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THE USE OF BIOCHEMICAL MARKERS, ECHOCARDIOGRAPHY AND ELECTROCARDIOGRAPHY IN THE ASSESSMENT OF CARDIOTOXICITY IN PATIENTS TREATED FOR ACUTE LEUKEMIA

J.M. Horacek, R. Pudil, M. Tichy, L. Jebavy, P. Zak, A. Strasova, L. Slovacek, M. Blazek, J. Maly

Faculty Hospital, Charles University, HRADEC KRALOVE, Czech Republic

Background. Cardiotoxicity is a relatively frequent and potentially serious complication of hematooncology treatment. Anthracyclines (ANT) represent the greatest risk. Cardiotoxicity of ANT may develop during the treatment (acute cardiotoxicity) and during the follow-up (chronic and late cardiotoxicity). Various methods including biochemical markers have been recommended for monitoring of cardiotoxicity of treatment in hematooncology. *Aims.* Monitoring of cardiotoxicity of ANT in patients treated for acute leukemia with biochemical markers ' N-terminal pro brain natriuretic peptide (NT-proBNP), cardiac troponin T (cTnT); echocardiography (ECHO) and electrocardiography (ECG). *Methods.* 26 adult acute leukemia patients (mean age 46.2±12.4 years, 15 males) treated with 2'6 cycles of ANT-based chemotherapy (CT) were studied. Cardiac evaluation was performed at the baseline (before CT), after first CT (cumulative ANT dose 136.3±28.3 mg/m^2), after last CT (cumulative ANT dose 464.3±117.5 mg/m^2) and circa 6 months after completion of CT (6 Mo after CT). *Results.* The results are summarized in the Table. Six months after CT, NT-proBNP concentrations correlated with systolic and diastolic LV dysfunction on ECHO ' (r=0,514; p<0.01) and (r=0,587; p<0.01). Decreased QRS voltage on ECG correlated with systolic and diastolic LV dysfunction on ECHO ' (r=0.660; p<0.001) and (r=0,592; p<0.01). *Conclusions.* Our results demonstrate acute and chronic cardiotoxicity of ANT. Clinical manifestation of cardiotoxicity in terms of heart failure developed in 2 (7.7%) patients. In asymptomatic patients, abnormal cardiac findings represent subclinical cardiotoxicity, which indicates a risk for development of heart failure (NT-proBNP elevations, diastolic LV dysfunction) and malignant ventricular arrhythmias (QTc prolongation). In regard of late ANT cardiotoxicity, further cardiology follow-up is warranted in all acute leukemia survivors.

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Table 1. Abnormal cardiac findings in patients treated for acute leukemia (n=26).

abnormal cardiac findings	*before CT*	*after first CT*	*after last CT*	*6 Mo after CT*
NT-proBNP elevation	3 (11.5%)	23 (88.5%)	23 (88.5%)	16 (61.55)
cTnT positivity	0	0	0	3 (11.5%)
systolic LV dysfunction	0	1 (3.8%)	1 (3.8%)	2 (7.7%)
diastolic LV dysfunction	1 (3.8%)	5 (19.2%)	6 (23.1%)	12 (46.2%)
QTc interval prolongation	1 (3.8%)	3 (11.5%)	7 (26.9%)	9 (34.6%)
QRS voltage lowering	-	31 (11.5%)	5 (19.2%)	6 (23.1%)

NT-proBNP elevation - NT-proBNP 100 pg/mL for male, 150 pg/mL for female; cTnT positivity - cTnT above 0,01 ng/mL; systolic LV dysfunction - EF bellow 55%; diastolic LV dysfunction - E/A inversion, DT above 220 ms; QTc interval prolongation - QTc above 440 ms; QRS voltage lowering - decrease in QRS voltage more than 1,0 mV.

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THE GROWTH AND SURVIVAL OF AML CELLS WITH T(8;21) ARE DEPENDENT ON VEGF/VEGF RECEPTOR TYPE2 AND PHOSPHORYLATION OF AKT

H. Miwa, N. Imai, M. Shikami, A. Hiramatsu, M. Watarai, E. Tajima, H. Yamamoto, K. Suganuma, A. Satoh, M. Itoh, A. Imamura, H. Mihara, M. Nitta

Aichi Medical University, NAGAKUTE, Japan

Background. We have recently shown that AML cells having t(8;21) chromosome abnormality had augmented expression of vascular endothelial growth factor (VEGF) and type 2 receptor of VEGF (VEGFR2) (Leuk Lymphoma 47: 89-95, 2006). *Aims.* In this study, we examined the biological significance of VEGF/VEGFR2 system in AML cells. *Methods.* Two AML cell lines, Kasumi-1, having t(8;21) chromosome abnormality and NB4, having t(15;17) chromosome abnormality were studied. AML patient samples were also studied after the informed consent. Akt phosphorylation was determined by western blotting. The effects of VEGF165 and/or VEGF receptor2 kinase inhibitor were examined by MTS assay, cell count or annexin/PI assay. *Results.* First, we examined the phosphorylation of Akt, which is thought to be activated by VEGF, by different concentration of fetal calf serum (FCS). Kasumi-1 showed distinct Akt phosphorylation in a dose-dependent manner, although NB4 had undetectable level of Akt phosphorylation. Then, the Akt phosphorylation was almost completely inhibited by VEGF receptor2 kinase inhibitor, suggesting that Akt phosphorylation of Kasumi-1 by FCS was due to VEGF in FCS. Next, we checked the phosphorylation of Akt by the addition of VEGF165 in the culture of low FCS concentration (3%). Akt phosphorylation was augmented by the addition of VEGF165 in Kasumi-1, which was comparable to the phosphorylation status seen in Kasumi-1 cultured in 10% FCS. Finally, we examined the effect of VEGF165 and/or VEGF receptor2 kinase inhibitor on the growth of the cell lines. The addition of VEGF slightly augmented the growth of Kasumi-1. The addition of VEGFR2 kinase inhibitor greatly suppressed the growth of Kasumi-1 through induction of apoptosis although simultaneous addition of VEGF165 rescued the suppressive effect. On the other hand, the addition of VEGF165 with or without VEGFR2 kinase inhibitor did not show any significant influence on the growth of NB4. Then, patient samples were cultured with or without VEGF receptor2 kinase inhibitor. In accordance with the cell line study, all five AML cells with t(8;21) showed marked reduction of viable cells in proliferation (MTS assay) and increase of apoptosis cells by the addition of VEGF receptor2 kinase inhibitor. On the other hand, all four examined samples with t(15;17) did not show significant reduction of viable cells in proliferation by VEGF receptor2 kinase inhibitor. AML cells without chromosome abnormality showed various responses to VEGF receptor2 kinase inhibitor. *Summary.* These data strongly suggested that the growth and survival of AML cells with t(8;21) were dependent on VEGF through type 2 receptor of VEGF on leukemia cells, resulted in activation of PI3 kinase /Akt pathway. It is also demonstrated that the growth of AML cells with t(15;17) is not considered to be dependent on VEGF/VEGFR / PI3 kinase /Akt pathway. This kind of approach will be necessary to evaluate the effects of VEGF on leukemia patients for the VEGF-targeted therapy.

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A NOVEL MUTATION IN THE EXON 11 OF NUCLEOPHOSMIN (NPM1) GENE LEADS TO A TRUNCATED FORM OF THE PROTEIN LACKING THE C-TERMINAL NES-MOTIF

E. Albiero, D. Madeo, I. Giaretta, C. Borghero, C. Visco, F. Rodeghiero

San Bortolo Hospital, VICENZA, Italy

Background. Aberrant cytoplasmatic expression of nucleophosmin (NPM1) due to mutations occurring at exon 12 of NPM gene has been associated to acute myeloid leukemia (AML). These mutations are the most frequent genetic abnormalities found in *de novo* AML with normal karyotype. They are often associated with Internal Tandem Duplication (ITD) of the FLT3 gene. All predicted mutant proteins carry at least one tryptophan amino acid at position 288 or 290. All but one showed the terminal amino acidic sequence VSLRK. Falini *et al.* recently demonstrated that all NPM1 mutant proteins carry a short stretch of hydrofobic amino acids, the nuclear export signal (NES) motif. The acquisition of the NES motif at the C-terminal and the loss of the tryptophan residue 290, are considered relevant to the cytoplasmic localization of NPM1 aberrant protein. *Aims.* DHPLC-based screening and sequence analysis of mutant products of the exons 9 to 12 of NPM1 gene in 102 AML patients, excluding M3 subtype. *Methods.* Bone marrow or peripheral blood samples were collected at diagnosis. RT-PCR for BCR-ABL, PML-RARalfa, AML1-ETO and CBFB-MYH11 were done. cDNA was used for the analysis of NPM1. Exon 12 of the NPM1 gene was screened using the NPM1_870F and NPM1_1112R primers. DHPLC analysis was conducted at 55.3 °C. Exons 9 to 11 were subsequently screened using the couple of primers NPM1_658F and NPM1_1112 R. DHPLC runs were performed at 54 °C and 55 °C. Only the homoduplex samples at the first round of DHPLC were tested. *Results.* All 26 mutant samples were negative for the searched translocations. Twenty-six (25.5%) amplicons were sequenced since they showed an heteroduplex profile. Type A (960_963dupTCTG) was the commonest observed change, occurring in 21/26 samples, followed by Type B (960_963insCATG) in 2 cases and Type IΔ (960_963insCAGA) in 1 case. We identified 2 novel sequence variants (VI1 and VI2), one of which in exon 11 of the gene (Table 1). Variant VI1 exhibited a 4 nucleotides insertion at position 958, leading to the acquisition of the most frequent NES motif type (LxxxVxxVxL). The mutation type VI2 showed an insertion of 8 nucleotides at position 902, in the middle part of exon 11. Nucleotidic insertion led to the creation of a stop codon at the level of the amino acid number 275 (Met274Stop). So, the truncated protein consisted of 274 amino acidic residues instead of 294 of the wild type. *Conclusions.* Variant VI2 is the only mutation described to date mapping outside the NPM1 exon 12. The predicted aberrant protein lacks the NPM C-terminal NES motif and do not contain neither tryptophan 288 nor 290. Further investiga-