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Akutne mijeloične leukemije

TETRAPLOID ACUTE PROMYELOCYTIC LEUKEMIA WITH DOUBLE T(15;17)/PML-RARA: FIRST REPORT IN CROATIA, EUROPE

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Keywords: Acute promyelocytic leukemia, tetraploid, PML-RARA, All trans-retinoic acid (ATRA)

Introduction: Acute promyelocytic leukemia (APL) is characterized by the t(15;17)(q22;q21) cytogenetic abnormality in the majority of cases. In most of the cases the cells of APL have normal, diploid karyotype. Very few cases have been presented with very rare tetraploid karyotype with double translocation t(15;17)(q22;q12). Tetraploidy has only been reported in 16 cases of APL in the literature, with cases reported in the Far East countries, Australia, United state of America and Dominican Republic. To our knowledge, our case is the first to reported tetraploid APL with double t(15;17)/PML-RARA in an adult from Croatia.

Case report: We present a case of a 66-year old male patient who presented with dyspnea, and dental bleeding. Blood work showed the white blood count of 1×109/L with 39% neutrophils, 49% lymphocytes and 5% monocytes. The hemoglobin was 124 g/L and the platelet count was 61×109/L. The protrombin and activated partial thromboplastin time were normal but level of fibrinogen level was low (1.4 g/L). The bone marrow showed numerous large promyelocytes (54 %) that contained irregular bilobed nuclei, abundant cytoplasm with granularity and few Auer Rods. Flow cytometry showed a population of large immature cells phenotypically positive for CD13, CD33, myeloperoxidase (MPO), CD117, CD 56, CD64, CD 2 and negative for HLA-DR and CD11c which refered to promyelocytes. Cytogenetics showed a tetraploid karyotype as follows: 46,XY,t(15;17)(q22;q21)/92,XXYY,t(15;17)(q22;q21)x2. Fluorescence in situ hybridization (FISH) analysis proved existence of clonal cells with translocation t(15, 17) in 15% of metaphase nuclei and tetraploid subclonal cells with the same translocation t (15, 17) in 70% of metaphase nuclei. PML/RARA copies were identified by a reverse transcriptase-polymerase chain reaction (RTPCR).

Findings were consistent with APL, tetraploid variant. Induction therapy with all trans-retinoic acid (ATRA) and idarubicin were completed with no complications. Post induction bone marrow cytogenetics revealed a normal male karyotype and FISH and PCR studies showed no PML/RARA fusion products, consistent with APL in molecular remission. The patient achieved a complete remission in 2 months and completed three consolidation therapy cycles with ATRA, idarubicin or mitraxontrate.

Conclusion: We report the first case of tetraploid APL in Europe. Based on the previous reports, APL with tetraploid karyotype appears to have a similar clinical outcome to diploid APL. Nevertheless, it is difficult to draw a firm conclusion in regards to the long-term prognosis of APL patients with a tetrapolid karyotype due to the very small number of such cases reported. We encourage others to report cases of tetraploid APL in order to understand this rare cytogenetic subgroup better.

DVOGODIŠNJI REZULTATI LIJEČENJA AKUTNE MIJELOIČNE LEUKEMIJE U BOLESNIKA STARIJIH OD 65 GODINA: ISKUSTVO ZAVODA ZA HEMATOLOGIJU KLINIKE ZA INTERNU MEDICINU KLINIČKOG BOLNIČKOG CENTRA RIJEKA

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Ključne riječi: akutna mijeloična leukemija, "staračka" AML, kemoterapija, niske doze citarabina, azacitidin

Cilj: prikazati rezultate liječenje bolesnika s akutnom mijeloičnom leukemijom (AML) starijih od 65 godina na našem Zavodu dvogodišnjem periodu.

Metode: retrospektivnim istraživanjem obuhvaćeni su bolesnici s AML koji su u trenutku dijagnoze bili stariji od 65 godina te su od 1.1.2017. do 31.12.2018. aktivno liječen na našem Zavodu. Bolesnici koji nisu aktivno liječeni te koji nisu primili barem 1 ciklus terapije isključeni su iz istraživanja.

Rezultati: analizirano je 18 bolesnika, 13 muškaraca i 5 žena. Medijana praćenja iznosio je 9,25 mjeseci. Medijan dobi bio je 75, a raspon 66–83 godine. 3 bolesnika imalo je sekundarnu AML. Prema WHO klasifikaciji 1 bolesnika je imao AML s minimalnom diferencijacijom, 11 sa sazrijevanjem, 4 mijelomonocitnu, 1 monoblastično/monocitnu i 1 megakarioblastičnu AML. 10 bolesnika imalo je citogenetiku intermedijarnog, a 8 visokog rizika (kompleksni kariotip 3 bolesnika). Raspon primijenjenih linija terapije bio je 1–4, a medijan 1 linija terapije. U 1. liniji liječenja 12 bolesnika liječeno je niskim dozama citarabina (NDC), 3 bolesnika azacitidinom (AZA), 2 bolesnice intenzivnom kemoterapijom („3+7" protokol), a 1 bolesnica hidroksiurejom. U 2. liniji 3 bolesnika liječeno je AZA, 1 bolesnik NDC, 1 bolesnica HAM protokolom i 1 bolesnik tiogvaninom. U 3. i 4. liniji 2 bolesnika liječeno je venetoklaksom, po 1 bolesnik hidroksiurejom i tiogvaninom. Odgovor na terapiju (kompletna hematološka remisija ili smanjenje broja blasta u koštanoj srži za barem 50 %) nakon 1. linije liječenja postignulo je 11 bolesnika (6 liječenih NDC, 3 AZA i 1 liječen intenzivnom terapijom). U 2. liniji liječenja samo je 1 bolesnica liječena HAM protokolom postignula remisiju. Raspon broja ciklusa za NDC iznosio je 2–24, a medijan 4 ciklusa. U bolesnika liječenih AZA raspon je bio 3–21, medijan 8 ciklusa. 13 bolesnika je preminulo, a 5 je živo (27,8%). Medijan preživljenja čitave grupe iznosi 12,5 mjeseci, isti je u grupi bolesnika liječenih u 1. liniji s NDC, a u grupi liječenih AZA u 1. liniji iznosi 14 mjeseci.

Zaključak: naši rezultati, iako se radi o vrlo maloj grupi bolesnika, odgovaraju literaturnima. Liječenje bolesnika s AML starijih od 65 godina i dalje je nezadovoljavajuće te zahtjeva nove pristupe.

ASYMPTOMATIC INCREASE OF SERUM LIPASE AND AMYLASE LEVELS DURING TREATMENT OF ACUTE PROMYELOCYTIC LEUKEMIA WITH ALL-TRANSRETINOIC ACID AND ARSENIC-TRIOXIDE

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Keywords: arsenic-trioxide, acute promyelocytic leukemia, lipase, adverse effect

Arsenic trioxide (ATO) is very potent as a single agent against acute promyelocytic leukemia (APL). It can induce complete remission when used for remission induction with or without the addition of chemotherapy or all-trans-retinoic acid. Commonly reported side effects of arsenic trioxide include: differentiation syndrome, QTc prolongation and hepatotoxicity (serum bilirubin and/or SGOT and/or alkaline phosphatase but not lipase and/or amylase). Pancreatitis due to ATO is extremely rare and preclude subsequent therapy until resolution of symptoms.

39-year old male was reffered to our hospital due to coagulopathy and suspected acute leukemia. As APL was considered in differential diagnosis, he received ATRA from the day of arrival. After diagnostic work up, low-risk acute promylocytic leukemia was confirmed. Treatment with ATRA+ATO was initiated according to APL0406 regimen. On the 8th day of therapy he complained of abdominal pain. Lab results were normal. Pain completely

regressed in 2 days. ATO was applied in same dose, and his complaints were considered as part of differentiation which occured in that time. Surprisingly, 2 days after complete regression of any symptoms, we noticed elevation in serum lipase and amylase levels. As this was completely asymptomatic we deicided to continue ATO in the same dose. Serum levels of lipase and amylase were steadily increasing in subsequent days, and peaked about 950 and 500, respectively. After that they started to slowly decrease, and complete normalization was seen in about 3 weeks. During whole episode, patient didn't have any complaints.

Asymptomatic increase in serum lipase and amylase levels was probably connected with ATO. Continuous application of therapy in induction is extremely important. In our case, with close monitoring, continuation of full dose ATO therapy was feasible and without subsequent adverse events.

IMMUNOEXPRESSION OF BONE MARROW HAEMATOPOIETIC CELLS 5-METHYLCYTOSINE (5-mC) IN PATIENT WITH ACUTE PROMYELOCYTIC LEUKEMIA

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Ključne riječi: acute promyelocytic leukemia (APL), DNA methylation, 5-methylcytosine (5-mC), cytomorphology, immunocytochemistry

Acute promyelocytic leukemia (APL) is characterized by a unique chromosome translocation t(15;17)(q24;q21), which leads to the formation of the promyelocytic leukemia (PML)–retinoic acid (RA) receptor-alpha (RARA) fusion protein. However, it is acknowledged that this rearrangement alone is not able to induce the whole leukemic phenotype. In addition, epigenetic processes, such as DNA methylation, may play a crucial role in leukemic pathogenesis. Accentuated DNA hyper- and hypomethylation were both identified in t(8;21)-AML1/ETO, inv(16)-t(16;16)-CBFB-MYH11 and in t(15,17)-PML-RARa. Some reports point that cells from APL patients showed increased genome-wide DNA methylation with higher variability than healthy CD34+ cells, promyelocytes, and remission bone marrow (BM) cells. DNA methylation, catalyzed by DNA methyltransferases (DNMTs), involves the covalent transfer of a methyl group (-CH3) resulting in the formation of 5-methylcytosine (5-mC). Moreover determination of DNA methylation could be also a basis for therapy while for example decitabine, "epigenetic drug" exerts its effect by hypomethylating DNA and reduces the global levels of 5-mC.

The aim of this study was to present a patient with acute promyelocytic leukemia and high immunocytochemical expression of 5-mC in BM haematopoietic mononuclear cells (HMCs).

Methods: BM and peripheral blood of APL patient were cytologically analysed after standard Pappenheim staining and cytochemical stainings for Sudan black, PAS, myeloperoxidase, and alpha-naphtyl acetate esterase. Immunocytochemical Envision method with monoclonal antibody for 5-mC was also done, as well as flow cytometry immunophenotyping, cytogenetics and molecular analyisis of BM.

Results: Blasts and atypical promyelocyts with coarse cytoplasmic granules comprise 50% of all BM nucleated cells. All cytochemical reactions were positive in 30% leukemic cells. Immunophenotyping, cytogenetic and molecular analyses confirmed APL by detecting negativity of HLA-DR and CD34 and positivity of CD33, t(15;17) and PML-RARa fusion protein. High 5-mC immunoexpression was found in 60% of BM leukemic cells.

Conclusion: In patient with cytomorphology, immunophenotype, cytogenetic and molecular characterists of APL was found high 5-mC immunoexpression of BM leukemic cells. This finding refelect complex leukemic transformation of APL. Also, although epigenetic modulation is not a common treatment strategy in APL, targeting this pathway may have some clinical utility in refractory or relapsed APL cases.

C-MYC AMPLIFICATION ON DOUBLE MINUTE CHROMOSOMES IN ACUTE MYELOID LEUKEMIA PATIENT

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Keywords: C_MYC, double minute, AML, G-banding, aCGH

Double minute chromosomes (dmins) are small extrachromosomal amplified material without centromeres. Although found in variety of solid tumors, they are a rare finding in hematological malignancies.

We present a case of double minutes in a 69-year-old male patient, who was diagnosed with acute myeloid leukemia with multilineage dysplasia. Anamnestic data showed a history of chronic kidney disease that was accelerating into acute phase.

Bone marrow sample was sent to cytogenetic laboratory for G-banding, FISH and array-CGH workup. G-banding showed complex karyotype, with hyperdiploid clone with multiple structural alternations in chromosomes 2, 17 and 20 with aneuploidy of chromosomes 7 and 13. In addition, another subclone was found, that in addition to structural and numerical aberrations, had a marker chromosome. Further on, a hipertriploid clone was detected, again, with multiple chromosome alterations. Interesting finding was a various number of double-minute chromosomes in all pathologic metaphases. FISH analysis revealed deletion of chromosome 20 in 42% interphase nuclei (IN), 3–4 copies of chromosome 11 in 45% of IN and 4 copies of chromosome 1 in 53% of IN.

Further on, array comparative genomic hybridization with single nucleotide polymorphism (aCGH+SNP) revealed loss of genetic material on chromosomes 2p, 3q, 9p (with a double deletion of CDKN2A and CDKN2B genes), 9q, 17p (p53 deletion) and 20q. Loss of heterozygosity was detected on chromosomes 1p, 2q, 4q (TET2 gene), 12q and 13q. 4.3 MB amplification (x10) of 8q24.13-q24.21 region comprising C-MYC gene was found, that could account for double minutes found by G-banding. To corroborate this, FISH analysis was performed with C-MYC break-apart probe. We detected multiple C-MYC signals that were indeed located on double minute chromosomes as proven by metaphase FISH.

Two weeks into the treatment, the patient passed away.

TREATMENT OF FLT3-ITD POSITIVE AML WITH MIDOSTAURIN IN COMBINATION WITH 3+7 INDUCTION THERAPY CONSISTING OF IDARUBICIN AND CYTARABINE

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Keywords: acute myeloid leukemia, FLT3 mutations, midostaurin, standard induction therapy, anthracyclines

Mutations in FLT3 in acute myeloid leukemia (AML) are usually associated with adverse outcomes. In the phase 3 CALGB 10603/RATIFY trial AML patients were randomly assigned to 3+7 chemotherapy consisting of daunorubicin and cytarabine, plus midostaurin versus placebo. Midostaurin significantly prolonged overall survival. Clinical trials of anthracyclines and dose-intensity have generally shown outcomes with idarubicin or daunorubicin induction to be equivalent. However, the safety and efficacy of idarubicin that is commonly utilized anthracycline in AML induction, in combination with midostaurin, has not yet been evaluated in a randomized prospective trial.

Here we present a case series of FLT3 positive patients treated with induction therapy consisting of idarubicin and cytarabine plus midostaurin in our centre, from January 2018 to January 2020.

We analyzed 6 patients. They all exhibited internal tandem duplication (ITD) on diagnosis and all were classified as poor risk leukemia by ELN criteria. Median age was 44.5 years. There were 3 male and 3 female patients. Patients were treated with induction therapy consisting of idarubicin 12mg/m2 for 3 days and cytarabine 200mg/m2 for 7 days. Midostaurin was initiated on day 8 of induction and only one patient did not receive all FLT3 inhibitor doses due to early fatal outcome. The median time from day 1 of induction to neutrophil (>0.5×109/l)

and platelet (>100×109/l) recovery was 29 days and 31 days, respectively. Bone marrow aspiration was performed on day 28 and in 5/6 patients morphologic leukemia-free state was verified. Four patients achieved complete remission after one cycle of treatment and one patient achieved complete remission after two cycles of induction therapy. One patient died having not completed treatment. In 4 patients FLT3-ITD was negative on day 28. All 6 patients experienced febrile neutropenia. One patient had maculopapular rash attributed to midostaurin. Four patients proceeded to allogeneic transplantation and are still alive at the time of reporting.

In our case series midostaurin in combination with idarubicin-based induction 3+7 therapy appears to be safe. However, it should be noted that in this patient cohort neutrophil and platelet recovery time was longer than expected, although patient numbers are limited.