VIA MEDICA

CASE REPORT

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Is it leukaemia? Haematological disorders in paediatric patients with Down syndrome — case report and literature review

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ABSTRACT

Introduction: Down syndrome (DS) is the most common chromosomal aberration. DS is characterized by a higher incidence of many disorders, including those involving the haematopoietic system. The risk of developing acute myeloid leukaemia is up to 150 times higher in this group. Also, characteristic is the presence of transient abnormal myelopoiesis (TAM), which can precede the development of malignancy. These phenomena are primarily associated with mutation of the GATA1 gene, cohesin complex and signalling pathways genes, as well as overactivity of foetal liver stromal cells and intensification of inflammation, stimulating the expansion of blastic cells.

Case report: A 15-month-old patient diagnosed with DS was admitted to the Clinic for diagnosis of neutropenia and thrombocytopenia. The myelogram showed no features of proliferative disease; however, TAM was suspected based on the bone marrow biopsy result. Six months later, based on the evaluation of the myelogram and immunophenotype of tumour cells, myeloid leukaemia (ML-DS) with megakaryoblastic differentiation was diagnosed. Genetic testing revealed a mutation in the GATA1 gene. The girl was qualified for treatment according to the AML-BFM 2019-ML-DS Protocol. The treatment was carried out as planned and the patient has achieved remission.

Conclusions: The pathogenesis of myelopoietic disorders in children with DS is mainly due to a cascade of mutations and genetic abnormalities. For this reason, DS patients must have appropriate molecular testing and regular haematologic follow-up to closely observe the evolution of myelopoietic disorders.

Keywords: leukaemia, Down syndrome,children, haematology, genetics

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Introduction

Down syndrome (DS) is the most frequent aneuploidy in the population, with an increasing prevalence. It is estimated to occur in up to 1 in 779 children [1]. This is caused by trisomy of the 21st chromosome. In addition to its well-defined phenotypic features, DS is also characterized by an associated increased incidence of other medical conditions, including oncological and haematological diseases [2]. It is suggested that this is related to chromosomal instability, resulting in intensified tumorigenesis. The most common haematologic malignancies accompanying DS are acute myeloid leukaemia (AML) (46–83 times more frequent than in the general population), including acute megakaryoblastic leukaemia (AMKL) (500 times more frequent) and acute lymphoblastic leukaemia (ALL) (10–20 times more frequent) [3–5]. Myeloid leukaemias occurring in patients with Down syndrome are called Myeloid Leukaemia of Down Syndrome (ML-DS) [3, 4]. A particular phenomenon in patients with DS is transient abnormal myelopoiesis (TAM), developing in 10% of children, which can transform into AML or even lead to death in the future [4]. Noteworthy is the fact that solid tumours of childhood (neuroblastoma, Wilms' tumour, brain tumours) occur far less frequently in DS patients than in

the general population, which is probably related to the triple replication of the *DSCR1* gene and its protective effect against increased angiogenesis, stimulating tumour growth [3]. The above data suggest the extremely important role of vigilant haematologic observation in DS patients from the first days of life.

The main aim of the following study is to present the transformation of TAM in ML-DS based on a case report and review of literature along with the molecular mechanism involved.

Case report

A 15-month-old child with Down syndrome, a congenital heart defect (type II atrial septal defect) and hypothyroidism was admitted to the Clinic in February 2023 for diagnosis of neutropenia and thrombocytopenia. There was a history of consultation by a paediatric haematologist at 11 months of age due to the presence of blasts in her peripheral blood. At admission, physical examination on admission showed phenotypic features of Down syndrome, an audible murmur over the heart (2/6 on the Levine scale) and a perceptible liver 3 cm below the costal arch, but otherwise no abnormalities. At that time, a bone marrow aspiration biopsy was performed twice. The results of these examinations exclude the formation of proliferative disease. Also, interestingly enough, the patient was not within the diagnostic age for TAM, which is 6 months. However, TAM was suspected due to the lack of leukaemic features. Moreover, specialists from two separate pathomorphological centres, including a reference centre for myeloid leukaemia, concluded that the bone marrow pattern was consistent with TAM. The patient was discharged home in good general condition and was under observation with suspected transient disruption of myelopoiesis, a component of TAM. In June 2023, the patient aged 19 months was hospitalized in another local hospital for oedema of the left periorbital region and pneumonia confirmed by imaging studies. During hospitalization, anaemia (haemoglobin 8.9 mg/dl), thrombocytopenia ($43 \times 10^3/\mu L$) and the presence of blasts in the peripheral blood smear (11.6%) were incidentally detected in laboratory tests, and the patient was referred to the Clinic for this reason. Table 1 shows particular results from laboratory tests at the time of admission. A wide range of additional tests were performed at the Clinic — bone marrow biopsy and genetic testing. The presence of 19.2% blasts was detected. In this examination, promyelocytes accounted for 0.8%, myelocytes for 1.6%, metamyelocytes for 1.2%, band cells for 1.6%, and segmented granulocytes for 14% of marrow cells. The immunophenotype of the tumour cells was positive for: CD4, CD7, CD13, CD33, CD36, CD38, CD41a, CD42b CD45, CD58, CD61, CD71, CD81 and CD117. Based on the assessment of the myelogram and immunophenotype of tumour cells, ML-DS with megakaryoblastic differentiation was diagnosed. Genetic testing identified a mutation in the *GATA1* gene (c.114dupT variant). BCR-ABL, RBM15-MKL1, ETV-RUNX1 translocations and KMT2A rearrangement were not found. The girl was qualified for treatment according to the AML-BFM 2019 — ML-DS Protocol. The administration of four blocks of chemotherapy was planned in the treatment: AIE (cytarabine, idarubicin, etoposide), AI (cytarabine, idarubicin), haM (cytarabine, mitoxantrone), HA (HD-cytarabine). The tolerance of the AIE block was quite good. The complications of treatment were as follows: deep bone marrow aplasia, pneumonia and grade I stomatitis. A myelogram from the 28th day after the start of the AIE Block showed 9.6% blasts. A large percentage of lymphocytes (62%), monocytes (9.6%) and neutrophils (14.8%) were also detected, but no megakaryocytes were found. Band cells accounted for 2.8%, segmented granulocytes — 14.8%, and metamyelocytes — 0.4% of marrow cells. On the 35th day after the start of the AIE Block, residual disease (MRD)

Table 1. Results of some laboratory tests of the patient on the day of admission to the hospital

Parameter	Result	Range of reference
Leukocytes [$10^3/\mu L$]	8.09	6–11
Erythrocytes [$10^6/\mu L$]	3.55	3.7–6
Haemoglobin [g/dL]	9.4	10.5–13.5
Platelets [$10^3/\mu L$]	103	140–410
Neutrophils [$10^3/\mu L$]	2.33	0.7–4.4
Lymphocytes [$10^3/\mu L$]	3.26	2.4–11
Monocytes [$10^3/\mu L$]	2.45	0.3–1.7
Eosinophiles [$10^3/\mu L$]	0.01	0–0.7
Basophiles [$10^3/\mu L$]	0.04	0–0.2
AST [U/L]	28	< 56
ALT [U/L]	20	< 39
CRP [mg/dL]	18.29	0–0.5
D-dimers [ng/mL]	2565	< 500
Creatinine [mg/dL]	0.21	0.24–0.4
Uric acid [mg/dL]	2	1.8–5

ALT — alanine aminotransferase, AST — aspartate aminotransferase, CRP — C-reactive protein, LDH — lactate dehydrogenase

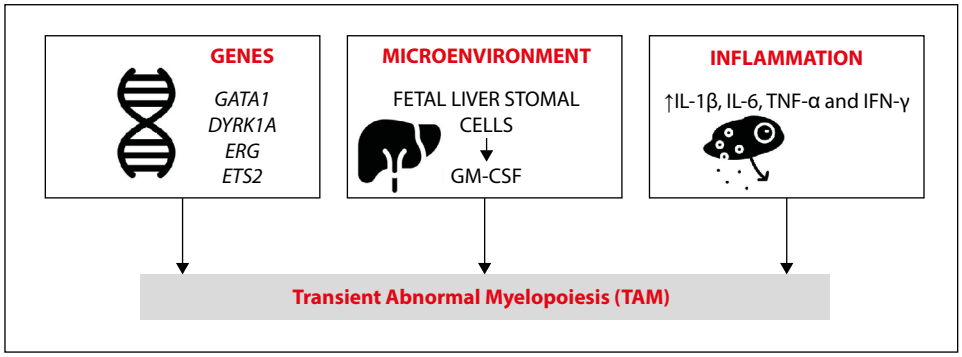

Figure 1. Presumed pathogenetic pathways of TAM [3, 8, 12]. Abbreviations: GM-CSF — Granulocyte-macrophage colony-stimulating factor, IFN-γ — interferon γ, IL-1β — interleukin 1β, IL-6 — interleukin 6, TGF-β — transforming growth factor β

was < 0.1% on cytometric assessment. Chemotherapy was continued as scheduled. Bone marrow examination before AI Block confirmed remission of the proliferative process (no blastic cells). The patient underwent AI Block with good tolerance. Treatment was complicated by the occurrence of a toxic-allergic rash, bone marrow aplasia and grade I stomatitis. Before hAM Block, a bone marrow examination was performed, and MRD was < 0.1% in cytometric evaluation. Tolerance to the treatment was satisfactory again, with only bone marrow aplasia. In December 2023, the patient received the next scheduled Block of chemotherapy — HA. Treatment was complicated by bone marrow aplasia. Currently, the patient is in remission of proliferative disease.

Discussion and literature review

DS is associated with haematological abnormalities from the first several days of life. This is due to the localization on chromosome 21 of many genes that regulate haematopoiesis, including *ERG*, *ETS2* and *RUNX1* [6]. In neonates with DS, peripheral blood counts often show elevated levels of haemoglobin, high mean corpuscular volume (MCV), erythroblastosis (34% of patients), high leucocytosis with neutrophilia (80% of patients) and thrombocytopenia (80% of patients) [6, 7]. In most cases, these conditions are mild and regress spontaneously within 1–2 months [4, 7].

Hematologic disorders in DS are a multistaged process. One of the first abnormalities found in the prenatal period or within the first few days of life is TAM, referred to by some as transient myeloproliferative disorder (TDM) [6, 8]. It affects about 10–30% of children with DS and is diagnosed within the first 2 months of life, usually in the first week [3, 9, 10]. The definition of TAM includes diagnosis within the first 6 months of life. The patient discussed above was older, but a previously undiagnosed disorder was assumed, which also did not fit the diagnosis of leukaemia. He was initially assumed to have developed so-called "silent" TAM, which occurs in 20% of children [3, 10]. Such diagnostic uncertainties occurred for the first time in the Clinic's history. It is probable that in all cases of TAM, a mutation is present in the *GATA1* gene, whose products determine the normal maturation of erythrocytes and thrombocytes. Its mutation results in pathological proliferation of erythroblasts and megakaryoblasts, determining the AMKL development pathway [10, 11]. In people without trisomy 21, a mutation in this gene is responsible for the development of anaemia and neutropenia, not TAM or ML-DS [12]. It has been shown that the presence of a *GATA1* mutation alone cannot induce TAM [3]. In addition to it, an extra copy of the *ETS*, *ERG* and *DYRK1A* genes (21q22.2), promotion of blast proliferation by foetal liver stromal cells, and inflammation (expressed by increased levels of the cytokines: transforming growth factor β [TGF-β], interferon γ [IFN-γ] and the interleukins IL-1β and IL-6) play an important role in the pathogenesis of this disorder [3, 8, 12]. The putative pathogenesis of TAM is shown in Figure 1.

Interestingly, in the WHO definition of TAM, it is necessary to find an increased number of blasts, although the minimum number of blasts required to make the diagnosis is not precisely stated [10]. According to Bhatnagar et al. at a threshold of > 10% of blasts, a typical clinical picture of TAM is observed [13]. In the milder type of TAM, no symptoms are noticed. The most common clinical sign in the course of TAM is hepatosplenomegaly. Furthermore, in the more severe course, body cavity effusions, multi-organ failure (mainly liver) and disseminated intravascular coagulation (DIC) are observed. Moreover, in 10–20% of TAM cases, there is hydrops fetalis, pleural effusions and irreversible liver fibrosis in the prenatal stage, often leading to stillbirth [3]. The majority of patients with TAM (up to 80%) have

Table 2. Factors suggesting the necessity of introduction of therapy for TAM [1, 3, 8, 9]

- Preterm birth
- Hyperleukocytosis (> 100 × $10^3/\mu$L) (according to Berlin-Frankfurt-Münster group > 50 × $10^3/\mu$L)
- Hepatosplenomegaly, ascites
- Hydrops fetalis
- Pleural or pericardial effusion
- Kidney and/or heart and/or liver failure, not due to congenital defects
- Symptomatic coagulopathy with bleeding

spontaneous resolution of the disease without the necessity of introducing therapy, within 2–194 days, with an average of 58 days [3, 7]. The implementation of treatment is indicated in the cases described in Table 2. The most effective treatment regimen is the use of low-dose cytosine arabinoside (0.5–1.5 mg/kg of body weight) for 3–12 days [3, 9].

About 10–20% of TAM cases result in death, and 20–30% of patients progress to ML-DS within 4–5 years after birth [3, 8]. Up to now, no risk factors for such transformation have been identified, except for the presence of *GATA1* mutations. However, it is known that treatment with cytosine arabinoside does not reduce the number of cases of transformation to leukaemia [3]. After trisomy 21 and the *GATA1* mutation, involved in the pathogenesis of TAM, the next step in the development of ML-DS is additional mutations, usually two to five in total, mainly of genes encoding the cohesin complex and JAK pathway kinases [3, 8, 14]. Mutations of genes encoding the cohesin complex affect up to half of ML-DS patients. This is mainly referring to *STAG2*, *RAD21*, *SMC1*, *SMC3*, and *NIPBL* genes. The coexistence of *STAG2* and *GATA1* mutations increases the number of megakaryocytes, by enhancing cell proliferation and impairing cell differentiation, further conditioning the immunophenotype of tumour cells [15]. *SMC3* mutation enhances the abovementioned mechanism [16]. Mutations of *NIPBL*, through the Wnt signalling pathway, and *RAD21*, by impairing *RUNX1* expression, impair haematopoiesis and induce leukaemia development [14]. One in five ML-DS patients also has a mutation of the *CTCF* gene, whose product is a tumour suppressor, influencing RNA splicing, chromatin organization and myelopoiesis. Abnormal expression of this gene downregulates *MYC* and is responsible for modifying DNA methylation patterns, thus being an inducer of haematopoiesis disruption [14]. Nearly half of patients (48%) with ML-DS are found to have mutations in signalling pathways. The most common is mutation of *JAK3* (13.5%) and *JAK2* (9.9%) genes. Interestingly, gain-of-function mutations of the JAK-STAT pathway are found only in patients with ML-DS and not TAM, indicating a role of this pathway in the transformation to leukaemia [17]. Megakaryopoiesis is stimulated by thrombopoietin-mediated STAT5 activation through the MPL receptor. *MPL* mutation is also found in ML-DS [14]. In 14% of patients, mutations of the RAS pathway genes are found, particularly *NRAS* and *KRAS*, whose mutation conditions uncontrolled cell proliferation and increased cell survival [14, 17]. The *EZH2* and *SUZ12* genes, which, as components of PRC2, naturally act as tumour suppressors by regulating chromatin binding, have also been attributed a role in the pathogenesis of ML-DS. However, when they are mutated, megakaryocyte proliferation is stimulated, and their differentiation is inhibited [14]. Moreover, aberrations of chromosomes other than 21 are also found in patients with ML-DS [14]. Their role in the pathogenesis of this particular type of leukaemia has not been demonstrated to date, but it has been observed that trisomy of chromosome 8 is associated with a higher risk of relapse (71% of patients with relapse) and a worse prognosis (73% 5-year event-free survival [EFS] in patients with trisomy vs. 91% without it) [18]. The progression pathway of ML-DS along with the mutations involved is shown in Figure 2.

Leukaemias in about 60% of patients are myeloid and among them, the majority is AMKL [5, 7]. Overall, the incidence of AMKL in patients with DS is estimated to be about 1% [10]. The median age of onset for ML-DS is 2 years, and for ALL is 4 years [7]. ML-DS has a lower leukocyte count and higher blast count than AML without DS, while ALL in DS (DS-ALL) has higher haemoglobin levels than ALL in the general population [6, 7]. The immunophenotype of blasts in AMKL, corresponding to those in TAM, is positive for stem cell (CD34, CD117), myeloid (CD13, CD33), and megakaryocytic lineage (CD41, CD61) markers [3]. Interestingly, more than half of DS-ALL cases are associated with the presence of mutations in the *CRLF2* gene, while there is a lower incidence of chromosomal rearrangements (including prognostically favourable ones — *ETV6-RUNX1*, hyperdiploidies) and greater toxicity of standard induction chemotherapy compared to ALL without DS. This is reflected in a worse prognosis for DS patients (56% 10-year EFS for DS-ALL vs. 74% for ALL, and 55% 10-year disease-free survival [DFS] for DS-ALL and 73% for ALL) [3, 8]. Furthermore, relapses and treatment-related mortality (TRM) are more common in DS-ALL [3]. ML-DS has a much better prognosis than "typical" AML (90% 5-year EFS for ML-DS patients vs. 49–62% for

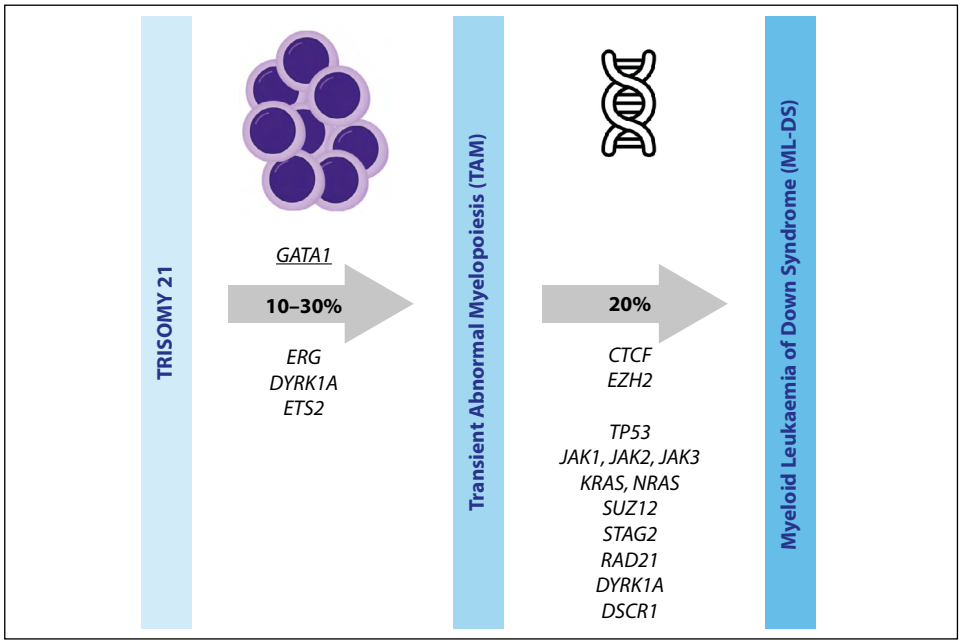

Figure 2. ML-DS progression pathway [1, 3, 7–9, 14]

AML) [3, 19]. Favourable prognostic factors for ML-DS include only younger age of onset (86% 6-year EFS for diagnosis at < 2 years vs. 64% for age > 2 years) [7]. Adverse prognostic factors include the aforementioned chromosome 8 trisomy and unsatisfactory early response to treatment (58% 5-year EFS for unsatisfactory response vs. 88% for good response) [18]. A reduction in the intensity of chemotherapy is necessary for ML-DS due to the higher incidence of infections, oral mucositis and TRM [8]. Chemotherapy (especially using cytarabine and etoposide) is the best treatment for ML-DS, and haematopoietic cell transplantation is of limited use here [8]. The course, prognosis and treatment of DS-ALL and ML-DS are therefore different compared to their "typical" equivalents in the non-DS population.

Conclusions

The DS course has a lower risk of childhood solid tumours, but up to several hundred times higher risk of haematologic malignancies. Therefore, vigilant haematologic observation of children with DS is necessary, involving performing complete blood counts from the first days of life and verifying all conditions of concern, especially nonspecific symptoms not susceptible to routine treatment. Even an initially innocent-looking TAM-like disorder can eventually turn out to be a developing leukaemia that requires treatment.

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