

IDENTIFICATION AND TREATMENT OF LATE ONSET FANCONI'S ANEMIA

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ABSTRACT

We diagnosed Fanconi's anemia (FA) in a 34-year-old lady, daughter of consanguineous parents, from a small Southern Italian town. The patient was pancytopenic when she was 31, and was found to be aplastic at the age of 34. Spontaneous chromosomal break were not evident in peripheral blood lymphocyte cultures but the diepoxybutane (DEB) test, carried out during the aplastic phase, was clearly positive. Danazol treatment significantly improved her hematological condition, yielding a Hb peak value of 13.4 g/dL. Four years later moderate pancytopenia has reoccurred. This case demonstrates that even adult pancytopenic patients may have FA and that a test detecting chromosomal hypersensitivity to cross-linking agents is the only key for a correct diagnosis, which in turn is essential to avoid improper treatment.

Key words: chromosomal instability, danazol, diepoxybutane, Fanconi's anemia, pancytopenia

Fanconi's Anemia (FA) is a rare genetic syndrome first described in 1927 by G. Fanconi,¹ characterized by pancytopenia, congenital malformations, chromosomal instability and proneness to leukemia. In 1947 Estren and Dameshek described an FA variant without malformations in childhood.² Adult patients are very rare and show pancytopenia as the only sign of FA; in these cases chromosome hypersensitivity to clastogenic agents such as diepoxybutane (DEB test) is the only reliable diagnostic tool.³ While the mode of inheritance is consistently monogenic autosomic recessive, the genetic defect is not always the same. Genetic heterogeneity was proven by cell fusion experiments among FA cell lines which demonstrated the existence of at least 4 complementation groups. Current prevalence estimates are from 1 in 22,500 to 1 in 350,000 in different countries. In Italy the reported incidence is 1 in 20,000 to 1 in 30,000, but this may be underestimated⁴ because diagnosis can be missed in cases not showing malformations, especially

those with late onset. We report the case of a woman in whom FA was diagnosed at the age of 34.

Case report and Results

A 37-year-old woman, the daughter of consanguineous parents (cousins) born in a small village near Benevento, a Southern Italian town, first came to our observation at the age of 31 (June 1989). The patient had had recurrent miscarriages since the age of 25; in one instance she had been transfused. At age 28 she was found to be moderately pancytopenic (Hb 11.8 g/dL; MCV 110 fL; WBC $3.8 \times 10^9/L$; neutrophil count $1.2 \times 10^9/L$; PLT count $55 \times 10^9/L$) with a hypoplastic marrow. The karyotype from peripheral blood lymphocytes was reported to be normal. Physical examination was negative. Blood counts showed Hb 11 g/dL, WBC $3.0 \times 10^9/L$; neutrophil count $0.7 \times 10^9/L$, PLT count $45 \times 10^9/L$. Bone marrow histology disclosed cellularity decreased to 20%. The Ham

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test was negative. Anti-erythrocyte (anti-Kell) and platelet-associated antibodies were present in the serum, probably related to previous transfusions.

Colony assay was carried out as previously described.⁵ Briefly, peripheral blood mononuclear cells (PBMNC), isolated by Ficoll-Hypaque (Flow Laboratories) density gradient centrifugation, were plated at a concentration of $5 \times 10^5/\text{mL}$ in methylcellulose with erythropoietin 3U/mL, phytohemagglutinin leukocyte conditioned medium 10% and pancytopenic plasma 20%. Erythroid (BFU-E), myeloid (CFU-GM) and mixed (CFU-GEMM) colonies were scored by inverted microscope after incubation for 14 days in 5% CO_2 at 37°C. Each experiment was carried out in duplicate. *In vitro* cultures by methylcellulose assay showed reduced growth of all circulating hematopoietic progenitors: BFU-E 1 (n.v. 32-54); CFU-GM 6 (n.v. 50-120); CFU-GEMM 0 (n.v. 10-30). HLA typing revealed no matched donor among her siblings. A diagnosis of non severe aplastic anemia was formulated and the patient was left untreated until March 1990, when symptomatic thrombocytopenia ($25 \times 10^9/\text{L}$) occurred, leading to a course of immunosuppression with antilymphocyte globulin (horse ALG, Merieux, 1.5 vial/10 kg/day for 5 days). This was followed by a worsening of the hematological condition that required regular erythrocyte and platelet transfusion support. In August 1990, danazol (Danatrol, Winthrop) at a dose of 400 mg/d was started; a progressive increase of Hb, WBC and platelets followed. A single cutaneous *café-au-lait* patch was seen on the right ankle.

Tests for chromosome instability were performed on peripheral blood lymphocytes. Heparinized blood samples were incubated for 72 hours at 37°C in RPMI 1640 medium supplemented with phytohemagglutinin, glutamin and penicillin-streptomycin. Cultures were set up in duplicate to study both spontaneous and DEB-induced chromosome instability. For the latter purpose, freshly diluted DEB (final concentration 0.1 $\mu\text{g}/\text{mL}$) was added 24 hours after culture initiation, thus exposing the cells to the clastogenic agent for 48 hours. Cultures were harvested and slides prepared following stan-

dard methods. Each metaphase was scored by two cytogeneticists for numerical and structural chromosome abnormalities, including any kind of rearrangement or break except for gaps (achromatic areas less than a chromatid in width). The results were expressed as: a) percentage of mitoses with one or more chromosome or chromatid breaks (aberrant cells); b) mean number of breaks per cell; c) mean of number breaks per aberrant cell. Tests for chromosome instability on peripheral blood lymphocytes showed a non significant rate of spontaneous breakages (5% of mitoses showing one or two chromatid breaks), but positivity for the DEB test was undoubtful (46% aberrant cells; 1.48 breaks per cell; 3.20 breaks per aberrant cell; 13% cells with rearranged typical figures)(Figure 1).

A diagnosis of Fanconi's Anemia was made and the patient continued danazol treatment at the same dose without suffering side effects. Peak values were reached in March 1991 (Hb 13.4 g/dL; WBC $4.2 \times 10^9/\text{L}$; neutrophil count $1.6 \times 10^9/\text{L}$; PLT count $58 \times 10^9/\text{L}$). The last follow-up data (August 1995) showed Hb 10.4 g/dL; WBC $3.0 \times 10^9/\text{L}$; neutrophil count $0.6 \times 10^9/\text{L}$; PLT count $29 \times 10^9/\text{L}$. While the possibility of bone marrow transplant from a matched unrelated donor was under evaluation, the patient developed a hemangiosarcoma in the left breast that required ablative surgery.

Discussion

FA is present as *hot spots* in some Italian regions, such as Campania and Sardinia, probably as a result by geographic isolation of inland districts; the Benevento area is probably the zone with the highest prevalence. FA phenotypes are so variable that a diagnosis on the basis of clinical manifestations only is often delayed and sometimes missed. Despite a lack of knowledge about the specific molecular defect in the majority of FA patients, the hypersensitivity of FA chromosomes to the clastogenic effect of oxidants with DNA cross-linking properties, such as DEB, provides a reliable marker for diagnostic purposes. Since a diagnosis of FA may change the therapeutic approach,



Figure 1. DEB test: a metaphase presenting chromosomal break (♣) and multiple rearrangements (◆).

especially the conditioning treatment in preparation for bone marrow transplantation, it is of paramount importance to perform the DEB test in all hypoplastic patients, even adults. In the case of bone marrow transplant from an HLA-identical sibling, a DEB test on donor cells is also mandatory in order to avoid donation by another FA patient.

We documented the therapeutic efficacy of danazol in this patient as we have in other patients with bone marrow hypoplasia.⁵ Progressive loss of efficacy of androgenic treatments after a few years is common in FA patients. Moreover, a course of ALG, which is highly effective in primary aplastic anemia,⁶ administered to our patient before diagnosing FA, was of no help.

The peculiarities of our case are the late onset of pancytopenia and the relatively mild disease, as indicated by the good quality of life with only an occasional need for blood derivative support. In a series of 838 FA patients reviewed by Alter,⁷ the median age at diagnosis was 7 years, range 0-48. Late onset of pancytopenia was reported by Liu *et al.*,⁸ in their case as well, the initial diagnosis of acquired idiopathic aplastic anemia was changed to FA a year later when chromosomal hypersensitivity to cross-linking agents was tested. Our patient has recently developed an

aggressive soft tissue tumor, which should probably be interpreted as a manifestation of the proneness to unusual neoplastic diseases in FA patients. It has already been stressed that the absence of malformations, a late occurrence of pancytopenia and even a successful bone marrow transplant do not prevent tumor development; in fact, the older the patient, the higher the risk of tumor.⁷ It is still unclear, in our patient as in others, whether late onset and milder hematological complications are associated with a particular genotype or complementation class. Even the most recent classification into 6 clinical groups⁹ does not take into account age at onset of pancytopenia.

Attempts to generate cell lines and to identify a gene mutation in samples from our patient are in process in properly equipped laboratories. Although preliminary correlations between mutation type and clinical course are envisaged among patients belonging to group C,¹⁰ at present there is no evidence that any phenotypical finding is characteristic of a specific subgroup of FA patients.^{7,11} Siblings⁸ or even identical twins,¹² who certainly share the same genetic abnormality, may have dissimilar phenotypes. The simplest explanation for the marked clinical heterogeneity, even in the setting of molecularly homogeneous groups, may be that the gene alteration

is not directly responsible for most of the late complications; these could originate from additional stochastic events to which FA patients are predisposed because of the basic genetic defect.

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