Serial Observations of the Fibrous Tissue in the Bone Marrow of Hematological Disorders

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The fibrous tissue in the biopsied bone marrow was serially observed in 157 patients with hematological disorders. An increase of argyrophil fibers was demonstrated in acute and chronic leukemias, erythroleukemia, malignant lymphomas, carcinoma with metastasis to the marrow, polycythemia vera and primary myelofibrosis. The increase of argyrophil fibers was generally correlated with the grade of proliferation of the hematopoietic cells and its duration. Argyrophil fibers were reduced in aplastic anemia.

The histological observations of the bone marrow have been confined mostly to specimens from autopsy; however, these may not be representative of the real pathological findings in hematological disorders because of examinations at the terminal phase of the diseases, the postmortem changes of the specimens and the pictures modified by intensive treatments. Recently, there have been several reports of bone marrow biopsy which attempted to observe essential histology of the marrow in diseases.1-6 Some take the view that proliferation of hematopoietic cells in the bone marrow fluctuates in the course of hematological disorders including leukemias. On the other hand, fibrosis seems to be slowly progressive in the development of the diseases, but it still remains obscure in many respects.

This research was undertaken to follow the increasing amount of the fibrous tissue, particularly argyrophil fibers of the bone marrow in hematological disorders. This is considered to be of significance for clarifying some aspects of pathogenesis of primary myelofibrosis.

MATERIALS AND METHODS

One hundred and forty-seven patients with various hematological disorders and ten normal controls were investigated (Table 1). Two hundred and forty-seven biopsies were performed on these patients.

Bone marrow samples about 10-20 mm in length and 3 mm in diameter were repeatedly obtained from the posterior iliac crest by our bone marrow biopsy method.7

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### TABLE 1. Patients investigated and pattern of the argyrophil fibers in the diseases

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number of cases</th>
<th>Number of biopsies</th>
<th>Types of pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute myelogenous leukemia</td>
<td>18</td>
<td>43</td>
<td>1 2 3 4</td>
</tr>
<tr>
<td>Acute lymphocytic leukemia</td>
<td>7</td>
<td>17</td>
<td>1</td>
</tr>
<tr>
<td>Chronic myelogenous leukemia</td>
<td>15</td>
<td>35</td>
<td>1 14 20</td>
</tr>
<tr>
<td>Other leukemias</td>
<td>3</td>
<td>6</td>
<td>1 1 1</td>
</tr>
<tr>
<td>Primary myelofibrosis</td>
<td>2</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Polycythemia</td>
<td>2</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Malignant lymphomas</td>
<td>11</td>
<td>20</td>
<td>7 7 4 2</td>
</tr>
<tr>
<td>Carcinomas</td>
<td>9</td>
<td>9</td>
<td>5 2 1 1</td>
</tr>
<tr>
<td>Hypochromic anemia</td>
<td>22</td>
<td>27</td>
<td>23 4</td>
</tr>
<tr>
<td>Aplastic anemia</td>
<td>15</td>
<td>22</td>
<td>21 1</td>
</tr>
<tr>
<td>Other anemias</td>
<td>3</td>
<td>3</td>
<td>2 1</td>
</tr>
<tr>
<td>Agranulocytosis</td>
<td>5</td>
<td>6</td>
<td>3 1 2</td>
</tr>
<tr>
<td>Purpuras</td>
<td>9</td>
<td>10</td>
<td>5 5</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>26</td>
<td>28</td>
<td>22 3 3</td>
</tr>
<tr>
<td>Normal</td>
<td>10</td>
<td>11</td>
<td>9 2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>157</td>
<td>247</td>
<td>104 59 56 28</td>
</tr>
</tbody>
</table>

All the specimens observed were decalcified with a mixture of aluminium chloride, hydrochloric acid and formic acid after fixation in 10% neutral formalin.

The decalcified tissues were embedded in paraffin and the sections were cut at 4µ in thickness. Hematoxylin and eosin stain, modified silver impregnation method (Watanabe, 1959), and Masson’s trichrome and PAS stains were used.

### RESULTS

Sections from all the patients were evaluated as to cellularity of the hematopoietic tissue, the amount, thickness and arrangement of the argyrophil fibers, presence of collagenous fibers and the findings of the sinuses of the marrow. The criteria for evaluating cellularity of the hematopoietic cells were proposed by Prof. M. Baba and his co-workers as follows: hypercellular, hematopoietic cells constituting 75 to 100% of the total bone marrow cells; normocellular, 25 to 75% and hypocellular, 0 to 25%. Patterns of argyrophil fibers were classified into four types: Type 1, normal; Type 2, slightly increased in fine fibers around the trabeculae and sinuses; type 3, moderately increased with abundant fiber networks; and Type 4, markedly increased with bundles of thick fibers.

**Normal subjects**

In the normal bone marrow the cellularity was considered to be essentially normocellular. The cells of the three series of the bone marrow, granulocytes, erythrocytes and megakaryocytes, were differentiated and no atypical cells were seen. Argyrophil fibers were fine and irregularly arranged in the medullary cavity of the bone with a fine network of fibers, which was rather variable in distribution from case to case. These were generally continuous with the bone trabeculae, the fat tissue and the sinuses (Fig. 5). Eight cases were those of Type...
1 and two, Type 2 in the pattern of the fibers. The sinuses appeared to be compressed by proliferating hematopoietic cells.

**Acute myelogenous leukemia**

Forty-three specimens from eighteen patients, treated chiefly with 6-mercaptopurine and steroid hormones, were observed before treatment, during response to treatment, in remission and in relapse. Comparative study revealed considerable discrepancies in the findings of argyrophil fibers depending upon these stages.

In nine of these cases studied before treatment seven showed hypercellularity with monotonous leukemic cells (Fig. 6), while two revealed hypocellular marrow. The argyrophil fibers were fine and irregularly arranged. The network of fibers was occasionally not clear. Slightly patchy foci of increased fibers around the trabeculae and sinuses occurred in three cases (Fig. 7), the finding being compatible with type 2 in the pattern of the fibers. Three cases were those of type 1 and other three, of Type 3. The sinuses appeared to be compressed by proliferating monotonous leukemic cells.

Six cases were observed during response to treatment. The cellularity of the marrow was normocellular to hypocellular in these cases (Fig. 8). The fibers, which were slightly increased around the trabeculae and sinuses before treatment, remained, and the network of fine argyrophil fibers tended to become evident in hypocellular regions of the medullary cavity (Fig. 9). There were no cases in which the fibers were considered to be increased in amount more than those before treatment.

Seven cases were observed in remission. In normocellular bone marrow, normal hematopoietic cells were seen (Fig. 10). Generally, argyrophil fibers were still fine and irregularly arranged (Fig. 11). Five cases were those of Type 2 and two, Type 3 in the pattern of the fiber. Patients who developed relapse showed a quantitatively greater increase of argyrophil fibers than in remission, but there were no tendencies in their behavior to aggregate into bundles of thick fibers and no collagenous fibers were demonstrated (Fig. 12). One case was of Type 2 and two, of Type 3 in the pattern of the fibers. In seven cases, which were resistant to treatment, the pattern of the fibers was almost similar to that of the cases in relapse.

Summarizing the findings of eighteen cases of acute myelogenous leukemia, it appears obvious that argyrophil fibers increased in amount proportionately with development of the disease as well as with its duration, although two cases showed no increase during response to treatment (Figs. 1 and 2).

**Acute lymphocytic leukemia**

Seventeen specimens from seven patients were evaluated. The marrows were extremely hypercellular, consisting chiefly of small round cells with nuclei rich in chromatin before treatment. They became normocellular to hypocellular
Fig. 1. Relationship between patterns of argyrophil fibers and development of acute myelogenous leukemia.

Fig. 2. Relationship between patterns of argyrophil fibers and duration of acute myelogenous leukemia.

after treatment with steroid hormones. Argyrophil fibers were fine, and increased slightly to moderately in amount even before treatment. They tended to increase during remission. In one case under observation for two years and five months argyrophil fibers formed an abundant network in the medullary cavity and some were arranged in bundles of parallel fibers. Two cases were of Type 2, one, of Type 3 and another one, of Type 4 in the pattern of the fibers. The sinuses became apparent and were dilated.

Chronic myelogenous leukemia

Thirty-five specimens from fifteen patients, treated with busulfan, 6-mercaptopurine and mitomycin C, were observed. All of the marrows were hypercellular. The pattern of the fibers was generally uniform. Argyrophil fibers were slightly to moderately increased in amount even before treatment. During remission, argyrophil fibers progressively increased and aggregated into bundles of thick fibers. Three cases had Type 3 and eight, Type 4 in the pattern
of the fibers. The sinuses were considerably deformed and dilated. Six cases developed relapse. Argyrophil fibers were thick and arranged in bundles (Fig. 13). The network of fibers was not clear in these cases. Collagenous fibers were demonstrated in two of six cases.

Summarizing the findings of fifteen cases of chronic myelogenous leukemia, it appears obvious that argyrophil fibers increased in amount proportionately with development of the disease as well as with its duration, although they had been moderately increased even before treatment (Figs. 3 and 4).

Fig. 3. Relationship between patterns of argyrophil fibers and development of chronic myelogenous leukemia.

Fig. 4. Relationship between patterns of argyrophil fibers and duration of chronic myelogenous leukemia.

Other leukemias

Two cases of acute erythroleukemia and one case of chronic erythroleukemia, characterized by abnormal proliferation of granulocytes and erythrocytes, were observed. The fibers were moderately increased with abundant fiber networks in
which monotonous leukemic cells proliferated. The acute cases had Type 3 and the chronic one, Type 4 in the pattern of the fibers. Collagenous fibers were demonstrated in the chronic case.

**Primary myelofibrosis**

Three specimens from two patients were observed. The first case was a 44-year-old male. Leukocytosis with immature cells, erythroblasts, and tear drop cells in peripheral blood and difficulty in aspirating bone marrow were characteristic. Bone marrow histology revealed considerably hypertrophied bone trabeculae and diminished hematopoiesis. Thick collagenous fibers were arranged in bundles with small numbers of hematopoietic cells along them (Fig. 14). The second case was a 40-year-old male with splenomegaly, slight anemia, presence of immature cells, erythroblasts, and tear drop cells in peripheral blood and difficulty in aspirating bone marrow.

Histology showed hypercellular bone marrow with abundant megakaryocytes. Argyrophil fibers were thick and arranged in bundles. Collagenous fibers were partially demonstrated. Both cases had Type 4 in the pattern of the fibers.

**Other diseases**

The patterns of the argyrophil fibers in other diseases are shown in Table 1. Moderate increases of argyrophil fibers with abundant fiber networks were demonstrated in six successive specimens from a patient of polycythemia vera; however, one case of secondary polycythemia showed no increased argyrophil fibers. Among the malignant lymphomas, three cases of Hodgkin’s disease, granuloma type, showed moderate to marked increase in argyrophil fibers (Fig. 15). Four cases of reticulum cell sarcoma were of Type 2, one of lymphosarcoma, of Type 1 and three of reticulosis, of Type 2 to Type 3 in the pattern of the fibers.

In one case of carcinoma of the stomach the metastasis in the marrow was surrounded by a markedly increased argyrophil fiber network (Fig. 16).

Six other cases of carcinoma were of Type 1, and two, of Types 2 and 3 in the pattern of the fibers. In iron deficiency anemia argyrophil fibers were slightly increased around the sinuses where erythropoiesis was conspicuous after iron treatment. In aplastic anemia the amount of argyrophil fibers was generally reduced and the network of fibers was not clear in the medullary cavity. Argyrophil fibers were not increased in two cases of aplastic anemia due to radiotherapy.

**DISCUSSION**

Most of the histological observations of the bone marrow have been performed in autopsy or experimental studies. Therefore, the development of argyrophil fibers of the marrow in hematological disorders are still obscure. In this study some changes of argyrophil fibers in the marrow were observed in the course of various diseases. As mentioned previously, the patterns of argyrophil
fibers were classified into four types. Type 1 represents normal bone marrow without any pathological increase of argyrophil fibers. Type 2 represents a slight increase of argyrophil fibers in the marrow around the trabeculae and sinuses. Even in the early stage, increase of argyrophil fibers is considered to be closely associated with proliferation of hematopoietic cells, since it is generally accepted that granulocytes proliferate around the trabeculae and erythrocytes, around the vessels including the sinuses. Type 3 represents a moderate increase of argyrophil fibers in the marrow in the form of abundant fiber networks. This may be the pattern in which argyrophil fibers spread throughout the medullary cavity from patchy foci of increased fibers. Type 4 represents a marked increase of argyrophil fibers with bundles of thick fibers. Fine argyrophil fibers are considered to aggregate into bundles of thick fibers, losing gradually their argyrophilia, and forming collagenous fibers. There is a tendency of argyrophil fibers to increase slowly from type 1 to Type 4 in an orderly pattern.

In two cases of acute myelogenous leukemia argyrophil fibers, which had slightly increased before treatment, and fiber networks, which were obscured owing to hypercellularity, tended to become evident in the hypocellular regions in remission. The disease in these two patients relapsed after seven to thirteen months of treatment. At that time argyrophil fibers increased throughout the medullary cavity with an abundant fiber network. Fig. 17 represents an increase of argyrophil fibers in close association with proliferation of hematopoietic cells.

Argyrophil fibers were found increased in fifteen (37%) of forty-one cases under serial observations, remained almost unchanged in twenty-one (51%), tended to decrease in two (7%) and fluctuated in amount in three (7%). These findings may suggest a tendency to gradual increase of argyrophil fibers with the advance of the diseases. Five of forty-one cases showed decrease of argyrophil fibers during observation. However, it was not certain whether there was really a decrease, because the biopsy specimen was so small that fortuitous sampling of foci unusually rich in argyrophil fibers in early stages could not be ruled out. Increase of argyrophil fibers was generally correlated with duration of hypercellularity in the marrow.

Fig. 17. Relationship between proliferation of hematopoietic cells and increase of argyrophil fiber in the marrow.

- increase of argyrophil fiber

Proliferation of hematopoietic cells
Whether argyrophil fibers are produced intracellularly or extracellularly has not been ascertained. Many authors are of the opinion that the argyrophil fibers are produced extracellularly. However, formation of the argyrophil fibers is considered to be associated with the cells with well developed endoplasmic reticulum and Golgi apparatus. The proliferation of reticulum cells in myelofibrosis has been well recognized and it is generally accepted that reticulum cells appear to have affinity for argyrophil fibers.

The etiology of primary myelofibrosis is still in controversy. The following three theories have been proposed for the concept of primary myelofibrosis. (1) Primary myelofibrosis is a form of chronic myelogenous leukemia. (2) Primary myelofibrosis is a form of chronic bone marrow failure with compensatory myeloid metaplasia. (3) Primary myelofibrosis is one of the groups of myeloproliferative diseases which are characterized by primary abnormal proliferation of one or several marrow elements. Myelofibrosis is that form of myeloproliferative disease in which fibroblastic activity is especially prominent. The third theory seems to be most prevalent. It is difficult to detect early pathological changes of the marrow in primary myelofibrosis because this progressive disorder may be of insidious onset. However, argyrophil fibers possibly increase in the fashion mentioned before.

One of our two cases of primary myelofibrosis with hypercellular bone marrow observed could be differentiated from chronic myelogenous leukemia, because of the presence of immature cells, erythroblasts and tear drop cells in peripheral blood, difficulty in aspirating bone marrow and leukopenia. In contrast to close association of reactive increase of argyrophil fibers with proliferation of hematopoietic cells, the fibrous tissue appears to increase independently in primary myelofibrosis. This is in favor of the view that primary myelofibrosis is one of the myeloproliferative disorders rather than a mere reaction of the bone marrow.

**CONCLUSION**

1) The fibrous tissue in the bone marrow obtained by our new biopsy method was serially observed in 247 biopsy specimens from 147 patients with hematological disorders and from 10 normal subjects.

2) The patterns of argyrophil fibers in the marrow could be classified into four types: Type 1, normal; Type 2, slightly increased around the trabeculae and the sinuses; Type 3, moderately increased with abundant fiber network; and Type 4, markedly increased with bundles of thick fibers.

3) Reactive increase of argyrophil fibers was demonstrated in acute and chronic leukemias, erythroleukemia, malignant lymphomas, carcinoma with metastasis to the marrow and polycythemia vera. Increase of argyrophil fibers was generally correlated with proliferation of the hematopoietic cells and its duration. The amount of argyrophil fibers was in contrast reduced in aplastic anemia.

4) Two cases of primary myelofibrosis were observed and could be
differentiated from chronic myelogenous leukemia in that these cases showed
signs of primary abnormal increase of the fibrous tissue among the several marrow
elements.

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Fig. 5. Normal bone marrow. Fine network of argyrophil fibers is seen. Silver impregnation. ×400.

Fig. 6. Bone marrow from acute myelogenous leukemia before treatment. Leukemic cells monotonously occupy the medullary cavity. H-E stain. ×200.

Fig. 7. Silver impregnation of the same specimen. A slightly patchy focus of increased fibers around the trabeculae and sinuses is demonstrated. ×200.

Fig. 8. Bone marrow from the same patient during response to treatment. The cellularity of the marrow is hypocellular. H-E stain. ×200.

Fig. 9. Silver impregnation of the same specimen. Fibers, which have slightly increased around the trabeculae and sinuses, still remain to be seen. ×400.

Fig. 10. Bone marrow from the same patient during remission. Normocellular bone marrow contains normal hematopoietic cells. H-E stain. ×200.
Fig. 11. Silver impregnation of the same specimen. Argyrophil fibers are still fine and irregularly arranged. ×400.

Fig. 12. Silver impregnation of the bone marrow from the same patient in relapse. Abundant networks of argyrophil fibers are seen. ×400.

Fig. 13. Chronic myelogenous leukemia. Argyrophil fibers are arranged in thick bundles, and the sinus is dilated. Silver impregnation. ×200.

Fig. 14. Primary myelofibrosis. Collagenous fibers are demonstrated. Masson's trichrome stain. ×400.

Fig. 15. Hodgkin's disease. Argyrophil fibers are arranged in thick bundles. Silver impregnation. ×200.

Fig. 16. Carcinoma of the stomach with metastasis to the bone marrow. Argyrophil fibers are arranged in thick bundles. Silver impregnation. ×400.