CASE REPORT

Bone marrow fibrosis and radiological changes of the long bones in children with acute megakaryocytic leukaemia

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The diagnosis of acute megakaryocytic leukaemia (AMkL) may be difficult to establish owing to difficulties in obtaining adequate bone marrow aspirates secondary to bone marrow fibrosis. We describe three children without Down’s syndrome under 2 y of age with AMkL. Although none of the patients had the non-random t (1;22) (p13;q13) translocation, bone marrow cells from all patients exhibited chromosome abnormalities with complex karyotypes, including trisomy 21 in two cases. All patients had profound bone marrow fibrosis and characteristic lamellar diaphyseal radiological changes of the long bones.


Acute megakaryocytic leukaemia (AMkL) is a disease that frequently was misclassified. The disease has been formally recognized as a separate leukaemic subtype with strict diagnostic criteria since 1985 (1). Previously the diagnosis was based primarily on morphologic characteristics of the blast cells e.g. large cell size, abundance of basophilic cytoplasm, cytoplasmic blebbing and clustering of blast cells. At present the use of monoclonal antibodies in detecting megakaryocyte/platelet specific antigens has greatly improved the possibility of diagnosing AMkL. The number of reported cases has increased and some authors believe that AMkL may constitute 10% of all cases of acute non-lymphocytic leukaemia in children (2). Almost 50% of primary AMkL occurs in patients younger than 3 y and there is a strong association with Down’s syndrome in this age group (3). We here describe three Swedish children with AMkL who presented during an 18-month period at our centre, which annually admits around 30 patients with acute childhood leukaemia. The patients had a varying clinical presentation, but all had bone marrow fibrosis and a characteristic radiological appearance of the long bones.

Case 1

A previously healthy 15-month-old boy developed persistent low-grade fever with frequent bruising 3 weeks after an apparently uncomplicated varicellae infection. On admission, examination showed a pale boy with bruises on his legs and trunk. Laboratory investigations revealed anaemia, thrombocytopenia and a normal leukocyte and differential count. There was no hepatosplenomegaly. Aspirates from the bone marrow were extremely difficult to obtain and bone marrow biopsy demonstrated fibrosis and a decreased representation of all haematopoietic cell lines. No fatty infiltration was observed. Radiographs of the tibia and femur showed lamellar periosteal thickening with new bone formation in the diaphyseal regions (Fig. 1). Constitutional chromosomes were normal.

Although AMkL could not be excluded, a preliminary diagnosis of acute myelofibrosis was established. During the following 3 months his clinical condition deteriorated slowly with increasing fatigue, anorexia and weight loss as well as episodic fever. Repeated bone marrow biopsies demonstrated the same characteristics as the initial biopsy with no morphological signs of blastic proliferation of any cellular origin and only few cells of megakaryocytic lineage were detected despite staining with megakaryocyte-specific antibodies.

A course of prednisone (40 mg/m²/d) was instituted with a rapid improvement of the clinical condition. After 2 weeks of prednisone therapy, bone marrow aspiration demonstrated a blastic population of 40% with morphological characteristics of megakaryocytes. This was verified by flow cytometric analysis demonstrating presence of glycoprotein IIb/IIIa complex in 40% of the cells in the aspirate.

Cytogenetic analysis of the bone marrow cells at first admission demonstrated a normal karyotype. At time of diagnosis an abnormal clone (49,XY,+19,+21,+22) was found in three of six cells possible to analyse. Bone marrow biopsy revealed a population of malignant blastic cells expressing glycoprotein Ib and IIb/IIIa. Blood cell counts
and serum immunological parameters at time of diagnosis are given in Table 1.

The patient was treated according to the Nordic protocol for acute non-lymphocytic leukaemia including cytosine arabinoside, etoposide, thioguanine, mithoxantrone and doxorubicin (4). After two courses he achieved a complete remission and bone marrow biopsy demonstrated a marked decrease in fibrosis and essentially normal haematopoiesis. Radiographs of the long bones showed regression of the diaphyseal abnormalities. During consolidation therapy he relapsed, salvage therapy with amecrine, etoposide and cytosine arabinoside was without effect, and he died from progressive disease at the age of 24 months.

Case 2
A previously healthy 16-month-old girl developed an intensely pruritic macular rash over the face, trunks and extremities. Allergic urticaria was suspected and she received a single dose of β-methasone and a course of an antihistamine preparation. The symptoms persisted and 2 d later a differential blood cell count was obtained which surprizingly demonstrated 20% blast cells with malignant morphological characteristics. The concentrations of erythrocytes, leukocytes and platelets were initially normal. Bone marrow aspiration resulted in a dry tap and a bone marrow biopsy demonstrated numerous cells with cytoplasmic blebbing, abundant basophilic cytoplasm, polymorphism and clustering of cells. Immunohistochemical stains with glycoprotein Ib and IIb/IIIa were strongly positive and a diagnosis of AMKL was established. During the few days when her initial evaluation was performed, she became febrile and gradually developed anaemia and thrombocytopenia. Ultrasound of the abdomen and a chest radiograph were normal. Radiographs of the tibial and femoral bones demonstrated diaphyseal changes almost identical to those of case 1.

Cytogenetic analysis of bone marrow cells showed a karyotype 47,XX,-7,del (16) (q23),+21,+Mar (8)/46,XX (5), i.e. an abnormal clone in 62% of the cells analysed. Constitutional chromosomes were normal.

She was treated with the same chemotherapeutic drug regimen as case 1 and entered remission after two induction courses. Bone marrow biopsy demonstrated only a slight increase in reticulin and the radiological abnormalities of the long bones gradually resolved. She was subjected to one further induction course and two consolidation courses after which an allogeneic bone marrow transplantation, using her HLA identical older brother as donor, was performed. The conditioning regimen included busulfan twice daily (125 mg/m²/d) for 4 d followed by 2 d of intravenous cyclophosphamide (60 mg/kg/d). With the exception of a mild graft vs host reaction, which resolved upon a short course of steroids, the transplantation was uncomplicated. She is now in complete continuous remission and in excellent clinical condition 30 months post-transplantation.

Case 3
An 11-month-old girl presented with a 10-d history of high grade fever without any focal signs of infection. Physical examination showed a pale child with a few petechia on the trunk and extremities. No hepatosplenomegalia was observed. Laboratory investigations demonstrated anaemia and thrombocytopenia and a differential count showed the presence of blastic cell forms (17%). A bone marrow aspiration was difficult to obtain, but revealed a poly- morphic blast population which was impossible to classify morphologically. Bone marrow biopsy showed fibrosis with markedly decreased cellularity. Repeat bone marrow aspirations and biopsies were performed weekly and after 1 month a biopsy showed an increase in a blastic mononuclear cell population in the marrow. These cells stained positively for glycoprotein Ib and IIb/IIIa, as well as for von Willebrand factor antigen. Cyto genetic analysis of the

Table 1. Blood cell counts and immunological parameters at time of diagnosis in three children with AMKL.

<table>
<thead>
<tr>
<th>Blood parameter</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (g/l)</td>
<td>75</td>
<td>82</td>
<td>84</td>
<td>110–135</td>
</tr>
<tr>
<td>Platelet count (10⁹/l)</td>
<td>5</td>
<td>342</td>
<td>30</td>
<td>150–450</td>
</tr>
<tr>
<td>Leukocyte count (10⁹/l)</td>
<td>10.5</td>
<td>12.1</td>
<td>12.0</td>
<td>4–9</td>
</tr>
<tr>
<td>Blast cells (% of leukocytes)</td>
<td>19</td>
<td>15</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td>TNF-a (pg/ml)</td>
<td>2.3</td>
<td>21.5</td>
<td>100</td>
<td>&lt;20</td>
</tr>
<tr>
<td>Interleukin-6 (pg/ml)</td>
<td>25.1</td>
<td>26.5</td>
<td>147</td>
<td>&lt;50</td>
</tr>
<tr>
<td>Interferon-γ (U/ml)</td>
<td>1.1</td>
<td>0.8</td>
<td>2.4</td>
<td>&lt;1</td>
</tr>
<tr>
<td>IgG (g/l)</td>
<td>12.6</td>
<td>10.1</td>
<td>11.5</td>
<td>3.2–8.2</td>
</tr>
<tr>
<td>IgA (g/l)</td>
<td>1.5</td>
<td>1.3</td>
<td>0.9</td>
<td>0.1–0.3</td>
</tr>
<tr>
<td>IgM (g/l)</td>
<td>2.2</td>
<td>2.0</td>
<td>1.3</td>
<td>0.2–0.9</td>
</tr>
<tr>
<td>PHA (relative response)</td>
<td>0.7</td>
<td>1.1</td>
<td>0.3</td>
<td>0.7–1.3</td>
</tr>
<tr>
<td>ConA (relative response)</td>
<td>1.1</td>
<td>1.7</td>
<td>0.3</td>
<td>0.7–1.3</td>
</tr>
<tr>
<td>T cells (10⁹/l)</td>
<td>1.4</td>
<td>3.3</td>
<td>5.0</td>
<td>0.9–2.8</td>
</tr>
<tr>
<td>T helper cells (10⁹/l)</td>
<td>0.9</td>
<td>1.9</td>
<td>3.0</td>
<td>0.3–1.7</td>
</tr>
<tr>
<td>T suppressor cells (10⁹/l)</td>
<td>0.8</td>
<td>2.5</td>
<td>2.2</td>
<td>0.3–1.2</td>
</tr>
<tr>
<td>B cells (10⁹/l)</td>
<td>1.7</td>
<td>2.2</td>
<td>3.5</td>
<td>0.1–0.5</td>
</tr>
</tbody>
</table>

*Methods for cytokine analysis and normal values are described in reference 8.
bone marrow cells showed a karyotype 46,XX,-3,del (6) (q11),-12, +der (3;6) (q13q11), +der (3;12) (q21p12) (6)/46,XX (24) i.e. an abnormal clone in 20% of cells analysed. Radiographs of the tibia showed marked diaphyseal thickening with new bone formation. Constitutional chromosomes were normal.

She was treated according to the same protocol as the previous patients and entered remission after two induction courses. After three courses of consolidation treatment she underwent autologous bone marrow transplant. Three months after transplant, ten months after start of therapy, she relapsed and the disease was refractory to further treatment.

Discussion

The differential diagnosis of myelofibrosis in children is still difficult. Previously a large proportion of these patients was classified as having idiopathic myelofibrosis or malignant myelosclerosis. The introduction of immunohistochemical and flow cytometric methods using monoclonal antibodies directed against platelet-specific antigens has greatly facilitated the identification of immature cells of megakaryocytic origin. This has led to an increase in the reported frequency of AMkL. In fact, many authors consider idiopathic myelofibrosis as being AMkL (5). Two of our cases had myelofibrosis at least 1 month before the development of an increased megakaryoblastic population in the bone marrow. This could be explained by the presence of a preleukaemic condition or by blastic transformation of a chronic myelocytic leukaemia. However, no dysplastic morphological features were observed in either erythroid nor myeloid cells. Furthermore in case 2, PCR amplification failed to detect the presence of a bcr/abl rearrangement. Thus it is possible that myelofibrosis in many cases occurs as an early event in the pathogenesis of AMkL.

The cause of the myelofibrosis in AMkL is unknown. Several platelet-derived humoral factors, such as platelet-derived growth factor and platelet factor 4, have been proposed to be involved (6). Two of our patients had elevated serum levels of TNF-α, a cytokine capable of stimulating fibroblast proliferation (7). However, TNF-α is elevated also in children with acute lymphoblastic leukaemia, in whom only rarely myelofibrosis is observed (8).

All three patients had a characteristic radiological appearance of the long bones showing a diaphyseal thickening with lamellar periosteal bone formation. Similar features have previously been described in children with AMkL (9) but in a study of eight children with AMkL two cases exhibited lytic bone lesions but no diaphyseal thickening was observed (3). The new bone formation could depend on an increased osteoblastic activity, possibly caused by the same growth factors that induce fibrosis in the bone marrow. It could also be a stress response to an abnormal pressure inside the bone marrow due to increased fibroblast activity and malignant cell proliferation. The roentgenological findings are so characteristic that we now include radiological investigation of the tibia and femur in the diagnostic work-up of all patients with suspected acute non-lymphocytic leukaemia.

Acute megakaryocytic leukaemia is known to occur more frequently in children with Down’s syndrome. None of our patients had any constitutional chromosomal defects but trisomy 21 was observed in bone marrow cells from two of the patients. The non-random t (1;22) (p13;q13) translocation has, in other studies, been observed in a high proportion of infants with AMkL (10) and it has been proposed that c-sis gene expression in association with this translocation may be involved in the pathogenesis of marrow fibrosis in AMkL (11). Only one of our cases was under the age of 1 y at time of diagnosis and none exhibited the t (1;22) (p13;q13) translocation. Since all had myelofibrosis our data cannot verify that t (1;22) (p13;q13) is involved in the pathogenesis of fibrosis in AMkL. Nonetheless, all of our patients demonstrated complex cytogenetic abnormalities in the malignant clones.

Our patients all had laboratory evidence of activation of the immune system with elevated levels of all immunoglobulin isotypes, increases in several lymphocyte subsets including B cells, T helper cells and T suppressor cells. This is in contrast to patients with acute lymphatic leukaemia who usually exhibit low to normal serum immunoglobulin levels (12) and normal counts of mature lymphocyte subsets. At least one of the three pro-inflammatory cytokines investigated was elevated in each of the patients and it is possible that increased cytokine production in response to the leukaemic disease augments lymphocyte proliferation and immunoglobulin production.

All of our patients achieved remission when treated with an intensive protocol for acute non-lymphocytic leukaemia. Other studies have showed a remission rate of 80% when using protocols designed for ANLL whereas regimens for acute lymphoblastic leukaemia were considerably less effective (3). Whether the prognosis in childhood AMkL differs from that of the other acute non-lymphocytic leukaemias is as yet unknown. Large multicentre studies may in time resolve this issue as the number of patients with AMkL increases due to the currently available diagnostic methods.

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