Diamond-Blackfan syndrome and neutropenia

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Abstract

Neutropenia is a rare complication of Diamond-Blackfan syndrome (congenital hypoplastic anaemia). Three patients are reported: all had neutropenia as well as anaemia, and to investigate the cause of the neutropenia culture of bone marrow for granulocyte-macrophage colony forming cells (GMCFCs) was performed. Two cases had a low incidence of GMCFCs, but the third case had a high incidence. These findings suggest that myeloid precursors can be abnormal in Diamond-Blackfan syndrome and that the mechanism of neutropenia may, like that of anaemia, vary from patient to patient.

Congenital hypoplastic anaemia (Diamond-Blackfan syndrome) presents at or soon after birth with anaemia due to decreased erythroid precursors in the bone marrow. The white cell and platelet counts are typically normal: none of the 42 cases reported by Diamond, Wang, and Alter had neutropenia.¹ About a third of patients have one or more of a wide spectrum of physical abnormalities.¹ We report three patients with congenital hypoplastic anaemia all of whom had neutropenia as well as anaemia. Culture of bone marrow showed low numbers of granulocyte-macrophage colonies in two cases and raised numbers in the third.

Methods

Granulocyte-macrophage colony forming cells (GMCFCs) were assayed using Iscove's modified Dulbecco's medium, 20% fetal calf serum, 10% 5637 conditioned medium and 0.33% agar.² Cultures were set up in triplicate and plates were incubated at 37°C for 11 days. Colonies of 50 cells or more were counted and expressed as the number of colonies per 10⁵ cells. The mean of the three results was calculated: the range for 13 normal children was 16–130/10⁵ cells with a mean of 48/10⁵ cells.

Case reports

CASE 1

The patient was male, born in 1977 at 38 weeks' gestation, weighing 2.58 kg. The placenta was infarcted. He had a bifid thumb on the left hand with hypoplastic thenar eminences in both hands. The haemoglobin concentration was 7.0 g/l, mean corpuscular volume 109 fl, and a white cell count 6.6 ×

10⁹/l. Bone marrow showed severe hypoplasia of erythroid cells and Diamond-Blackfan syndrome was diagnosed. He was referred for further investigations in 1987 because of a fall in white cell count to 2.6 × 10⁹/l. In addition to the thumb abnormalities already described, physical examination showed pes cavus, a high arched palate, and small eyes set close together. A full blood count showed a haemoglobin concentration of 6.1 g/l, a mean corpuscular volume of 98 fl, and a white cell count of 2.5 × 10⁹/l—neutrophils 0.7 × 10⁹/l, lymphocytes 1.6 × 10⁹/l—a platelet count of 257 × 10⁹/l and reticulocytes at 4%. A bone marrow aspirate was hypocellular with a reduction of both myeloid and erythroid precursors. Bone marrow cultures showed GMCFC numbers just below the normal range. The mean of three plates was 15 GMCFC/10⁵ cells (mean of 13 normal children was 48, range 16–130). Chromosome studies of peripheral blood showed a normal male karyotype and addition of mitomycin-C showed no increased sensitivity to suggest Fanconi's anaemia. Neutrophil antibodies were absent. The haemoglobin concentration and neutrophil count increased to normal levels with 25 mg doses of prednisolone on alternate days.

The patient's father remarried and had another child by his new wife. This girl also had Diamond-Blackfan syndrome with incurved thumbs and small close-set eyes. She had an anaemia responsive to corticosteroids, but neutrophil counts were normal. Bone marrow cultures showed normal GMCFCs at 22 per 10⁵ cells.

CASE 2

The second case was a female infant born in 1987 by emergency caesarean section because of fetal distress, weighing 2.5 kg. The haemoglobin concentration at birth was 40 g/l and she required ventilation and exchange transfusion. A direct Coombs' test was negative. A bone marrow aspirate at 6 weeks of age showed erythroid and myeloid hypoplasia with normal megakaryocytes. She was treated with prednisolone and oxymetholone for four months but remained transfusion-dependent. She was referred when she was 12 months old. Physical examination showed a well, plump infant with no skeletal or other abnormalities. A full blood count (after transfusion) was as follows: haemoglobin 13.7 g/l, mean corpuscular volume 79 fl, and a white cell count of 4.1 × 10⁹/l—neutrophils 1.25 × 10⁹/l, lymphocytes 2.37 × 10⁹/l—a platelet

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count of $287 \times 10^9/l$ and reticulocytes at 0.1%. Over the next two months the neutrophil count varied between $0.2 \times 10^9/l$ and $2.4 \times 10^9/l$. No cycling was detected. A bone marrow aspirate was of normal cellularity with active granulopoiesis and normal megakaryocytes but with a noticeable reduction in erythroid precursors. Chromosome studies of bone marrow showed a normal female karyotype. A test for anti-nuclear factor was negative. Bone marrow cultures showed the same low GMCFC numbers (15) as the first case. By the age of 34 months, the neutropenia had resolved spontaneously and remained normal for 16 months (time of writing).

CASE 3

The third child was normal and born in 1981 at term. No physical abnormalities were present. He presented at 3 weeks of age with anaemia. The haemoglobin concentration was 3.3 g/l, mean corpuscular volume 89 fl, and white cell count was $9.0 \times 10^9/l$ —neutrophils $0.7 \times 10^9/l$ lymphocytes $8.0 \times 10^9/l$ —reticulocytes $12 \times 10^9/l$. A bone marrow aspirate showed complete absence of erythroblasts with a normal granulocyte series and normal megakaryocytes. Chromosome studies were normal. Diamond-Blackfan syndrome was diagnosed and he responded to transfusions and prednisolone. He had a remission the following year, and did not require further transfusions or steroids to maintain his haemoglobin concentration. He remained well until he was 3 years old when he had several episodes of upper respiratory tract infection, tonsillitis, and gingivitis. A blood count showed a haemoglobin of 12.6 g/l, a white cell count of $4.1 \times 10^9/l$ —neutrophils $0 \times 10^9/l$, lymphocytes $3.4 \times 10^9/l$ —and a platelet count of $414 \times 10^9/l$. A bone marrow aspirate showed normal erythropoiesis, much reduced granulopoiesis, and normal megakaryocytes. Bone marrow cultures showed raised GMCFC (168) numbers. Serum adenosine deaminase activity was high at 141 IU (normal 50–100). Multispecific HLA antibodies were detected but granulocyte agglutinins were only very weakly positive. The neutropenia responded initially to low doses of prednisolone, rising to $1 \times 10^9/l$, but subsequently required much larger doses and eventually became refractory. He remained severely neutropenic and died when he was 5 years old from septicæmia.

Discussion

We believe that all three cases have Diamond-Blackfan anaemia on the grounds that long term anaemia presented at birth or up to three weeks later. The unusual feature of all three cases is the low neutrophil count. The marrow from our first case, taken when he was neutropenic and anaemic, was hypocellular and when cultured showed a low incidence of granulocyte-macrophage precursors. The fact that his half sister did not have neutropenia suggests that the low neutrophil count was caused by a different mechanism from that of

the anaemia, or that a defective gene showed variable penetrance in the two children.

Bone marrow from our second case, taken when she was 6 weeks old, showed both erythroid and myeloid hypoplasia, and although the myeloid component had improved when the marrow was repeated, when she was 12 months old she was still neutropenic and culture of her marrow also showed a reduced incidence of granulocyte-macrophage precursors. Later resolution of neutropenia occurred in this patient which suggests that the myeloid abnormality was not an intrinsic feature of her disease. Both cases 1 and 2 raise the possibility that the neutropenia is an epiphenomenon and is not a genetic feature. The third case, in contrast, showed increased numbers of granulocyte-macrophage colonies at a time when the bone marrow showed virtual absence of myeloid precursors, suggesting that the defect here arose soon after the granulocyte-macrophage progenitor stage.

Our cases do not have the physical or typical haematological and cytogenetic features of Fanconi's anaemia. In both disorders, however, there may be a defect of the myeloid stem cell which can predispose to the development of leukaemia which is known to occur in Fanconi's anaemia and which has also been reported in Diamond-Blackfan anaemia.³ Patients in whom neutropenia is persistent need to be considered separately as they may be at further risk of leukaemic change. A patient reported by Halperin and Freedman developed pancytopenia and marrow culture showed decreased numbers of granulocyte-macrophage precursors.⁴

The neutropenia may have been due to subclinical virus infection. Virus infections are common in young children. Several viruses are associated with neutropenia, but long term anaemia due to virus infection has been described only in patients who are immunosuppressed.^{5,6} If chronic virus infection is associated with neutropenia in Diamond-Blackfan anaemia, it seems likely that it could be responsible only for the neutropenia, and not for both the anaemia and neutropenia.

The primary defect in Diamond-Blackfan anaemia is not known. Reduced BFU-E have been reported.^{5,6} In adults red cell aplasia is usually immunologically mediated, and it is possible that a similar process occurs in some childhood cases. This would explain the occasional cases of spontaneous remission and the response to corticosteroids. It has been suggested that cellular inhibitors of erythropoiesis have a primary role in the pathogenesis,⁸ but other studies have suggested that there is either a quantitative deficiency of erythroid stem cells or reduced responsiveness to erythropoietin.⁹ A recent study proposed that an intrinsic progenitor defect accounted for the failure of erythropoiesis.¹⁰ A defect in the bone marrow microenvironment has also been postulated.¹¹ The anaemia, like the acquired red cell aplasia of adults, is probably due to multiple causes.⁴ For children, these would arise during intra-uterine life, some being of genetic origin and others acquired. One or more of these mechan-

isms may also apply to the myeloid series and may be expressed at different stages of myeloid maturation, resulting in neutropenia which may only be expressed temporarily, as in case 2, and which may be associated with a widely varying incidence of GMCFCs.

We conclude that neutropenia in Blackfan-Diamond syndrome is of variable aetiology. In some cases, such as our first two cases with low GMCFCs, it may be due to inadequate granulocyte precursors. In others, such as our third case, the defect may affect maturation. It is not associated with the presence or absence of other congenital abnormalities, and, like failure of erythropoiesis, may remit spontaneously.

Further studies of myeloid progenitors in patients with and without neutropenia are needed to elucidate the mechanisms involved. The possibility that Diamond-Blackfan syndrome in some patients may be a clonal disorder also requires investigation.

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