Sideroblastic Anemia in Multiple Myeloma: A Preleukemic Change

By M. Khaleeli, W. M. Keane, and G. R. Lee

In a 10-yr observation period, 75 patients with multiple myeloma have been studied, and 55 of these were treated daily with low dose melphalan by mouth. Of these, four developed sideroblastic anemia, manifested by anemia, thrombocytopenia, aniso-poikilocytosis, and hypochromic stippled cells in the blood, and ringed sideroblasts in the bone marrow. In two patients, this complication occurred after more than 7 yr of therapy and at a time when there was no evidence of myeloma. In a third patient, it occurred when the myeloma relapsed, and in the fourth, myeloma was active but stable. The anemia did not respond to either folate or pyridoxine. In all four patients, acute myelomonoblastic leukemia developed within 6 mo after sideroblastic anemia had been detected. These observations suggest that sideroblastic anemia in myeloma is a preleukemic manifestation.

SINCE 1970, ACUTE MYELOMONOBLASTIC LEUKEMIA has been reported as a terminal complication in 18 patients with multiple myeloma treated with cyclophosphamide (Cytoxan) or melphalan. Sideroblastic anemia has also been observed in patients with myeloma, but a relationship between these two complications has not been recognized.

We have observed four patients with multiple myeloma (MM) who developed sideroblastic anemia while receiving melphalan. In all four, acute myelomonoblastic leukemia was detected about 6 mo after the onset of sideroblastic anemia. Because of the possible prognostic implication of this sequence of events, these patients are presented.

MATERIALS AND METHODS

Over a 10-yr period, 75 patients with multiple myeloma were referred to the Hematology Division of the Department of Medicine, University of Utah, Salt Lake City, Utah. Of these, 55 patients were treated with melphalan according to a daily, low-dose regimen. Therapy was instituted with a dose of 4-6 mg/day, and this dose was reduced if leukopenia (leukocyte count less than 2000-2500/µl) or other signs of hematologic toxicity were observed. Ultimately the daily maintenance dose, having been adjusted to each patient's tolerance, ranged from 0.5 to 4 mg/day. Among the 55 melphalan-treated patients, four developed sideroblastic anemia and are reported herein. Bone marrow iron stains were available for 35 of the remaining 51 patients: 18 prior to therapy, 13 during melphalan therapy, and 4 both before and during therapy. Ringed sideroblasts were searched for in all these specimens and none were found.
Routine hematologic determinations and special stains on bone marrow were performed according to standard techniques. Serum iron and iron-binding capacity were measured by the method of Peters et al. The paraprotein in each patient was characterized at the Immunoglobulin Reference Center, National Cancer Institute, NIH, Bethesda, Maryland, and quantitative immunoelectrophoresis was performed by Dr. Bram Rose (Royal Victoria Hospital, Montreal, Canada) in two of the patients. Immunoglobulins were quantitated by immunodiffusion in cases 3 and 4. Serum folate and vitamin B₁₂ levels were assayed by Bioscience Laboratories, Los Angeles, Calif. Erythrocyte porphyrins were quantitated by the method of Heller or of Wranne. Cytogenetic studies on bone marrow were performed with the technique of Moorhead et al. Serum muramidase was assayed by the method of Osserman and Lawlor.

**CASE REPORTS**

**Case 1. C.C.**

A 48-yr-old male plumber was evaluated in 1963 after a 2-year history of recurrent fever, cough, otitis media, pyelonephritis, and rib fractures from trivial trauma. The physical examination was negative except for pallor. He was mildly anemic.

A diagnosis of MM was made (Table 1), and therapy with oral, low-dose melphalan was instituted, with a significant improvement. He remained asymptomatic for 7 yr. In December, 1970, mild anemia and thrombocytopenia were detected and during the next 6 wk, the patient complained of progressive weakness and pallor. On blood smear, distinct abnormalities of the erythrocytes were observed (Table 2), and occasional promyelocytes (2%) and myelocytes (4%) were found among the leukocytes. Serum immunoglobulins were normal. On marrow examination, there was no evidence of either myeloma or leukemia, but typical signs of sideroblastic anemia were detected (Table 2).

The anemia did not improve when pyridoxine was administered in a dose of 200 mg/day for 6 wk, and blood transfusions were required. Over a 5–6 mo period, the leukocyte count gradually increased, reaching 10,500 sl. The bone marrow contained 35% myelomonoblasts and 16% promyelocytes. Peroxidase and Sudan black stains were positive, and the PAS stain was negative in both myeloid and erythroid precursors. On the basis of these findings, the diagnosis of acute myelomonoblastic leukemia was made, and therapy with 6-mercaptopurine, 75 mg/day, was instituted. This was discontinued after 5 days because of severe pancytopenia and gastrointestinal hemorrhage. After 1 mo, therapy with cyclophosphamide, vincristine, cytosine arabinoside, and prednisone (COAP) was begun, but 3 days later the

Table 1. Evidence for the Diagnosis of Multiple Myeloma

<table>
<thead>
<tr>
<th>Case 1 (C.C.)</th>
<th>Case 2 (R.L.)</th>
<th>Case 3 (L.M.)</th>
<th>Case 4 (K.V.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>History</td>
<td>Pathological fracture</td>
<td>Bone pain</td>
<td>Asymptomatic</td>
</tr>
<tr>
<td>Paraprotein</td>
<td>Type</td>
<td>IgG-k</td>
<td>IgG-k</td>
</tr>
<tr>
<td></td>
<td>Total γ-globulin (g/100)</td>
<td>5.8</td>
<td>9.7</td>
</tr>
<tr>
<td>Bence Jones</td>
<td>None</td>
<td>None</td>
<td>Trace*</td>
</tr>
<tr>
<td>Bone x-ray</td>
<td>Diffuse</td>
<td>Multiple osteolytic lesions</td>
<td>Multiple osteolytic lesions; fractures</td>
</tr>
<tr>
<td></td>
<td>demineralization</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marrow plasma cells</td>
<td>26%</td>
<td>6%</td>
<td>12.6%</td>
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*By concentration and electrophoresis.
**See text.
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patient died of a massive gastrointestinal hemorrhage. At autopsy, infiltration of the liver and spleen with immature cells was observed. The gastrointestinal hemorrhage was accounted for by the presence of numerous, superficial mucosal ulcers throughout the large and small intestines. There was no evidence of myeloma.

Case 2. R.L.

This 50-yr-old white male, with a known previous history of alcoholism, was evaluated in 1962 because of weakness, fatigability, and bone pain of 2 mo duration. Physical examination was negative except for mild pallor and a questionably palpable spleen. He had mild anemia and thrombocytosis. Following the diagnosis of MM (Table 1), he was given melphalan, with an excellent remission extending over 8½ yr. Between March and December, 1970, he noted an unexplained 13-lb weight loss. The VPRC fell from 45 to 39.5 ml/100 ml, and mild thrombocytopenia developed. Occasional promyelocytes (1%) and myelocytes (4%) were found on blood smear. In the bone marrow, eosinophilia (17.8%) was detected, along with typical signs of sideroblastic anemia (Table 2). There was no response to a short course of folic acid (5 mg/day for five days). Serum globulins by immunodiffusion remained qualitatively and quantitatively normal. By August 1971, the patient had lost an additional 12 lb and complained of epistaxis and increasing symptoms of anemia. There was no response to 6 wk of therapy with pyridoxine, 200 mg/day, and blood transfusions were required. By September the number of myeloblasts and monoblasts in the blood reached 8% and a diagnosis of leukemia was made on the basis of bone marrow findings. No improvement was observed after courses of therapy with 6-mercaptopurine, vincristine with prednisone, or cytosine arabinoside with thioguanine. Five-day courses of intravenous cyclophosphamide every 3 wk controlled the leukocytosis and may have decreased the transfusion requirement; however, no remission was achieved and the patient died in May 1972, probably of a cerebral hemorrhage. No autopsy was permitted.

Case 3. L.M.

This 55-yr-old white female was evaluated in 1967 after a homogeneous protein "spike" on electrophoresis was detected elsewhere. Her brother had died at age 45 of MM. Physical examination and routine hematologic values were within normal limits. Following the diagnosis of MM (Table 1), therapy with melphalan was instituted, and she remained asymptomatic for 3½ yr. After an episode of pneumonia in March 1971, pancytopenia

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<tbody>
<tr>
<td>Stippled, hypochromic cells</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anisocytosis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Poikilocytosis</td>
<td>+</td>
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<table>
<thead>
<tr>
<th>Bone Marrow</th>
<th>Cellularity (biopsy)</th>
<th>Erythroid maturation</th>
<th>Total sideroblasts (%)</th>
<th>Ringed sideroblasts (%)</th>
<th>RE iron</th>
<th>Myeloblasts (%)</th>
<th>Promyelocytes (%)</th>
<th>Plasma cells (%)</th>
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<tr>
<td></td>
<td>Hyperplastic</td>
<td>Hyperplastic</td>
<td>60</td>
<td>12</td>
<td>Increased</td>
<td>1.2</td>
<td>7.2</td>
<td>1.2</td>
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<tr>
<td></td>
<td>3.7</td>
<td>0.4</td>
<td></td>
<td></td>
<td></td>
<td>4.8</td>
<td>3.4</td>
<td>0.6</td>
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<tr>
<td></td>
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<td></td>
<td>70</td>
<td>21</td>
<td>Increased</td>
<td>1.2</td>
<td>5</td>
<td>8.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>50</td>
<td>5</td>
<td></td>
<td>&quot;Sheets&quot;</td>
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Table 2. The Blood and Bone Marrow at the Onset of Sideroblastic Anemia
developed, and the typical blood and marrow findings of sideroblastic anemia were demonstrated (Table 2). There was no response to folic acid, 10 mg/day. Four months later, she became considerably weaker, was found to be anemic, and was given blood transfusions. Serum IgA was increased to 2000 mg/100 ml, and IgG and IgM were subnormal. Her leukocyte count was 12,000/µl of which 5% were monoblasts and 73% were monocytes. In a bone marrow aspirate, excessive storage iron and sideroblasts were again evident but, in addition, 6% of the cells were myeloblasts and 9.4% were promyelocytes. These were positive on peroxidase and Sudan black staining; PAS stains were negative. Immunofluorescent stains for IgA were also negative. A diagnosis of myelomonoblastic leukemia was made. She was treated with cytosine arabinoside and thioguanine to the point of marrow hypoplasia, but failed to achieve a remission. By January 1972, she had developed adenopathy, hepatosplenomegaly, fever, and a leukocyte count of 26,000/µl, mostly monoblasts. She was given intermittent courses of cyclophosphamide, vincristine, cytosine arabinoside, and prednisone (COAP).17 This was associated with a decrease in the size of the liver and spleen and in the leukocyte count, but no remission developed. Because of steroid myopathy and vincristine neuropathy, COAP was discontinued and 6-mercaptopurine was begun. On this therapy, blasts have disappeared from the blood, but she continues to have thrombocytopenia and to require transfusions.

Case 4. K.V.

A 50-yr-old white male was referred in December 1968, with fatigue and bone pain. Physical examination was within normal limits except for rib tenderness. Routine hematologic studies were within normal limits. The marrow was infiltrated by sheets of immature cells which were difficult to identify and initially were thought to be lymphosarcoma cells. With a provisional diagnosis of lymphosarcoma complicated by paraproteinemia, treatment was begun with cyclophosphamide, 150 mg/day, for 1 mo. At the end of this period, the blood urea nitrogen rose to 81 mg/100 ml and the creatinine to 7.8 mg/100 ml. Cyclophosphamide was discontinued, and renal function improved. In February 1968, therapy with melphalan was instituted with symptomatic improvement and disappearance of the protein abnormalities. The patient remained asymptomatic until December 1971, when he noted the onset of bone pain and pallor. Evidence of sideroblastic anemia was found in blood and bone marrow (Table 2), but, in addition, many “myeloma” cells were observed. Bence Jones protein (3.5 g/day) was detected in the urine and progressive bone lesions were found on x-ray. The patient was given 8 mg melphalan, and 180 mg prednisone per day for 4 days without benefit.

By February 1972, large doses of prednisone were needed to control hypercalcemia. He again developed renal failure with massive proteinuria. Because of continued severe bone pain he required local radiation. After four courses of therapy with melphalan, procarbazine, prednisone, and vincristine, the proteinuria decreased, bone pain disappeared, and the hypercalcemia could be controlled with low doses of prednisone. He developed a severe pseudomonas pulmonary infection which was controlled with Carbenicillin and Gentamicin. After occasional myeloblasts were observed in buffy-coat preparations, the bone marrow was examined, and 20% of the cells were either myelomonoblasts or promyelocytes.

SUMMARY OF CLINICAL AND LABORATORY OBSERVATIONS

Characteristics of the Multiple Myeloma (MM)

These are summarized in Table 1. In three of the four patients, the first evidence of the disease was pain or pathologic fracture of the bones. In case 3, a homogeneous paraprotein was detected at the time of examination for an unrelated problem. A circulating, homogeneous myeloma protein, type G in three patients and type A in one, was found in all. All four paraproteins
carried kappa-type light chains. Bence Jones protein was present in large amounts in one patient, was detectable only by electrophoresis in another, and was absent in two. Decreased amounts of normal immunoglobulins were measured in two patients. Characteristic, osteolytic, bony lesions were found in two patients and diffuse demineralization of the bones was observed in the other two. Myeloma was evident in marrow examinations in two of the patients. In case 2, only 6% atypical plasma cells were found in a dilute aspirate. The diagnosis in case 4 was complicated by difficulty in identifying the cells which infiltrated the marrow. These were very immature and initially were thought to be lymphosarcoma cells. In both of these cases the response to melphalan and the subsequent course leave little doubt regarding the diagnosis of myeloma.

Response to Therapy

All patients received melphalan therapy in a daily, low-dose regimen as reported elsewhere. The total cumulative dose of the drug ranged from 2.73 to 8.1 g given over periods from 3 to 8.5 yr (Fig. 2). All patients responded to the medication. The best responses were observed in cases 1 and 2. In these, performance status improved, abnormal proteins disappeared, volume of packed red cells (VPRC) increased (Fig. 1), and myeloma cells disappeared from the marrow. These two patients remained asymptomatic for over 7 yr (Fig. 2). In case 4, performance status improved, Bence Jones protein disappeared from the urine, and bone lesions stabilized and did not progress; however, the remission lasted only 3 yr. It is more difficult to evaluate the response in patient 3. She was asymptomatic when therapy began and remained asymptomatic for 3½ yr. A fall in serum gamma globulin was observed (Fig. 2), but the IgA spike never disappeared from the electrophoretic pattern. Although there was no progression of bone disease by x-ray, she continued to have myeloma cells in the marrow. The VPRC decreased somewhat on therapy (Fig. 1).

Characteristics of the Sideroblastic Anemia

In retrospect, the onset of sideroblastic anemia in all four patients was heralded by a slight (2–3 ml/100 ml) fall in the VPRC from previously stable levels. This mild change was overlooked or attributed to melphalan toxicity and not investigated more fully. In the subsequent 3 mo a greater decrease in VPRC (7–10 ml/100 ml) was observed. In all cases, mild to moderate aniso- and poikilocytosis along with a population of stippled, hypochromic cells were observed on blood smear. The anemia was accompanied by moderate thrombocytopenia and, in two of the patients, leukopenia (Fig. 1). Normoblastic erythroid hyperplasia with ringed sideroblasts and increased reticuloendothelial iron were found in bone marrow aspirates (Table 2). Transferrin saturation was close to the upper limit of normal in cases 1, 2, and 3, and clearly increased only in case 4 (Table 3). Thus the changes in plasma iron were not as great as has been observed in other varieties of sideroblastic anemia. Free erythrocyte protoporphyrin was increased in cases 3 and 4,
Fig. 1. Performance status, serum gamma (γ) globulin levels, and values for the volume of packed red cells (VPRC), white cell count (WBC), and platelets at three points in time: (1) at the time of diagnosis of multiple myeloma, (2) after optimal response to melphalan, and (3) at the onset of sideroblastic (S.) anemia. Dashed lines enclose the normal range. Performance status was graded as follows: A, able to carry on normal activity; B, unable to work, but able to live at home; C, unable to care for self, requiring institutional care.18

and near normal in cases 1 and 2. Serum folate was subnormal in three of the four cases (Table 3), but in none was the marrow megaloblastic. Two were treated with folic acid without response. Leukocyte alkaline phosphatase was normal.

When sideroblastic anemia was first observed, there was no evidence of myeloma in two patients (numbers 1 and 2). In patient 3 sideroblastic anemia was discovered after an infection (pneumonia) and the myeloma neither worsened nor improved; the marrow contained 6.8% myeloma cells and serum IgA was increased to 2000 mg/100 ml. In the fourth patient, sideroblastic anemia occurred at the time of a myeloma relapse, manifested by bone pain, Bence Jones proteinuria, and sheets of plasma cells in the marrow. The sideroblastic anemia did not respond to 200 mg of pyridoxine daily by mouth in any of the patients.

The Development of Acute Leukemia

Acute leukemia was observed about 6 mo after sideroblastic anemia was first documented (Fig. 2). A possible clue to the preleukemic nature of the picture was the presence of myeloid immaturity in the blood smear; occasional myelocytes and even promyelocytes were observed, even though a diagnosis of leukemia could not be made from marrow examination (Table 2). In all cases, the leukemia was classified as myelomonoblastic. Monocytoid features predominated in cases 2 and 3. Serum muramidase was found to be 72, 107, and 70 μg/ml, respectively, in cases 2, 3, and 4. (normal: 25–65 μl/ml). The leukemic cells were peroxidase and Sudan black positive and PAS negative.
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Fig. 2. Clinical course and cumulative dose of melphalan in each of the four patients. Leukemia has also been detected in KV, but is not of sufficient duration to appear in the figure.

The erythroid cells also were negative to the PAS stain. Cytogenetic studies were normal in cases 2 and 4 but in 1 and 3, hypodiploidy (modal count 45) was found. No remissions were induced in any of the first three patients. As yet, case 4 has not received antileukemic therapy.

DISCUSSION

These four cases suggest that the development of sideroblastic anemia in patients with melphalan-treated myeloma should be regarded as an ominous prognostic sign, and that acute leukemia may follow. Only four instances of sideroblastic anemia were found among 75 patients with myeloma, and all four developed acute myelomonoblastic leukemia within 6 mo. We have been able to find reports of only five other myeloma patients who developed sideroblastic anemia.\textsuperscript{8,20-22} In none of these was the subsequent course described.

That acute leukemia may be a terminal event in patients with other forms of sideroblastic anemia is well known.\textsuperscript{19,23,24} In Dameshek’s experience, “approximately 50%” of patients with acquired sideroblastic anemia developed leu-

Table 3. Biochemical Abnormalities Accompanying Sideroblastic Anemia

<table>
<thead>
<tr>
<th></th>
<th>Normal range</th>
<th>Case 1 (C.C.)</th>
<th>Case 2 (R.L.)</th>
<th>Case 3 (L.M.)</th>
<th>Case 4 (K.V.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum iron ((\mu g/100) ml)</td>
<td>55–155</td>
<td>132</td>
<td>107</td>
<td>111</td>
<td>157</td>
</tr>
<tr>
<td>Iron-binding capacity ((\mu g/100) ml)</td>
<td>200–400</td>
<td>281</td>
<td>240</td>
<td>272</td>
<td>275</td>
</tr>
<tr>
<td>Transferrin saturation (%)</td>
<td>20–45</td>
<td>47</td>
<td>45</td>
<td>41</td>
<td>57</td>
</tr>
<tr>
<td>Free erythrocyte protoporphyrin ((\mu g/100) ml)</td>
<td>15–36</td>
<td>50</td>
<td>40</td>
<td>229</td>
<td>163</td>
</tr>
<tr>
<td>Serum folate (ng/ml)</td>
<td>5–21</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Serum Vitamin B(_{12}) (men) (pg/ml)</td>
<td>170–760</td>
<td>160</td>
<td>1180</td>
<td>481</td>
<td>943</td>
</tr>
<tr>
<td>(women)</td>
<td>90–630</td>
<td>71</td>
<td>55</td>
<td>114</td>
<td></td>
</tr>
<tr>
<td>Leukocyte alkaline phosphatase</td>
<td>40–139</td>
<td>—</td>
<td>71</td>
<td>55</td>
<td>114</td>
</tr>
</tbody>
</table>
kemia, which led him to regard the illness as a form of the DiGuglielmo syndrome. In a review of patients with the idiopathic refractory form of sideroblastic anemia, the incidence of leukemia was lower, but still substantial (7.4%).

Recognition of sideroblastic anemia in patients with myeloma may not be easy because a number of other types of anemia may occur in this complex illness. Furthermore, the early signs of the complication were subtle, and in particular, evidence of pronounced iron overload was lacking. Thus, the number of ringed sideroblasts and the serum iron alterations did not deviate from normal to the same extent as in the usual patient with the idiopathic form of sideroblastic anemia. These differences probably are a consequence of the fact that iron accumulation requires time; thus, when patients are detected early in the course, those manifestations which result from iron overload may not have developed. In myeloma patients, the diagnosis of sideroblastic anemia may not be appreciated when routine hematologic and biochemical data are analyzed. Of the routine observations made by ourselves, the most useful was the appearance of a population of hypochromic, stippled erythrocytes on blood smear. Definitive diagnosis required the detection of ringed sideroblasts in bone marrow aspirates stained for iron.

Prior to the availability of effective chemotherapy for myeloma, especially melphalan, acute leukemia appears to have been a very rare complication of the disease. Of the 18 cases of leukemia reported in myeloma patients since 1970, 11 were treated with melphalan regimens similar to ours, and two (Refs. 2, 6) responded to cyclophosphamide. In the remaining five patients, the therapeutic regimen was not presented. In all 18, the leukemia was judged to be myeloblastic, myelomonoblastic, or monoblastic. It is noteworthy that the leukemia was observed in patients who have survived for relatively long periods of time (2½–7 yr) usually those in whom excellent remissions had been achieved and were being maintained until the time of onset of leukemia. Our cases 1 and 2 are typical examples of this type of course. In our cases 3 and 4, and in only two others, leukemia occurred while myeloma was active. As in our cases, the leukemia was usually refractory to therapy, only one remission having been reported.

The reasons for the development of either leukemia or sideroblastic anemia in myeloma patients are unknown. The observation that these disorders occur chiefly in patients who have received and responded to melphalan therapy suggests that the drug itself induces the complication. Alternatively, the prolonged survival induced by therapy may increase the possibility of observing a hitherto unrecognized aspect of the natural history of myeloma. One suggested mechanism for the induction of sideroblastic anemia is somatic mutation. Drug or disease induced chromosome damage might result in such an effect as well as in leukemia. Some support for such a mechanism is derived from the cytogenetic abnormalities in two of our patients. Hypodiploidy was observed in them, and has also been observed in other patients with melphalan-treated myeloma in whom leukemia developed.
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REFERENCES


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