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Case Report

The Dilemma of Diagnosis of Acute Megakaryoblastic Leukemia in a Saudi Arabian Down Syndrome Patient with Mosaic Trisomy 21 Pattern-Case Report

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Introduction

Acute myeloid leukemia (AML) is the second most common type of leukemia diagnosed in adults and children; it occurs in about 15 % of childhood acute leukemia [1]. Acute megakaryoblastic leukemia (AMKL) is a rare subtype of AML that occurs in approximately 4-15% of newly diagnosed pediatric AML and is considered the most frequent type of AML in children with Down syndrome (DS) [2]. While the diagnosis of cases of trisomy 21 Down syndrome may not be difficult, yet, the diagnosis of mosaic Down syndrome is still challenging due to the unequal distribution of the third chromosome 21 among the different tissues type of the human body (Prudowski Z et al., 2020) [3]. It is of great importance to identify those patients with acute myeloid leukemia and mosaic Down syndrome, especially when they are phenotypically normal, because of clinical evidence of their response to reduced-intensity chemotherapy protocols [4] similar to the cases of myeloid leukemia of Down syndrome who are known to have favourable event-free and improved outcomes and hence avoiding intensified protocols and minimizing the treatment-related toxicities and mortalities that are also known to be associated with down syndrome patients [5]. Here, we report a case of AMKL with Trisomy 21 and tetrasomy 21 clones who was subsequently diagnosed with mosaic trisomy 21.

Case Report

A male patient was referred to our centre at the age of 9 months with new onset of pancytopenia four months earlier associated with anorexia and hypo-activity though no significant weight loss for further evaluation. He is known case of G6PDD with no significant past medical history apart from neonatal physiological jaundice that was treated with phototherapy for few days with normal developmental and vaccination history. The physical exam revealed a choppy child with his weight over the 90 centiles. He had some subtle dysmorphic features in form of brachiocephalic, broad forehead, slightly depressed nasal bridge, epicanthal fold, up slanted eye, low set ears, abnormal convolution of the ears, prominent incisor, tented mouth, retro-micrognathia, multiple café la spots (right upper thigh - lower abdomen - left shoulder) with no palpable lymphadenopathy or hepatosplenomegaly. His labs showed pancytopenia of WBC: 4.1x10^9/L, HGB: 7.0 g/dL, platelets: 96 x10^9/L, and ANC: 340 (8.2%) with no abnormal cells – blasts- seen on peripheral blood. He had normal HGB Fractionation pattern. His renal and hepatic profiles, Uric acid, Lactate dehydrogenase levels, and iron profiles all were within normal. Chest X- ray and Abdominal US were both unremarkable. The cardiac structures and function evaluated by echocardiogram showed Mild tricuspid regurgitation, Mild

pulmonary hypertension 36 mmhg but otherwise normal ejection fraction=80% and fractioning shortening = 47%. MRI brain showed diffused calvarial, skull base, facial bone leptomeningeal, pachymeningeal, and paranasal sinus as upper neck space leukemic infiltration with soft tissue and extra-axial components. Initial Bone marrow biopsy showed diffuse and dense increase in reticulin deposition with extensive intersections and coarse bundles of thick fibers consistent with collagen, leading to streaming of the marrow obliterating the cellular morphology in the majority of the inter-trabecular regions. Only one area showed residual trilineage hematopoiesis and another small reactive lymphoid aggregates. Flow cytometry is totally negative to CD34 in the CD45 dim to negative region, and the extensive fibrosis is noted morphologically. Lumbar puncture showed a CSF cytology was positive for blast cells. The patient was put under close monitoring. His CBC showed persistent pancytopenia but with the appearance of blast cells in peripheral blood. Four weeks later, a repeated bone marrow studies revealed variable cellularity with marked streaming/ fibrosis and focal areas of blast infiltration. The megakaryocytes are small and hypolobated with frequent micro megakaryocytes. Rare granulocytes are seen. The erythroid elements are markedly decreased. Trichrome highlights increased collagen fibrosis. Immunohistochemical stains are performed with proper controls on the biopsy section; interpretation is limited by marked fibrosis and crush artifact. CD34 and CD117 highlights areas of increased blasts; these blasts are positive for CD7 and focally positive for CD61. The blasts are negative for MPO, CD3, CD79a and E-cadherin. CD61 also highlights marked megakaryocytic hyperplasia with many dysplastic forms and micro megakaryocytes. CD20 highlights a small interstitial lymphoid aggregate. Flow Cytometry was performed on peripheral blood. It showed gated population of interest in the CD45 dim (Blast gate) accounts for 20% of total analysed events. The population is positive for CD34 partial, CD117, CD7 bright, CD35 dim, CD36, TdT dim, CD71 dim and partial, CD38, CD61 dim and partial and negative for CD19, cyCD79a, MPO, surface and cytoplasmic CD3, CD13, CD33, CD15, CD4, CD64, HLA-DR, CD14, CD11b and the rest of the tested myeloid/lymphoid markers. This immunophenotype is suggestive of acute megakaryoblastic leukemia AMKL. Repeated lumber puncture showed infiltration by blast cells. Chromosomal studies confirmed the Normal 46, XY karyotype. Trisomy 21 FISH analysis showed 15% of cells having trisomy 21 and tetrasomy 21 but 6 days later a repeated blood sample showed 90% of cells having trisomy 21. The Conclusion after discussion with the cytogeneticist that it is most likely that the cells which showed the trisomy 21 are of the malignant clone and the patient does not have trisomy 21 syndrome. Other common cytogenetics and molecular abnormalities that was known to be associated with AML or AMKL were negative including GATA1 mutation. Based on the previously collected data, the initial diagnosis was AMKL in non -down syndrome patient with positive CNS infiltration by leukemic cells and the consent was obtained, and the patient was started on therapy as per AML-MRC-15 protocol. First induction cycle (ADE) consisted of IV.Cytosine Arabinoside 100 mg/m2 12hourly for 10 successive + IV. Daunorubicin 50 mg/m2 on days 1, 3 and 5 + IV Etoposide 100 mg/m2 daily on days 1-5 inclusive with twice weekly triple intrathecal chemotherapy with negative blasts in CSF was obtained after the first Triple intrathecal chemotherapy. Following the first cycle of chemotherapy, the patient developed severe complications in form of pancytopenia, severe lung infection and enteritis with severe diarrhea and ascites. His management required transient admission to PICU for intensive supportive care, the use of broad-spectrum antibiotics, antifungal therapy as well as blood component therapy. CSF analysis following the first cycle of chemotherapy was free of blast cells. Because of the patient`s poor general condition and the prolonged pancytopenia, bone marrow evaluation post the 1st cycle of chemotherapy was delayed approximately 52 days. The BM post 1st cycle of chemotherapy showed the presence of 0.2% blast population by flow cytometry (Positive, AML MRD cut-off is 0.1%). In addition to the presence of circulating blasts in CBC. Chromosome Analysis at our centre and at Mayo clinic (On BM post 1st cycle of chemotherapy) showed 46, XY. Cytogenetics RUNX1 – FISH (On the same BM sample) were positive in 3% of analysed nuclei. (Abnormal chromosome 21). Based on the collected results post the 1st cycle of chemotherapy, the patient was considered in partial remission and a second induction cycle was given as per FLA protocol (IV Fludarabine 30 mg/m2/daily for 5 days + IV Cytarabine 2000 mg/m2 /daily for 5 days). This cycle was followed by minor febrile illness but subsequent prolonged myelosuppression which allowed an optimum bone marrow evaluation after recovery by day 40 and the results of BM post the 2nd cycle of chemotherapy showed a cellular marrow with trilineage hematopoiesis, myeloid hypoplasia with no increase in blast cells (By morphology= 3-4%) - Patient is in complete remission with incomplete hematological recovery, no residual malignant clone detected, confirmed by flow- cytometry. CSF analysis Following the second cycle of chemotherapy continued to free of blast cells. Cytogenetics for RUNX1 – FISH (On the same BM sample) were positive in 2% of analysed nuclei. (Abnormal chromosome 21). Patient was given the 3rd cycle of chemotherapy again as per FLA chemotherapy considering that he was not on full molecular remission yet. He tolerated this cycle very well with no major complications. Bone marrow evaluation following the 3rd cycle on day 29 showed Cellular marrow for the patient age with trilineage hematopoiesis, mild myeloid hypoplasia, no increase in blast cells. Patient in hematological remission with no residual clone detected by Flow-cytometry. Cytogenetics for RUNX1 – FISH were positive for trisomy RUNX1 gene in 3% of analyzed nuclei. The persistent positivity of the RUNX1 by FISH and

molecular studies for the presence of trisomy 21 despite full remission status and eradication of the malignant clone proved by bone marrow morphology and flow-cytometer results raised the suspicion that this patient might be a mosaic down syndrome rather than being an AMKL with associated +21 who is refractory to therapy. To confirm this postulation, the patient was subjected to a skin biopsy/fibroblast culture and FISH analysis with an Abbott Vysis probe was performed for copy number changes of chromosomes 13 (RB1) and 21 (D21S259/D21S341/D21S342 on fibroblast-cultured sample. 45% of nuclei had 3 D21S259/D21S341/D21S342 signals suggesting presence of 3 copies of chromosome 21. The hybridization pattern observed is consistent with the clinical diagnosis of mosaic Down's Syndrome. Based on this new information the diagnosis was revisited and accordingly the patient was diagnosed as AMKL in a mosaic DS and considered to be in complete remission based on the complete disappearance of the malignant clone from the bone marrow by flow-cytometer. The persistent +21 chromosome which was seen several times in the bone marrow evaluation during chemotherapy and thought to be as residual malignant clone was then considered as part of his mosaic Down Syndrome condition. The plan of therapy was changed from intensive non-DS-AML therapy to reduced- intensity chemotherapy for DS-AML according to COG protocol BFM-98 protocol (AML in DS) and started on maintenance phase of the protocol (Oral Thioguanine 40 mg/m2 daily + Subcutaneous Cytarabine 40 mg/m2 x 4 days monthly for one year). Thought he still has few months more to complete his chemotherapy protocol but so far, he had perfect tolerance to chemotherapy and excellent clinical condition with no further complications.

Genetics Approach

Chromosomal and FISH analysis

During the 8 months since diagnosis time and after induction therapy evaluation, the chromosomal and FISH analysis detected the presence of abnormal cells with trisomy/tetrasomy 21 (Figure 1). The important variation and the progression curve of the abnormal trisomy/tetrasomy 21 cells suggest strongly that these abnormal trisomy/tetrasomy 21 cells are more belonging to a leukemic clone rather than a constitutional process, from 90% during diagnosis time period, reaching a 2% 9 months later (Figure 2). The persistence of this extremely low percentage and WES analysis evidences, both raised the possibility of a trisomy of chromosome 21 in a mosaicism pattern. Chromosome 21 FISH analysis on fibroblasts extracted from the patient skin biopsy confirmed the presence of this mosaicism.

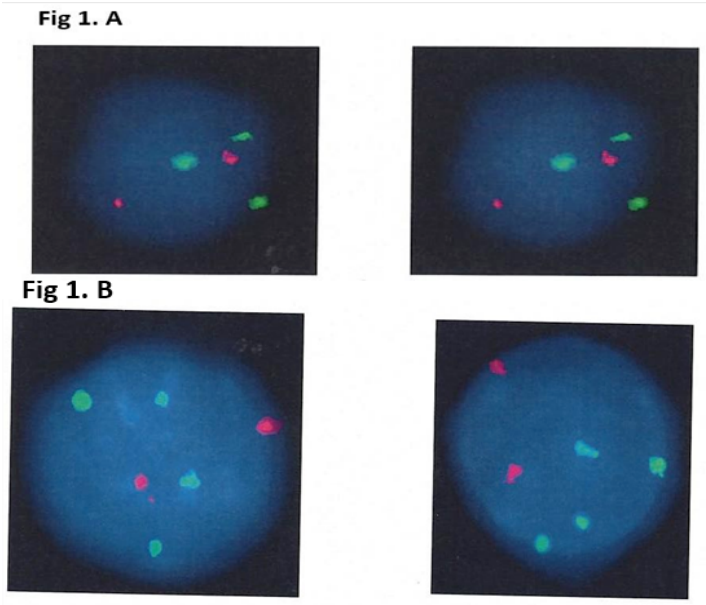

Figure 1: RUNX1 FISH analysis on the patient bone marrow during diagnostic time period. A. Two nuclei, each one with three green signals represent trisomy of RUNX1 gene and two red signals representing two copies of RUNX1T1 gene. B. Two nuclei, each one with four green signals represent tetrasomy of RUNX1 gene and two red signals representing two copies of RUNX1T1 gene.

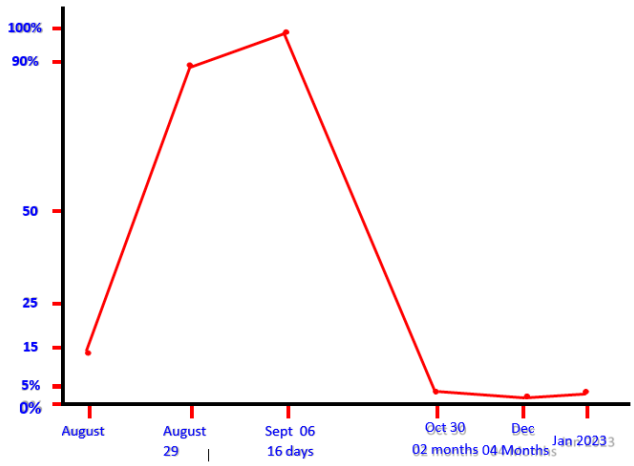

Figure 2: RUNX1 FISH analysis on the patient bone marrow during diagnostic time period. The progression of the abnormal leukemic trisomy/tetrasomy 21 cells during a period of 8 months.

Microarray Analysis

Revealed the presence of only two VUS without any evidences of the presence in a mosaic pattern of trisomy/tetrasomy 21.

Whole Exome Sequencing Analysis

Indicates the presence of a 48 Mb gain in the 1q32 to 1q44 region in a mosaic state. WES reported that this clonal chromosomal abnormalities such as the reported one (1q+) are common features in Fanconi Anemia patients and are associated with progressive bone marrow failure and/or a pre-leukemia condition. In addition, this analysis raised the possibility of presence of trisomy of chromosome 21 in a mosaic pattern. This possibility must be confirmed by a constitutional FISH analysis approach.

Discussion

Acute leukemia is the most common childhood cancer. It accounts for about one- third of all childhood malignancies. Pediatric acute myeloid leukemia (AML) represents 15%–20% of all pediatric acute leukemias and is far less than acute lymphoblastic leukemia (ALL), which accounts for almost 80% [1]. The latest improvements in AML survival have been achieved through the role of intensification of therapy, including the use of allogeneic hematopoietic cell transplantation (HCT) and the improvements in supportive care. The overall survival for children with AML ranges between 65 to 70 percent, yet still inferior to that for childhood ALL [1]. Acute megakaryoblastic leukemia (AMKL) is a subtype of acute myeloid leukemia (AML) characterized by abnormal megakaryoblasts that express platelet-specific surface glycoprotein. Bone marrow biopsy usually showed extensive fibrosis, often making aspiration difficult among those patients [6]. Although AMKL represents 4–15% of newly diagnosed pediatric AML, it is considered the most frequent type of AML in children with Down syndrome (DS) [7]. Interestingly, young children (<4 years) with DS have a 500-fold increased incidence of AMKL [8]. The median age at diagnosis for AML-DS patients is 1.8 years vs. approximately eight years for AML in non-DS cases [9]. AMKL in DS is associated with mutations in the transcription factor GATA1 which is thought to be the key factor linked to both leukemogenesis and the high cure rates of DS AMKL [10]. In Saudi Arabia, the prevalence of Down syndrome has been reported to be 18 per 10 000 live births [11]. In cases of mosaic Down syndrome, there is an extra copy of chromosome 21 in some and not all of the body cells. In the USA, the frequency of mosaicism of Down syndrome has been estimated to range from 1 in 16,670 to 1 in 41,670 conceptuses/live births (4). Again, the prevalence of mosaic DS was reported to be 3.85% of the DS population, and an accurate clinical diagnosis was made in only 37.5% of mosaic patients [12]. The risk of leukemia in Mosaic trisomy 21 patients was addressed in some studies and believed to have a lower chance for clinical consequences in them compared to non- mosaic forms of this condition, including the risk for leukemia, while Little is known about the propensity for people with mosaicism to develop solid tumors. Generally, individuals with non-mosaic forms of Down syndrome have been noted to have a reduced risk of developing most types of solid tumors [4]. The physical features in patients with mosaic DS may be milder as in our case. Thus, low-level mosaicism may have resulted in the unrecognition and underdiagnoses of this clinical entity [4], .In the current report, because of the slight clinical features of the patient, the diagnosis of his mosaic condition took more than six months to be established from the onset of his pancytopenia. AMKL is often associated with myelofibrosis, which delays the diagnosis since usually no sufficient leukemic blast cells could be obtained in this situation by bone marrow aspiration [7]. Studies showed no difference between the DS-AMKL and de novo AMKL groups in the frequency of myelofibrosis [13]. In the setting of myelofibrosis, an assessment for immunophenotypic, cytogenetic, and genetic abnormalities by bone marrow aspiration may yield false-negative results. The initial bone marrow assessment of our patient showed extensive fibrosis that obliterating the cellular morphology in the majority of the inter-trabecular regions and diagnosis was not made until the appearance of the classical AMKL blasts few weeks after the first bone marrow evaluation. About one-half of AMKL patients have DS. It is necessary to exclude mosaic DS when AMKL is diagnosed [14]. Due to the inability to exclude mosaic T21 by physical examination alone and the low frequency of somatic T21 in pediatric non-DS-AML, Some studies recommend that patients without known DS but with T21-positive AML, should undergo an evaluation for germline T21 via skin biopsy, even in the absence of phenotypic features of DS. Our patient required skin biopsy/ fibroblast culture and FISH analysis to establish the diagnosis of his mosaic trisomy 21 condition , Since there was variation in the level of the abnormal trisomy/tetrasomy 21 cells that were detected at diagnosis time and when evaluated after starting therapy, that initially strongly suggests that these abnormal trisomy/tetrasomy 21 cells belong to a leukemic clone rather than a constitutional process and the variation in their level from 90% during a diagnosis time, reaching a 2% nine months later after commencing therapy may reflect the response to treatment. The conflicting results we got between the MRD from flow cytometry that confirmed the remission status after the second cycle of induction and the conventional cytogenic tests on the same bone marrow sample that was still positive for the presence of RUNX 1, raised the suspicion of considering mosaic trisomy 21 and lead us to confirm that by skin fibroblast as well. As it is well known, the most important factors predicting clinical outcomes for pediatric acute myeloid leukemia are the presence of specific (molecular) genetic aberrations and early response to treatment. Similarly, AMKL is

caused by a heterogeneous group of mutations that stratifies this clinical entity into high-risk and standard-risk groups. Translocation t(1;22) (p13;q13), resulting in a chimeric fusion of RBM15 and MKL1, was thought for a long time to be the only recurrent aberration described in pediatric AMKL, occurring in around 10% of the patients, with Conflicting results about its prognosis as some studies reported, outcome to be poor and others favorable [15]. NUP98/KDM5A, CBFA2T3/GLIS2, KMT2A-rearranged lesions, and monosomy 7 (NCK-7) independently predict a poor outcome, and AMKL patients with these genetic alterations should receive intensified therapy. all those rearrangements that commonly seen in non-DSAMKL do not occur with DS-AMKL [15]. On the other hand, DS-AMKL, characterized by GATA1 mutations, has a good prognosis when treated with reduced-intensity chemotherapy. In addition, GATA1 mutations (GATA1mut) were identified in children with trisomy 21 mosaic [16]. GATA1 mutations occurred in 11% of children with AMKL without symptoms or evidence of trisomy 21 or trisomy 21 mosaics. GATA1 mutations are associated with a trisomy 21 within the leukemic blasts, and generally, the prognosis was significantly better compared to other AMKL [17]. The prediction of AMKL In pediatric non-Down syndrome AML has been reported to be associated with poor outcomes and may be an indication for allogeneic hematopoietic stem cell transplantation (HSCT) in first complete remission; however, risk group stratification and treatment protocols are not yet optimized for this subtype of pediatric AML [15]. The event-free survival rates for DS patients with AMkL range from 80% to 100%, compared with <30% for non-DS children with AMkL [10]. The 10-year overall survival estimate was 79% for DS-AMKL and 76% for non-DS-AMKL with a median follow-up of 78 months [18]. In the literature, few studies addressed the prognosis of AMKL in mosaic Down syndrome. In a study by Kudo K et al., their data revealed that patients with mosaic DS and AMKL have a good prognosis. Reduction in intensity may work in patients with mosaic DS as well as with AML-DS [14]. Another case report by Eric Won et al. treated a two years old child with mosaic DS with reduced-intensity chemotherapy for DS-AML with good tolerance to therapy with the achievement of remission status without evidence of treatment-related toxicity or relapse [7]. In conclusion, diagnosing AMKL in children with down mosaic syndrome is challenging, mainly when associated with the absence of the classic phenotypic DS features. It may be delayed until after starting full-intensity conventional protocols for treating acute myeloid leukemia in some patients, as in our case. Our case report highlights the importance of proper testing for mosaic Trisomy 21 in any child with AML and Trisomy 21-positive clones since low-level mosaicism may be unrecognized and underdiagnosed and not uncommonly, the Conventional cytogenetic technology for confirmation of the clinical diagnosis of Down syndrome provides only limited information concerning T21 mosaicism. Fortunately, clinical evidence showed that Pediatric patients with mosaic Trisomy 21 and AML might receive a reduced-intensity regimen with good outcomes and decreased treatment-related morbidity and mortality, similar to the patients of AMKL and Down syndrome.

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