang DY, Yoon HJ, et al. (2010) Cytogenetic features of 5q deletion and 5q- syndrome in myelodysplastic syndrome in Korea; marker chromosomes proved to be chromosome 5 with interstitial deletion by fluorescence in situ hybridization. Cancer Genet Cytogenet 203: 193-202.

17. Patnaik MM, Lasho TL, Finke CM, Knudson RA, Ketterling RP, et al. (2011) Isolated del(5q) in myeloid malignancies: clinicopathologic and molecular features in 143 consecutive patients. Am J Hematol 5: 393-398.

18. Gurney M, Patnaik MM, Hanson CA, Litzow MR, Al-Kali A, et al. (2017) The 2016 revised World Health Organization definition of 'myelodysplastic syndrome with isolated del(5q)'; prognostic implications of single versus double cytogenetic abnormalities. Br J Haematol 178: 57-60.

19. Geyer JT, Verma S, Mathew S, Wang YL, Racchumi J, et al. (2013) Bone marrow morphology predicts additional chromosomal abnormalities in patients with myelodysplastic syndrome with del(5q). Hum Pathol 44: 346-356.