

## Chromosomal Abnormalities in Myelodysplastic Syndromes Detected With Multiplex Ligation Dependent Probe Amplification

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**Abstract:** Detection of chromosomal abnormalities in myelodysplastic syndromes enables classification according to WHO Classification and risk stratification according to IPSS and R-IPSS, providing physicians with data regarding personal therapy approach. Detection of the chromosomal abnormalities in our cohort study was performed with the method Multiplex Ligation-dependent Probe Amplification is similar to multiplex PCR reactions, but in this case the target-sequences are amplified, but MLPA probes hybridized for them. 70 patients, chromosomal abnormalities were detected in 32.9% patients, while 67.1% patients had normal findings. Patients were classified according to WHO and stratified according to IPSS and R-IPSS. Data obtained enabled us to make treatment strategy at diagnosis. There are different treatment options for different risk groups including allogeneic transplantation for high and very high risk patients as well as hypomethylating agents for those who are not candidates for transplantation. For low risk groups supportive treatment and growing factors were most often the choice. We can conclude that MLPA reliable method for detection of chromosomal abnormalities in myelodysplastic syndromes.

**Keywords :** chromosomal abnormalities , myelodysplastic syndromes, MLPA

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### I. Introduction

Myelodysplastic syndromes are heterogeneous group of diseases, with cytopenias as a result of an ineffective hematopoiesis and dysplastic morphological characteristics in one or more of the cell lines, and an increased risk of progression in acute myeloid leukemia (1).

Classical cytogenetics (karyotype) was a gold standard for a long period of time for detection of chromosomal abnormalities. But, it was introduced in the late fifties. Meanwhile, a lot of other methods were used such as FISH, PCR based methods and sequencing techniques. Multiplex Ligation-dependent Probe Amplification (MLPA) is a molecular method similar to multiplex PCR reactions. Detection of chromosomal abnormalities in myelodysplastic syndromes (MDS) is of great importance both for classification of patients according to FAB (2) and WHO (3) and stratification according to IPSS (4) and IPSS-R (5). It enables physicians to make personal therapy approach according to the NCCN Guidelines (6).

### II. Aim Of The Study

The aim of the study was refinement of the diagnosis of patients with MDS by introduction of the molecular cytogenetic analysis 'Multiplex ligation-dependent probe amplification' (MLPA) for detection of chromosomal abnormalities, enabling contemporary classification, prognostic risk stratification, and personalized therapy for MDS patients.

### III. Material And Methods

Our cohort consisted of 70 adult patients (>18 years), 33 women (47.1%) and 37 men (52.9%), 66 pts with 'de-novo' and 4 pts with therapy-related MDS (t- MDS), diagnosed at the University Clinic of Hematology, "Ss Cyril and Methodius" University, Skopje, Macedonia, from January 2011 till April 2015, with the follow up period of 52 months. Diagnosis was based on the criteria according to the recommendations of the International Consensus Working Group from 2007 (7). Patients signed informed consent for entrance in the study. Statistical analysis was performed with statistical programs STATISTICA 7.1 and SPSS 17.0.

#### 3.1 Collection Of Material And DNA Isolation

We collected 1 mL of bone marrow aspirate from each patient and put in the test tube with an anticoagulant - K<sub>3</sub>EDTA. Deoxyribonucleic acid (DNA) was isolated with standard phenol chloroform extraction in the Faculty of Pharmacy, Department of biomolecular sciences, Skopje, Macedonia. The material

was centrifuged at 3000 rpm, 4°C, 5 min. The watery upper layer was decanted, and the precipitated cells were washed with 0.9% NaCl, and later washed with lysed solution (1.55M NH<sub>4</sub>Cl, 0.1M NH<sub>4</sub>HCO<sub>3</sub> and 1mM EDTA Ph 7.4, final concentration), centrifuged at 3000 rpm, 4°C, 5 min, in order to lyse the rest of red cells. The rest of the other cells were digested over night at 37°C with 0.1M NaCl, 0.05 M Tris, 1mM EDTA in final concentration. The next day fluid-fluid extraction was performed with saturated phenol (pH=8) and Chloroform : Isoamyl alcohol 24:1, after centrifugation at 3000 rpm, 4°C, 5 min. and removal of the upper watery layer, the precipitation of the DNA with cold 100% ethanol was performed and dissolved with TE puffer (Tris 10mM и EDTA 1 mM, final concentration). The isolated DNA was incubated overnight on 37°C for homogeneous dissolution in the puffer. The intactness of the isolated DNA was examined on gel electrophoresis. With NanoDrop 2000 spectrophotometer (Thermo Scientific) was determined the concentration of the isolated DNAs.

## 1.2 MLPA Analysis

MLPA is a multiplex polymerase chain reaction (PCR)-based technique that can quantify up to 50 different genomic targets simultaneously in a single experiment through amplification of specific hybridizing probes (8). As the sequence recognized by an MLPA probe is only 50-70 nucleotides long, this assay is very useful for detection of deletions or amplifications of single exons (9). One of the major advantages is the high specificity of MLPA, because this method is able to distinguish sequences differing in length by only one nucleotide. Another advantage is the low amount of input DNA (minimum of 20-50 ng) required for a successful MLPA reaction (9). Up to 40-50 small specific probes are directed at DNA regions of interest and to reference regions not associated with the disease, providing a resolution greater than FISH or BAC – based aCGH (10). Each probe consists of two oligonucleotides (5' and 3' end-probes), that hybridize to adjacent sites of the target sequence. The short oligonucleotide contains a target-specific sequence and a universal PCR primer X. The long probe consists of a target-specific sequence, a universal PCR primer Y and a stuffer sequence of variable length in between (19-370 nucleotides) to generate the size differences necessary for electrophoretic resolution (9,11). The MLPA reaction comprises five steps and requires a thermocycler and automated genetic analyzer for fragment analysis by capillary electrophoresis. A crucial step of the MLPA assay, especially when used in the diagnostic setting. Screening of big gene deletions/duplications on different chromosome regions such as: 3, 5q (EGR1, MIR145, SPARC, MIR146A), 7q (EZH2), 8q (MYC), 11q (KMT2A), 12p (ETV6), 17 (TP53, NF1, SUZ12), 19, 20p (ASXL1) и Y. Analyses were performed with the application of P414-A1 MDS MLPA kits of MRC Holland, according to their protocol. The most widely used is the Coffalyser software, an Excel-based program able to perform data normalization steps and necessary corrections (12,13). The utility of MLPA assay in the testing of acute leukemias and myelodysplastic syndromes has been analyzed, confirming excellent accuracy and specificity of MLPA as compared to FISH (14). MDS MLPA assay is sufficiently specific and reproducible to be used in routine diagnostic settings as the first-line genetic screening too.

## IV. Results

Of 70 patients, 33 women (47.1%) and 37 men (52.9%) participated in the study, aged 64.3 years (range 22-86 years). Chromosomal abnormalities were detected in 32.9% patients, while 67.1% patients had normal findings (Table 1).

According to FAB Classification 39 pts(55.7%) belong to the subtype refractory anemia (RA), 2 pts (2.9%) to refractory anemia with ring sideroblasts (RARS), 13 pts (18.5%) to refractory anemia with excess of blasts (RAEB), 6 pts (8.6%) to refractory anemia with excess of blasts in transformation (RAEB-t), 6 pts (8.6%) in chronic myelomonocytic leukemia (CMML) and 4 pts (5.9%) in t-MDS. According to WHO almost half of the patients, 34pts (48.6%) to the subtype refractory cytopenia with unilineage dysplasia, 2 pts (2.9%) to 5q syndrome, 5 pts (7.9%) to refractory cytopenia with multilineage dysplasia, 1pts (1.4%) to RARS, 6 pts (8.6%) to RAEB-1, 6 pts (8.6%) to RAEB-2, 2 pts (2.9%) to CMML-1, 3 pts (4.3%) to CMML-2, 4 pts (5.7%) to t-MDS, and 1 pts (1.4%) to unspecified MDS (u-MDS).

**Table.1** Chromosomal abnormalities in our cohort

Chomosomal abnormalities	Number	%
Normal finding	47	67.1
5q-, 7q-, 12p-, 17p-, 19p+	1	1.4
+8	2	2.9
12p-	2	2.9
5q-	2	2.9
11p-	1	1.4
5q-, 7q-	2	2.9
5q-, 7q-, 17p-	1	1.4
19p-	1	1.4
7q-, 20q-	1	1.4

5q-, 12p-	1	1.4
5q-, 8q-	1	1.4
Yp-	2	2.9
t(3;3)(q21,q26)	1	1.4
5p+, 5q-, 7p-, 7q-, 8p+, 8q+, 12p-, 19p+	1	1.4
17p-, 17q+	1	1.4
20q-	1	1.4
inv(9)(p12q13)	1	1.4
+9	1	1.4
Total	70	100.0

According to IPSS, distribution was as follows: low risk - 16 patients (22.9%), intermediate 1 - 37 (52.9%), intermediate 2 – 8 (11.3%) and high risk - 9 (12.9) (Table 2).

**Table.2. Risk Stratification according to IPSS**

IPSS	Number	%
Low risk	16	22.9
Intermediate-1 risk	37	52.9
Intermediate-2 risk	8	11.3
High risk	9	12.9
Tortal	70	100.0

According to R-IPSS, distribution was as follows: very low risk - 5 (7.1%), low risk 22 (31.4%), intermediate risk - 24 (34.3%), high risk - 10 (14.3%) and very high risk - 9 (12.9%) patients. (Table 3)

**Table.3. Risk Stratification according to IPSS-R**

R-IPSS	Number	%
Very low risk	5	7.1
Low risk	22	31.4
Intermediate risk	24	34.3
High risk	10	14.3
Very high risk	9	12.9
Total	70	100.0

There are different treatment options for different risk groups including allogeneic transplantation for high and very high risk patients as well as hypomethylating agents for those who are not candidates for transplantation. In our cohort allogeneic transplantation was performed in 4 pts (5.7%). Azacitidine received only one patient (1.4%). Chemotherapy received 20% of pts.

For low risk groups supportive treatment and growing factors were the choice. Packed red blood cells (PRBCs) received 44 pts, < 18 units received 33pts (47.1%), while > 18 units received 11pts (11.7%). Erythropoietin was given in 10% of pts, while Granulocyte-Colony Stymulating Factor was given in 17,1%. Immunosuppressive therapy received 30% of pts. Chelating agents were given in 8.6% of pts.

## V. Conclusion

Data obtained from detection of chromosomal abnormalities are very important because they enable physicians to make treatment strategy at diagnosis. We can conclude from this study that MLPA is reliable method for detection of chromosomal abnormalities in MDS. It ought to be mentioned that there is still a significant percentage of patients with normal findings of chromosome analysis. Despite, they are heterogeneous and belong to different FAB and WHO subtypes and different prognosis according to I PSS and R - IPSS. Therefore, the researches lately are focused on gene mutations, aiming to detect mutations with impact on prognosis. That would lead to their incorporation in prognostic systems, enabling refinement of patient’s risk stratification. Due to population, and especially economic reasons, such researches are possible only in developed countries.

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