Detection of nucleophosmin (NPM1) gene mutations with different methods in patients with AML

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INTRODUCTION
Mutations in exon 12 of the nucleophosmin (NPM1) gene are the most common genetic lesions found in acute myeloid leukemia (AML). They occur in about 50-60% of adult AML with normal karyotype. NPM1 gene is located on chromosome 5q35 and encodes nuclear multifunctional proteins (Figure 1)1. Patients positive for mutations in NPM1 gene and negative for internal tandem duplication mutations in FMS-like tyrosine kinase 3 (FLT3) gene have favourable prognosis in this group2,3.

THE AIM OF OUR STUDY
The aim of this study was to compare the results of the NPM1 gene mutations detection by PCR-gel detection and sequencing of the amplified PCR products. Furthermore, we want to introduce the real-time quantitative polymerase chain reaction (RQ-PCR) assay for quantitative assessment of the most frequently observed mutations (type A and B).

RESULTS
The concordance between PCR-gel and sequence detection was 100% (Figure 7). NPM1 mutations were identified in 22 (23%) of the 95 AML patients. The most common detected mutation type was insertion of the TCTG tetranucleotide (type A, 77%), followed by insertion of the CATG tetranucleotide (type B, 9%), CCTG (type D, 5%) and CCAG (type C, 9%) (Figure 5). These are in agreement with recently published literature data.4

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Bone marrow samples from 95 patients with AML were analyzed. The diagnosis of AML was established Followed World Health Organization classification.

Bone marrow aspirates were used for the isolation of mononuclear cells (MC) by ficoll density centrifugation.

RNA was isolated from MC by High Pure RNA Reagent Kit (Roche).

cDNA was performed by SuperScript II reverse transcriptase (Invitrogen).

Amplification of the exon 12 of the NPM1 gene was performed with the forward and reverse primer as described on Gene Amp® PCR System 9700 (Applied Biosystems)5.

The PCR products were visualized after agarose gel (4%) electrophoresis by ethidium bromide staining.

All PCR-amplified samples were purified and sequenced on ABI PRISM 310 Genetic Analyser (Applied Biosystems).

RQ-PCR reaction assay for mutation type A and B were performed with MutaQuant™ Standards Kit (Cancer Profiler, Ipsogen) on ABI PRISM 7000 SDS.

The concordance between PCR-gel and sequence detection.

Table 1. Reproducibility: results of experiments repeated three times. We tested 5 plasmid dilutions in duplicate.

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Figure 1. The NPM1 gene encodes for a protein involved in multiple functions.

Figure 2. Nucleotide sequence of NPM1 wild type.

Figure 3. Nucleotide sequence of NPM1 mutation type A.

Figure 4. Nucleotide sequence of NPM1 mutation type B.

CONCLUSION
Our study shows, that PCR-gel detection is suitable screening method for identification of NPM1 mutations. A sequence detection assay is suitable for confirmation of presence mutations. RQ-PCR method was found out as sensitive and reliable method. On the basis of variation coefficient was also found out that RQ-PCR method is suitable for monitoring minimal disease in AML patients.

REFERENCES

3. Marija J. Mandelc Mazaj, Eva Surina, Uroš Mlakar, Tadej Pajič, University Medical Centre Ljubljana, Hematology Department, Zaloška 7, 1000 Ljubljana, Slovenia
4. INTRODUCTION