CASE REPORT

Tetraploidy acute myeloid leukaemia after chromosome 16 inversion

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SUMMARY

Our patient is a 36-year-old man referred by his general physician to the Department of Hematology because of mild neutropenia in a routine analysis at work. There was no history of previous diseases, and examination was normal. Blood investigations confirmed the neutropenia and showed elongation of prothrombin time. A bone marrow examination was performed revealing about 10% of myeloblasts on the aspirate smears. A cytogenetic study showed chromosome 16 inversion in all of these cells and tetraploidy only in some of them, which were extremely large in size. According to the revised WHO classification of tumours (2008), the patient was diagnosed as a case of acute myeloid leukaemia with chromosome 16 inversion.

BACKGROUND

Acute myeloid leukaemia (AML) is a highly heterogeneous disease. Many cytogenetic and molecular abnormalities have been described in recent years with an important prognostic significance, and they are a strong predictor of outcome in this disease.^{1 2} However, the implications of many abnormalities remain unknown. Chromosome 16 inversion [inv(16)(p13.1q22)] is a recurrent genetic abnormality in AML with a favourable prognosis that is identified in 5–8% of patients, and it is usually the primary mutational event in AML that is associated with many secondary cytogenetic abnormalities.³ However, tetraploidy or near-tetraploidy which is well described in other malignancies is extremely rare in AML. In the few reported cases, this abnormality has been associated with genetic instability and therefore, it confers very poor outcome even if it is not the trigger mutation of the disease. That is the reason why it is important to consider the trigger mutation and the sequence of mutations in order to establish correctly the prognosis and the most appropriate treatment.

CASE PRESENTATION

A 36-year-old man, completely asymptomatic, was referred to the Department of Hematology by his general physician. His routine analysis at work revealed leucopenia with neutropenia (white cell count $3.85 \times 10^9/L$, neutrophils $1.21 \times 10^9/L$, haemoglobin 148 g/L, thrombocytes $195 \times 10^9/L$). Further investigations confirmed neutropenia and showed 1% blasts on the blood smear and elongation of prothrombin time. A bone marrow aspirate

revealed 10% myeloblasts, some of them large in size with a small cytoplasm. Their nuclei were large and bizarre in shape (figure 1). Immunophenotypic analysis of bone marrow cells also revealed 10% of blasts with monocytic and granulocytic differentiation. The cytogenetic study on bone marrow cells using G-banding showed a mosaicism in which 20 of 53 metaphases analysed were tetraploidy (92, XXYY) (figure 2). Pericentric inversion of chromosome 16 was apparently observed in the tetraploid clone. Interphase fluorescence in situ hybridisation (FISH) was performed using a break apart 16q22 probe (Kreatech KBI-10304) which detects the inversion of chromosome 16 involving CBFB gene region at 16q22 in a dual colour (red at distal CBFB and green at proximal CBFB) split assay. The patient's FISH analysis showed three clones: 25% of the cells revealed two colocalised red/green signals corresponding to the normal diploid clone, 37% of the cells with one colocalised red/green signal and one splitted signal on chromosome 16 corresponding to diploid cells with inv(16) and another clone with two colocalised red/green signals and two splitted signals, corresponding to the tetraploid clone, always with inversion of the chromosomes 16 (figure 3). The fusion gene CBFB (16q22) and MYH11 (16p13) (type D transcript) created by the inversion of chromosome 16 was also confirmed by molecular studies of RNA. On the basis of the revised WHO classification of tumours (2008), a diagnosis of AML with inv(16) (p13.1q22) was made.

Figure 1 Microphotograph at $\times 100$ magnification with Giemsa staining showing a tetraploidy cell from the bone marrow aspirate near a neutrophil showing the big size and bizarre shape of the blast cell.

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Figure 2 Cytogenetic findings that show G-banded karyotype: 92,XXYY,inv16(p13;q22).

Figure 3 FISH findings. (A) Normal diploid cells (2n). (B) Diploid cell (2n) showing the inversion of chromosome 16 involving CBFB gene region at 16q22 in a dual colour (red at distal CBFB and green at proximal CBFB) split assay. (C) Tetraploidy cell (4n) with chromosome 16 inversion.

TREATMENT

The patient was started on induction chemotherapy: 3 days of intravenous anthracycline (idarubicin 12 mg/m²) and 7 days of continuous-infusion cytarabine (200 mg/m²) ('3+7' induction) with no major complications. After recovery of counts, the patient did not achieve a complete morphological remission assessed by a repeat bone marrow examination. The inv(16) was found in 4% of metaphases with complete disappearance of tetraploidy. Then, the patient received second induction chemotherapy again with 3 days of intravenous anthracycline (idarubicin 12 mg/m²) and 7 days of continuous-infusion cytarabine (200 mg/m²). At the end of that induction phase, the patient achieved complete morphological and molecular remission without any cytogenetic abnormalities in the bone marrow studies.

OUTCOME AND FOLLOW-UP

One month after recovery of counts, bone marrow transplantation from an identical non-related donor was performed as

consolidation therapy. The decision was not easy because AML with chromosome 16 inversion is supposed to have a favourable prognosis and the patient did not have a sibling donor. However, according to the available literature, tetraploidy usually confers very poor outcome even if it is not the trigger mutation. Therefore, the transplant was considered to be the best option for our patient. However, he developed some complications such as a respiratory infection that required intensive care for 5 days with non-invasive ventilation, veno-occlusive hepatic disease treated with defibrotide for 4 weeks and cutaneous graft-versus-host disease solved by local corticosteroids therapy. The patient was discharged and is on regular follow-up without major complications. At the time of this report, bone marrow studies show complete remission after 8 months post-transplant with no evidence of disease assessed by molecular and genetic studies.

DISCUSSION

AML is a form of cancer with an enormous molecular and cytogenetic heterogeneity. This disease is characterised by infiltration of clonal cells from the hematopoietic system. In the last 20 years, studies have shown that many genes are recurrently mutated in AML such as (inv(16)(p13q22) or t(16;16)(p13;q11), CBFβ/MYH11X), t(8;21)(q22;q22), AML1(CBFα)/ETO, t(15;17)(q22;q11-12) and variants, PML/RARα.¹⁻³ Moreover, according to the WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, updated in 2016, new entities have been included such as AML with CEBPA mutation, RUNX1 mutation or BCR-ABL1 gene fusion and many others that are currently on studying.

It is also clear that each AML is genetically unique because of a different mutational composition. Multiple mutations appear in AML even creating different subclones,^{1 2} so it makes it very difficult to establish the individual prognosis for each patient. In our case report, we could differentiate three different cell clones: one of them corresponding to the normal diploid cells, another diploid cell clone with inv(16) and the last one with inv(16) and complete tetraploidy, so that the 100% of tetraploid cells also have the inv(16). Therefore,

the sequence of genetic abnormalities seems to begin with diploid cells with inv(16), followed by the tetraploidisation of some of them.

It is well described in the literature that inv(16) or t(16;16) is associated with secondary genetic abnormalities. In a study of the German–Austrian AML Study Group (AMLSG),⁴ they evaluate secondary genetic lesion of 176 patients with AML and they report that more than 90% of the patients with inv(16)/t(16;16) AML harbour secondary genetic abnormalities, and some of them are important prognosis factors such as trisomy 8 and 22, KIT and FLT3 mutations with poor outcomes. For the best of our knowledge, inv(16)/t(16;16) has not been described to date in relation to complete tetraploidy or near tetraploidy, that otherwise, is unusual in AML.

According to the literature, near tetraploidy, tetraploidy and massive hyperdiploidy are usually categorised as having an unfavourable prognosis, since they are associated with chromosomal instability and the presence of additional chromosomal abnormalities. Some studies have suggested that polyploidy affects cellular metabolic rates and might be a physiological response to metabolic stress.⁵ Tetraploidy can arise by different mechanisms: cell fusion, mitotic slippage or a failure to undergo cytokinesis. Moreover, it must be differentiated between hyperdiploidy, defined as more than 50 chromosomes; near-tetraploidy, defined as 80–104 chromosomes; and complete tetraploidy, defined as complete duplication of all chromosomes (4n).⁶ Despite these entities could result from different pathophysiological events, in general, are classified in the same unfavourable group.

The largest series of patients have been reported in a retrospective study by Béné *et al*⁷ which includes 25 patients with near-tetraploid AML. Most of them had undifferentiated morphology, and they were secondary to myelodysplastic syndrome or therapy-related AML. Therefore, the outcome of these patients was poor after standard chemotherapy. However, none of them had a recurrent abnormality in AML such as our patient.

Pang *et al*⁸ described the characteristics of 13 cases of near-tetraploidy and tetraploidy. In their revision, they concluded that most of the cases were associated with myelodysplasia and they also had large and bizarre blasts on the bone marrow studies. They observed a better prognosis than was expected for a complex karyotype AML with a median overall survival of 8.6 months, which is the same follow-up as our patient after transplant.

However, in general, AML with tetraploidy or near-tetraploidy is associated with a low-remission rate and short survival period. In a reported case by Wan *et al*⁹ with near-tetraploid AML the remission duration with standard chemotherapy was only 4 weeks.

After the revision of the available literature, it looks clear that tetraploidy has poor outcome and it is associated with chromosomal instability. In our case report, we were able to make an early diagnosis and started promptly on treatment. Moreover, the patient was a young man with good underlying conditions so possibly these issues are an important point in the long survival of our patient. In the literature, there are also other near-tetraploidy or tetraploidy AML reported cases with unusually long survival.¹⁰ In this case report for example, the authors highlight the absence of dysplasia at diagnosis as the main reason of a better overall survival.

Therefore, in the approach to an AML patient many aspects must be considered. Genetic and molecular characteristics are essential, and studies of minimal residual disease must be performed as well in order to establish an accurate prognosis. Moreover, we would like to highlight the importance of an early diagnosis to avoid the increase in genetic instability and mutational events in AML. However, many features in this disease remain unknown so new studies are necessary to improve our knowledge in this heterogeneous and complex disease to improve the overall survival of our patients.

Learning points

- ▶ Acute myeloid leukaemia (AML) is a disease with an enormous molecular and cytogenetic heterogeneity. It is important to study the clinical impact of each genetic abnormality in the approach to an AML patient.
- ▶ Multidisciplinary integration of histopathologic, cytogenetic, molecular biology, flow cytometry and clinical features is critical to make an adequate diagnosis in AML, and studies of minimal residual disease must be performed for an accurate prognosis.
- ▶ Tetraploidy and near-tetraploidy are rare genetic abnormalities in AML, but they confer a very poor prognosis so an early diagnosis and aggressive treatment are necessary to improve the overall survival of patients.
- ▶ We must individualise each AML case and consider the underlying conditions of each patient to offer the most appropriate treatment.

Contributors ADB and MTD performed the laboratory analyses; JS-C made interesting suggestions and ASV wrote the manuscript.

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Patient consent Obtained.

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REFERENCES

- 1 Longo DL, Döhner H, Weisdorf DJ, *et al*. Acute myeloid leukemia. *N Engl J Med* 2015;373:1136–52.
- 2 Grimwade D, Ivey A, Huntly BJP. Molecular landscape of acute myeloid leukemia in younger adults and its clinical relevance. *Blood* 2016;127:29–41.
- 3 Swerdlow SH, Campo E, Harris NL, *et al*. *WHO classification of tumours of haematopoietic and lymphoid tissue*. Geneva, Switzerland: WHO Press, 2008.
- 4 Paschka P, Du J, Schlenk RF, *et al*. Secondary genetic lesions in acute myeloid leukemia with inv (16) or t (16;16): a study of the German-Austrian AML Study Group (AMLSG). *Blood* 2013;121:170–8.
- 5 Storchova Z, Kuffer C. The consequences of tetraploidy and aneuploidy. *J Cell Sci* 2008;121:3859–66.
- 6 Iyer RV, Sait SN, Matsui SI, *et al*. Massive hyperdiploidy and tetraploidy in acute myelocytic leukemia and myelodysplastic syndrome. *Cancer Genet Cytogenet* 2004;148:29–34.
- 7 Béné MC, Castoldi G, Derolf A, *et al*. Near-tetraploid acute myeloid leukemias: an EGIL retrospective study of 25 cases. *Leukemia* 2006;20:725–8.
- 8 Pang CS, Pettenati MJ, Pardee TS. Clinicopathological analysis of near-tetraploidy/tetraploidy acute myeloid leukaemia. *J Clin Pathol* 2015;68:236–40.
- 9 Wan TS, Ma ES, Chen YT. Near-tetraploid acute myeloid leukaemia. *Br J Haematol* 2011;155:285.
- 10 Lemež P, Klamová H, Zemanová Z, *et al*. Unusually long survival of a 67-year-old patient with near-tetraploid acute myeloid leukemia m0 without erythroblastic and megakaryocytic dysplasia. *Acta haematologica* 2011;126:129–34.

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