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Case Report

A novel cryptic *CBFB-MYH11* gene fusion present at birth leading to acute myeloid leukemia and allowing molecular monitoring for minimal residual disease

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ABSTRACT

Acute myeloid leukemia (AML) with the inv(16)/t(16;16) karyotype is associated with a favourable prognosis, showing longer periods of complete remission and high overall survival rates. Here we report a four year old girl, who presented with pallor, a history of viral infections and pancytopenia, an abnormal karyotype, but initially no signs of leukemia. After one month, molecular diagnostics revealed a rare *CBFB/MYH11* fusion variant transcript type S/I, leading to the diagnosis of CBF AML. Additional FISH confirmed the presence of a cryptic *CBFB/MYH11* fusion. We developed a nested PCR test for the *CBFB/MYH11* fusion gene transcript S/I to monitor this patient for minimal residual disease. Eleven months after complete remission this transcript was still absent in peripheral blood samples.

Because at presentation this girl had no clinical signs of leukemia, but showed an abnormal karyotype with a cryptic *CBFB-MYH11*-fusion, we investigated whether this fusion was already present at birth. Therefore, the DNA fusion junction was cloned from diagnostic DNA and the patient-specific sequence was used to investigate the neonatal blood spot. Remarkably, the type S/I transcript of *CBFB/MYH11* was present in the neonatal blood spot, most likely being the first hit in leukemogenesis.

1. Introduction

The pericentric inversion of chromosome 16 inv(16)(p13.1q22) or a balanced translocation t(16;16)(p13.1;q22) is seen in about 4% of patients with a de novo acute myeloid leukemia (AML), with the inversion being much more common (95%) than the translocation (5%). Both cytogenetic abnormalities result in a fusion gene between *CBFB* (core binding factor beta subunit) at 16q22 and *MYH11* (smooth muscle myosin heavy chain 11) on 16p13.1, leading to a chimeric CBFB/MYH11 protein. AML patients with an inv(16)/t(16;16) have been reported in all age groups, but most patients are relatively young; the median age is roughly 35 years [1].

The bone marrow of these AML patients usually shows monocytic and granulocytic differentiation and a variable number of eosinophils at all stages of maturation, without significant maturation arrest [1,2]. This AML subgroup was denoted by the French-American-British (FAB) classification as AML M4eo, the World Health Organization (WHO) 2016 has classified this subtype as "AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22);*CBFB-MYH11*" [3]. Patients harbouring an inv(16)/t(16;16) have a favourable prognosis, showing long periods of complete remission and high overall survival rates up to 70% [4].

At the molecular level, the fusion gene is formed by a 5′ sequence from *CBFB* at 16q22 with a 3′ sequence from *MYH11* at 16p13. There

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are more than 10 possible *CBFB-MYH11* fusion transcript variants identified, of which 85% of patients show the transcript type A [5,6]. The other, more rare, fusions show a more atypical cytomorphology, mostly with absence of the pathologic eosinophils, and therefore not recognized as FAB subtype M4eo. Due to the limited number of cases with a rare *CBFB-MYH11* fusion type, the biological and prognostic implications are still unclear [7].

In this study, we report on a girl who presented with pancytopenia, and showed to have an abnormal karyotype with a cytogenetic cryptic *CBFB-MYH11* fusion in her bone marrow, and therefore, diagnosed as AML. The fusion transcript appeared to be a rare variant type I, also known as type S/I [8]. We developed a patient specific marker for minimal residual disease (MRD) monitoring. Finally, by retrospective screening of the Guthrie card blood spot we showed that the *CBFB-MYH11* fusion transcript type S/I was already present at birth.

2. Case report

A four year old girl presented with pallor, a history of viral infections and pancytopenia, with a leukocyte count of 2.8×10^9/l and platelets 126×10^9/l and hemoglobin of 3.3 mmol/l. The peripheral blood smear revealed pancytopenia with some atypical lymphocytes. Paroxysmal nocturnal hemoglobinuria (PNH) and Fanconi's anemia were excluded. A bone marrow aspirate demonstrated mild dysplasia and megakaryocytosis, not indicative for a diagnosis of myelodysplasia nor acute leukemia (Fig. 1A). Immunophenotyping showed no increase of blasts. Based on these results a differential diagnosis was made including several infectious causes. However, serology could not confirm any of them (CMV, EBV, hepatitis, HIV, HSV, Parvo B19, toxoplasmosis and varicella zoster).

After a month her blood values had normalized (Hb 7.6 mmol/l, platelets 195×10^9/l, leukocytes 4.5×10^9/l). However, morphological examination of peripheral blood showed 12% Sudan Black positive blasts. Immunophenotyping of the peripheral blood showed the presence of 9% myeloblasts (CD13+, CD34+, CD117+, MPO+ and HLA-DR+, CD33−, and TdT weakly positive). The bone marrow aspirate showed 16% blasts and dysplasia, leading to the preliminary diagnosis of Refractory anemia with excess of blasts (RAEB) (Fig. 1B).

2.1. Cytogenetic studies

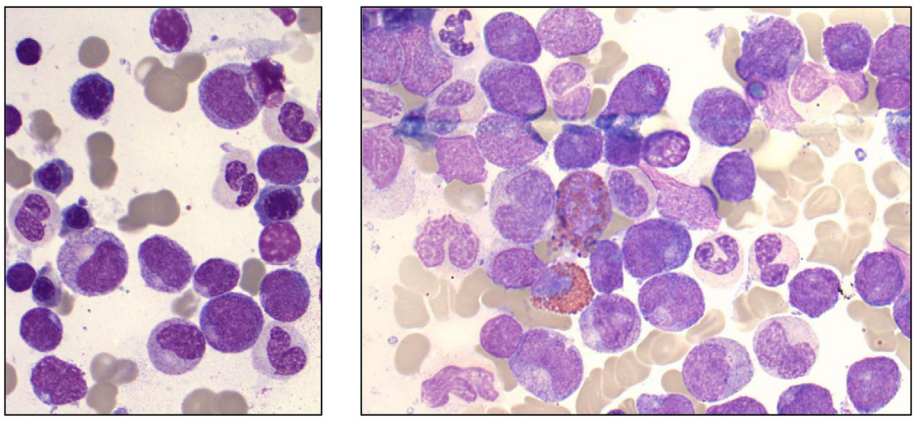
For karyotyping the patients' bone marrow aspirate were set up in two 24 h RPMI 1640 cultures, one unstimulated and one stimulated with G-CSF, IL3 and GM-CSF. After standard cytogenetic harvest and Giemsa-Trypsin-Giemsa banding 20 metaphase cells were analysed from both cultures. At presentation, the cytogenetic analysis showed in 6 of 20 cells an abnormal female karyotype with one aberrant chromosome 15 and two aberrant chromosomes 16 (Fig. 2A). Fluorescence in situ hybridisation (FISH) studies were performed according to the manufacturer's instructions in combination with our established laboratory protocol, using whole chromosome paints for chromosomes 15 and 16 (WC15 and WC16; Kreatech, Amsterdam, The Netherlands), and Telvysion probes 15q, 16p and 16q for the telomeres of chromosomes 15q, 16p and 16q, respectively (Molecular IL, Hoofddorp, The Netherlands). The aberrant chromosome 15 contained chromosome 16 material, including 16qter; one aberrant chromosome 16 had two 16pter signals, the other aberrant chromosome 16 contained 15q material. The karyotype was described according to ISCN 2016 [9] as: 46,XX,der(15)t(15;16)(?q22;?q24),?i(16)(p10),der(16)?i(16)(q10)t(15;16)(q?22;q?24)[6] /46,XX[14].

Since the meaning of this abnormal karyotype was unclear, we decided to repeat the investigations after one month. Cytogenetics revealed the same aberrant chromosomes 15 and 16 in 18 of 20 analysed cells. But now, also molecular studies were performed (see below) and showed a *CBFB-MYH11* fusion. Therefore, we performed additional FISH with LSI CBFB dual color break-apart rearrangement DNA probe (Abbott Molecular IL, Hoofddorp, The Netherlands) and CBFB MYH11 dual fusion translocation DNA probe (Cytocell Inc. Cambridge, UK). FISH demonstrated a CBFB-MYH11 fusion signal on the short arm of one aberrant chromosome 16, a CBFB signal on the derivative chromosome 15, suggesting a break within the CBFB-gene, and on the other chromosome 16, a CBFB signal on one chromosome arm and an amplified MYH11 signal on the other chromosome arm (Fig. 2B). The karyotype was described as: 46,XX,der(15)(15pter➔15q2?2::16q22➔16qter), der(16)(16pter➔16p13::16p13➔16q22::16p13➔16pter),der(16)(15qter➔15q2?2::16p13➔16qter).ish der(15)(3′CBFB+),der(16)(p13)(5′CBFB+,MYH11+) (q22)(MYH11++,3′CBFB-) ,der(16)(p13)(MYH11+)(q22)(CBFB+).

2.2. Molecular studies

Reverse transcriptase-polymerase chain reaction (RT-PCR) was performed using the *CBFB* and *MYH11* primer pairs as described [10]. One month after initial presentation RT-PCR revealed an amplification product of approximately 300 bp between exon 4 of *CBFB* and exon 35 of *MYH11*, the rare fusion transcript type S/I [6,8,10]. Other molecular tests showed no *NPM1*, *FLT3*, *JAK2*, *EVI1* and *KIT* mutations. There was no DNA available from the sample at presentation.

To confirm the presence of the rare inversion 16 transcript type S/I 10 ng diagnostic bone marrow DNA was subjected to long-distance PCR using



A B

Fig. 1. Representative images of the bone marrow of the patient. A). Bone marrow aspirate at first presentation: no excess of blasts. B). Bone marrow aspirate after one month: more blasts and also abnormal eosinophils with large basophilic granules (in the middle), typical for inversion 16.