Reticulin Fibrosis and Bone Infarction in Acute Leukemia. Implications for Prognosis

By Donald W. Kundel, George Brecher, Gerald P. Bodey and Geoffrey M. Brittin

While performing serial bone marrow examinations in leukemic patients for evaluation of their response to chemotherapeutic agents, repeated unsuccessful marrow aspirations were encountered with some frequency. In these instances, we employed a Vim-Silverman needle biopsy technic as described by McFarland and Dameshek1 and modified by Conrad and Crosby.2 This procedure was as readily performed and no more painful than marrow aspiration. An adequate sample of marrow was obtained in about three-fourths of such biopsies. In contrast to previous autopsy studies,3-6 the availability of Vim-Silverman sections made it possible to follow the increase in reticulin fibers and the occurrence of bone infarction during the patient's course. The observations indicated a high degree of association of reticulin formation with dry taps and of bone infarction with severe bone pain. Both reticulin formation and bone infarction were seen predominantly in patients unresponsive to therapy.

Materials and Methods

Forty consecutive cases of acute lymphocytic leukemia admitted to the National Cancer Institute between July 1, 1961 and July 1, 1962 were studied. They included all patients admitted during this period except four who expired before an adequate marrow biopsy could be obtained. All cases were followed until death or December 31, 1962.

Bone marrow was obtained from all patients at approximately 1-month intervals. Sections of all marrows were stained with H & E and evaluated as to adequacy of sample, degree of cellularity, proportion of leukemic versus normal hematopoietic elements, and patterns of tissue organization. Representative marrow sections were stained with a modified Hortega-Foot Stain and with the Masson Connective Tissue Stain to evaluate the amount of reticulin and collagen fibers respectively.

A total of 455 marrow aspiration biopsies were attempted on the 40 patients with acute lymphocytic leukemia. One hundred and ninety of the 455 resulted in dry taps, i.e., insufficient marrow obtained by aspiration to make a paraffin block section. One hundred of these 190 dry taps were immediately followed by a Vim-Silverman needle biopsy and adequate specimen of marrow was obtained in 78. The remaining 22 Vim-Silverman needle biopsies were considered failures, either because only cortical bone or cartilage was obtained or because the marrow was too crushed and distorted to evaluate. Reticulin stains were performed on 52 aspiration biopsies and 51 Vim-Silverman needle biopsies of patients with acute lymphocytic leukemia.

For comparison, reticulin stains were performed on normal bone marrow of five patients, on 10 marrows of eight patients with granulocytic or erythrocytic hyperplasia, on 11 marrows of six patients with chronic myelogenous leukemia, and on 29 marrows of 20 patients with acute myelogenous leukemia. Twenty of the patients with acute lymphocytic leukemia died during the period of study. Bone and marrow were available for examina-

From the Clinical Center and National Cancer Institute, National Institutes of Health, Bethesda, Md.
Submitted June 27, 1963; accepted for publication Nov. 9, 1963.

Blood, Vol. 23, No. 4 (April), 1964
tion on 18 of them, and reticulin stains on 12. In addition, autopsy material from all patients dying of acute leukemia at the Clinical Center between June 1, 1961 and October 1, 1962 was examined. This included 35 additional patients with a diagnosis of acute lymphocytic leukemia and 33 with acute myelogenous leukemia. Bones from a number of sites, generally sternum, vertebrae, pelvis, and femur were examined.

Criteria used for evaluating remission, partial remission, and relapse were those proposed by the clinical studies panel of the Cancer Chemotherapy National Service Center as modified by the Acute Leukemia Cooperative Group B.7

The criteria used for the differentiation of lymphocytic and myelocytic leukemia were those given in Wintrobe's or other standard texts. All leukemias in which Auer bodies were found or in which leukemic cells phagocytosed added starch particles or Staphalococci were classified as myelocytic, even though it was recognized that some of these would be classified as monocytic by others. This was done because most of these leukemias were of the Naegali type, in which the picture varied from myelocytic to monocytic on different examinations. The difficulty in distinguishing even the two major types of acute myelocytic and lymphocytic leukemia was fully recognized. Approximately 20 per cent of the leukemias could not be so classified when first seen. Since it has been the practice at the National Cancer Institute to obtain sequential bone marrow aspirations for the evaluation of new chemotherapeutic agents, the opportunity presented itself to reexamine these marrows at frequent intervals and to perform special studies of phagocytosis as well as peroxidase and PAS stains. Whenever possible, supravital preparations were also examined. It has been our experience that a certain degree of maturation frequently occurred during therapy, permitting classification as to type of leukemia. Unquestionably, the continued attempt to categorize the acute leukemias into one of two types carried some arbitrariness in cases where very immature forms predominated. The present observations, however, appear to justify the classification to some extent. They indicate a different biologic behaviour of the two types of leukemia as far as the incidence of bone infarction is concerned. It should be emphasized that at the time the diagnoses in the patients here presented were made, this relationship was not yet known and therefore could not affect classification.

The present observations, however, appear to justify the classification to some extent. They indicate a different biologic behaviour of the two types of leukemia as far as the incidence of bone infarction is concerned. It should be emphasized that at the time the diagnoses in the patients here presented were made, this relationship was not yet known and therefore could not affect classification.

The amount of reticulin in the bone marrow was graded trace (N) when only an occasional fiber was found. Increases were graded slight (1+), moderate (2+), or marked (3 or 4+). Sample of grading may be seen in figures 1, 2, 5, and 8. Marrow cellularity was graded normal, increased, or decreased. Increases were graded 1+ (slight hypercellularity with decrease in the number of fat cells) to 4+ (marrow solidly packed with cells and complete absence of fat).

**RESULTS**

**Controls**

In normal bone marrows, reticulin fibers were infrequent (fig. 1) and occurred mainly perivascularly. Only an occasional fiber was found adjacent to a bone spicule, crossing the marrow spaces, or near the cortex.

Of eight patients with granulocytic or erythrocytic hyperplasia, marrow reticulin was slightly increased (1+) in four. The distribution of the reticulin was similar to that of normal marrow. The additional fibers appeared randomly distributed, without selective increase near the bone cortex, a frequent site of biopsies (fig. 2).

All but one case of chronic myelogenous leukemia had easily obtainable, markedly (4+) hyperplastic marrow with slightly increased reticulin (1+). In one case with three consecutive dry taps, there was an associated myelofibrosis.

Twenty patients with acute myelogenous leukemia were studied. All had at
Figs. 1–3.—Reticulin stains. X500.

Fig. 1.—Normal marrow. Note absence of reticulin in this area. Only occasional reticulin fibers occur in normal marrows.

Fig. 2.—Leukemoid reaction. Slight increase (1+) in reticulin.

Fig. 3.—Acute lymphocytic leukemia with minimal reticulin (N).
least a slight increase in marrow reticulin (1+). Six patients had moderate or marked increase in reticulin at the time of admission and two more developed a similar increase during observation. Only in those patients with moderate (2+) or marked (3 or 4+) increase in reticulin was there any difficulty in obtaining marrow by aspiration. In two patients, moderately abundant reticulin decreased during a remission. In none of these patients was bone or marrow infarction observed.

Representativeness of Marrow Biopsies

In order to explore the representativeness of single marrow biopsies, multiple Vim-Silverman specimens were taken from six patients at autopsy. Both posterior iliac crests, one anterior iliac spine, a vertebra and the sternum were sampled and compared with corresponding trephine sections from these areas. In all instances, the cellularity and composition of the marrow and the amount of reticulin were similar in the trephine and Vim-Silverman specimens from various sites. In an additional nine patients with acute lymphocytic leukemia, sections of marrow obtained with a trephine from several sites at autopsy were compared with an aspiration or Vim-Silverman needle biopsy obtained 2-14 days before death. Again the amount of reticulin in the pre and postmortem marrow specimens was similar.

Marrow Reticulin in Acute Lymphocytic Leukemia

In 19 of the 40 cases of acute lymphocytic leukemia, reticulin was normal or only slightly increased (1+) on admission and did not increase further during the period of observation. Clinical data on this group are summarized in table 1-Group A.

In a typical case, the initial marrow aspiration prior to beginning therapy was solidly hypercellular, the marrow spaces being packed with leukemic cells. Abundant marrow was easily obtainable by aspiration biopsy regardless of the degree of hypercellularity. Reticulin stains demonstrated occasional strands of reticulin fibers with a distribution similar to that of normal patients (fig. 3). One hundred and seventy-seven aspirations were attempted and only 28 were dry taps. In no instance was there persistent difficulty in obtaining marrows.

The patients in this group were followed for an average of 8.4 months, and were in complete remission for an average of 5.8 months. Most of the time they were in complete remission (72 per cent) or frank relapse (22 per cent) and only 6 per cent of the time in an intermediate status of partial remission. Remission was usually induced rapidly and normal hemopoiesis replaced the leukemic marrow infiltrate 1-4 weeks after beginning of therapy.

Twenty-one of the 40 patients with acute lymphocytic leukemia developed a moderate (2+) to marked (3 or 4+) increase in marrow reticulin (table 1-Groups B and C). This occurred as a uniformly and densely arranged network of fine strands throughout the marrow spaces. The fibers generally separated and encircled individual or small groups of cells. There was condensation of fibers only about blood vessels. Only an occasional fiber was seen
adjacent to bone spicules or the cortex. In the subsequent data and discussion, “increased reticulin” will be used to indicate a grading of at least 2+.

The 21 patients with increased reticulin fell into two distinct groups. In 11 patients the reticulin was increased for less than 35 per cent of their course (Group B) and in 10 the reticulin was increased for over 80 per cent of the time (Group C). In 13 of the 21 cases (nine of Group C and four of Group B), reticulin was increased when the patient was first admitted to the National Institutes of Health. Nine of the patients were untreated or had a month or less of treatment differing in no respect from the treatment of other cases of acute lymphocytic leukemia. Reticulin was present when the marrow was packed with leukemic cells and before therapy produced any observed effects on the tumor (figs. 7 and 8). In only a third of the cases in which reticulin was increased on admission did it subsequently decrease in amount. In the others the reticulin persisted through phases of treatment-induced hypoplasia and recurrence of leukemic infiltration.

In eight of the 21 patients (7 of Group B and 1 of Group C), reticulin increased during the time of observation. In all instances the reticulin increased while the patient was in a prolonged relapse. The usual pattern was for the initial relapse marrow to be easily obtained, abundant, markedly hypercellular, and without increased reticulin. After a period of treatment, the marrow became considerably less cellular, its architecture obliterated and its cells scattered in an amorphous eosinophilic granular substance. The marrow lacked the component of fat cells usually seen in normal marrow with a similar degree of hypoplasia and marrow reticulin became increased (figs. 4 and 5). This was in contrast to the patients without reticulin fibrosis (Group A). In this group, effacement of architecture and amorphous eosinophilic background were less pronounced during the initial phase of treatment. Subsequently, fat cells appeared as the marrow became less cellular and usually normal hematopoietic elements rapidly regenerated. In six of the eight cases in which reticulin had increased under treatment, it decreased in subsequent marrows and often reverted to only slightly increased amounts (table 1). The reabsorption of reticulin was occasionally quite rapid, and marked increases of reticulin (3 or 4+) disappeared on occasion in 3 weeks. In all instances, reduction of increased reticulin occurred while the patient was in remission (fig. 6).

The frequent occurrence of reticulin fibrosis was generally unsuspected in sections stained with H. & E. In only four cases could an occasional collagen fiber be demonstrated by the Masson stain indicating that reticulin fibrosis rarely progressed to collagen formation. Increased reticulin occurred in all age groups; however, there was a larger number of adults (over 20 years old) in Group C. Fifty per cent of this group were over 20 years of age as compared with 9 per cent in Group B and 10 per cent in Group A.

The patients with increased reticulin (2-4+) were distinctly less responsive to therapy than patients without increased reticulin (N or 1+). The patients in Group A spent 72 per cent of their illness in remission; those in Group B 40 per cent, and those in Group C only 6 per cent.
Fig. 4.—Patient V. P. Hypoplastic marrow during early phase of chemotherapy. H & E stain. X500.

Fig. 5.—Same area as fig. 4. Reticulin stain. X500. Markedly increased reticulin (4+).

Fig. 6.—Same patient as figs. 4 and 5, 3 months later, during remission. Reticulin decreased to nearly normal amounts (1+). X650.
Remissions occurred in only one of 10 patients in Group C, compared to nine of 11 in Group B, and 18 of 19 in Group A. Despite their poor response to chemotherapy, patients with increased reticulin seemed to survive longer in their terminal relapse, which often lasted over 2 months. Only one of the patients in Group A had a terminal relapse of that duration. Persistent leukopenia was frequent in patients of Group C and rare in Group A. However, the highest recorded white blood counts in the patients of different groups were not significantly different.

**Incidence of Dry Taps**

*Failure of aspiration when reticulin elevated*

The 10 patients of Group C with persistently increased reticulin (2, 3 or 4+) provided the greatest difficulty in obtaining aspiration marrow. This group (table 1) accounted for 109 of the 189 dry taps, and 102 of these dry taps occurred when reticulin was demonstrated to be increased. In these patients, only 10 aspirations were successful and these were obtained when reticulin was shown to be normal.

On the 11 patients with transient increased marrow reticulin (Group B), 53 of 159 attempted aspirations of marrow were unsuccessful. Thirty-nine of these failures occurred when Vim-Silverman needle biopsies showed increased reticulin. These patients had normal amounts of reticulin when marrow was easily aspirated.

The patients without increased reticulin (Group A) had the fewest dry taps and in none was there persistent difficulty in obtaining marrows.

*Successful aspiration when marrow reticulin was increased*

In seven of the patients of Groups B and C, a single successful aspiration marrow was obtained at the time marrow reticulin was increased. In these cases the marrow clumps were generally compact and small and the marrow cells did not spill over into the surrounding blood clot. These successes probably represent "needle punch biopsies" or the tearing away of a single particle from an otherwise cohesive marrow. An example is that of D. L. who had only a single successful aspiration preceded and followed by a total of 24 unsuccessful attempts.

Except when reticulin was increased, abundant and markedly hypercellular bone marrow with nearly 100 per cent leukemic cells was readily obtained from new cases of acute lymphocytic leukemia. However, after 1–3 weeks of chemotherapy it was frequently difficult to aspirate marrow. This problem was transitory, and within a few weeks marrow of normal cellularity and composition or hypercellular marrow often with erythroid hyperplasia could again be readily aspirated. Adequate material was available in only a few patients during the interval when aspiration was difficult. In these marrows the normal architecture was obscured and fat cells were absent. Lipid-laden macrophages, normal and abnormal cells were loosely scattered in an amorphous, granular, eosinophilic material, presumably the result of massive cell destruction. Such marrows did not contain increased amounts of reticulin.
Bone Infarction

Six of the 40 patients with acute lymphocytic leukemia developed ischemic bone infarction diagnosed in Vim-Silverman needle biopsies (figs. 9 and 10). The infarction involved both marrow and bone spicules and occurred when the marrow was packed with leukemic cells, as evidenced by the persisting outlines of closely packed necrotic cells (figs. 11-14). The earliest change was pyknosis and karyorrhexis of densely packed leukemic cells. Bone spicules still had viable osteocytes, but the small vessels were filled with amorphous eosinophilic debris. Within 10 days, only cellular outlines of the eosinophilic, coagulated, closely packed cells were visible. Osteocytes in the bone spicules had disappeared. The infarcts were sharply circumscribed and surrounded by a zone of granulation tissue and later a fibrous scar. Frequently, metaplastic bone spicules formed a dense pattern of new woven bone in the reactive zone, followed later by osteoblastic new bone formation on both pre-existing infarcted spicules and the woven bone (figs. 11 and 12). All patients belonged to Group C, in which reticulin was increased through most of the course of the disease. Reticulin was always markedly increased (3 or 4+) both in the infarcted and non-infarcted areas. In only one of the six patients with infarction did reticulin decrease sufficiently to allow subsequent successful aspiration of marrow. The zones of infarction appeared to be both widespread and persistent, since they could be demonstrated on repeated biopsies from various sites. In one patient they persisted for 9 months, despite some healing at the edges, and in another they were demonstrated in a number of biopsies taken from various sites around the iliac crest. At autopsy, the greater portion of the vertebral column and pelvis was infarcted in two cases. Recurrent new infarction was demonstrated by biopsy in two cases and may have occurred in other patients in view of their repeated bouts of severe bone pain.

Infarcts may heal nearly completely. In one case (K. M., admitted to the National Institutes of Health in May 1960, hence not included in this series) we observed infarction in a biopsy, but 4 months later at autopsy the only evidence of infarction was several small fibrotic scars containing bone spicules devoid of osteocytes.

Bone infarction was followed in all cases by a period of pancytopenia. One patient (J. S.) had marked peripheral eosinophilia (14,000 mm.\(^3\)) and erythema multiforme while developing bone infarction. At that time his marrow smear contained 75 per cent leukemic cells, 13 per cent eosinophils, 10 per cent neutrophils, and 2 per cent normoblasts.

The occurrence of bone pain was evaluated from both doctors’ and nurses’ notes. Of the total group of 40 patients, 48 per cent (19) had bone pain. It was mild in four patients and required medication in seven. In eight the pain was severe and a major manifestation of the illness. In the 10 patients of Group C, only one had no pain and another was a 2 month old child in whom it could not be evaluated. One patient had moderate and seven had severe pain. Six of these seven had bone infarction. The onset of severe pain was compatible with the time of infarction.

In the intermediate group (B), no one had severe pain; in Group A, two
Table 1.—Group A

Nineteen Patients without Increased Reticulin

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Duration Followed (months)</th>
<th>Months Complete Remission</th>
<th>Months Partial Remission</th>
<th>Months Relapse</th>
<th>Treatment Prior to Admission</th>
<th>Reticulin on Admission</th>
<th>Greatest Amount of Bone Marrow Involved</th>
<th>Months Reticulin Increased</th>
<th>Number of Months Clinically Improved</th>
<th>Number of Days Transient Dry Tap After Treatment</th>
<th>Bone Pain</th>
<th>Bone Infection</th>
<th>Status as of December 31, 1932</th>
<th>Hemoglobin Blood Cell Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. B.</td>
<td>6</td>
<td>9</td>
<td>7 ½</td>
<td>1 ½</td>
<td>N</td>
<td>N</td>
<td>10</td>
<td>0</td>
<td>3+</td>
<td>0</td>
<td>remission 75,300</td>
<td>0</td>
<td></td>
<td>remission 8400</td>
<td>22,000</td>
</tr>
<tr>
<td>W. C.</td>
<td>5</td>
<td>8</td>
<td>1 ½</td>
<td>1 ½</td>
<td>1+</td>
<td>1+</td>
<td>9</td>
<td>2</td>
<td>+</td>
<td>2+</td>
<td>remission 8400</td>
<td>0</td>
<td></td>
<td>expired 6400</td>
<td>273,000</td>
</tr>
<tr>
<td>L. C.</td>
<td>7</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1+</td>
<td>1+</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>expired 89,000</td>
<td>0</td>
<td></td>
<td>expired 90,000</td>
<td>121,000</td>
</tr>
<tr>
<td>D. D.</td>
<td>6</td>
<td>13 ½</td>
<td>7 ½</td>
<td>5 ½</td>
<td>1+</td>
<td>1+</td>
<td>24</td>
<td>2</td>
<td>+</td>
<td>0</td>
<td>expired 260,000</td>
<td>0</td>
<td></td>
<td>remission 7400</td>
<td>24,000</td>
</tr>
<tr>
<td>S. F.</td>
<td>3</td>
<td>5 ½</td>
<td>3</td>
<td>2 ½</td>
<td>1+</td>
<td>1+</td>
<td>7</td>
<td>2</td>
<td>+</td>
<td>0</td>
<td>expired 24,000</td>
<td>0</td>
<td></td>
<td>remission 3200</td>
<td>3200</td>
</tr>
<tr>
<td>K. L.</td>
<td>18</td>
<td>6</td>
<td>2</td>
<td>4</td>
<td>5 mo.</td>
<td>1+</td>
<td>5</td>
<td>0</td>
<td>3+</td>
<td>0</td>
<td>expired 6500</td>
<td>0</td>
<td></td>
<td>relapse 19,000</td>
<td>36,900</td>
</tr>
<tr>
<td>H. L.</td>
<td>7</td>
<td>11</td>
<td>8 ½</td>
<td>1½</td>
<td>1½ mo.</td>
<td>1+</td>
<td>9</td>
<td>3</td>
<td>+</td>
<td>1+</td>
<td>expired 6500</td>
<td>0</td>
<td></td>
<td>expired 6500</td>
<td>3200</td>
</tr>
<tr>
<td>P. M.</td>
<td>2</td>
<td>16</td>
<td>8 ½</td>
<td>2</td>
<td>1½ mo.</td>
<td>1+</td>
<td>14</td>
<td>2</td>
<td>+</td>
<td>0</td>
<td>expired 6500</td>
<td>0</td>
<td></td>
<td>relapse 67,200</td>
<td>6300</td>
</tr>
<tr>
<td>N. M.</td>
<td>10</td>
<td>6½</td>
<td>3½</td>
<td>1½</td>
<td>1½ mo.</td>
<td>1+</td>
<td>9</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>remission 6500</td>
<td>0</td>
<td></td>
<td>remission 196,000</td>
<td>36,900</td>
</tr>
<tr>
<td>B. M.</td>
<td>11</td>
<td>3</td>
<td>2½</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>4</td>
<td>1</td>
<td>+</td>
<td>0</td>
<td>remission 6500</td>
<td>0</td>
<td></td>
<td>relapse 67,200</td>
<td>6300</td>
</tr>
<tr>
<td>C. M.</td>
<td>14</td>
<td>8</td>
<td>7 ½</td>
<td>N</td>
<td>5½ mo.</td>
<td>1+</td>
<td>9</td>
<td>0</td>
<td>N</td>
<td></td>
<td>remission 3200</td>
<td>0</td>
<td></td>
<td>expired 196,000</td>
<td>36,900</td>
</tr>
<tr>
<td>M. R.</td>
<td>5</td>
<td>18</td>
<td>13</td>
<td>2 ½</td>
<td>1+</td>
<td>1+</td>
<td>14</td>
<td>2</td>
<td>+</td>
<td>1+</td>
<td>remission 6500</td>
<td>0</td>
<td></td>
<td>remission 6500</td>
<td>3200</td>
</tr>
<tr>
<td>J. R.</td>
<td>27</td>
<td>7</td>
<td>4</td>
<td>1½</td>
<td>1+</td>
<td>1+</td>
<td>11</td>
<td>2</td>
<td>+</td>
<td>0</td>
<td>expired 6500</td>
<td>0</td>
<td></td>
<td>remission 6500</td>
<td>3200</td>
</tr>
<tr>
<td>R. S.</td>
<td>2</td>
<td>7½</td>
<td>6½</td>
<td>1</td>
<td>1+</td>
<td>1+</td>
<td>7</td>
<td>4</td>
<td>+</td>
<td>0</td>
<td>remission 6500</td>
<td>0</td>
<td></td>
<td>relapse 67,200</td>
<td>6300</td>
</tr>
<tr>
<td>M. S.</td>
<td>4</td>
<td>7</td>
<td>6</td>
<td>1½</td>
<td>1½ mo.</td>
<td>1+</td>
<td>10</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>relapse 67,200</td>
<td>0</td>
<td></td>
<td>relapse 67,200</td>
<td>6300</td>
</tr>
<tr>
<td>C. S.</td>
<td>3</td>
<td>12½</td>
<td>3½</td>
<td>1½</td>
<td>1+</td>
<td>1+</td>
<td>10</td>
<td>1</td>
<td>2+</td>
<td>0</td>
<td>relapse 67,200</td>
<td>0</td>
<td></td>
<td>relapse 67,200</td>
<td>6300</td>
</tr>
<tr>
<td>P. S.</td>
<td>2</td>
<td>12½</td>
<td>9½</td>
<td>2½</td>
<td>1+</td>
<td>1+</td>
<td>13</td>
<td>5</td>
<td>+</td>
<td>0</td>
<td>relapse 67,200</td>
<td>0</td>
<td></td>
<td>relapse 67,200</td>
<td>6300</td>
</tr>
<tr>
<td>D. S.</td>
<td>34</td>
<td>1½</td>
<td>0</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>1</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>remission 6500</td>
<td>0</td>
<td></td>
<td>relapse 67,200</td>
<td>6300</td>
</tr>
<tr>
<td>R. V.</td>
<td>2</td>
<td>8½</td>
<td>7½</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>8</td>
<td>2</td>
<td>+</td>
<td>1+</td>
<td>remission 6500</td>
<td>0</td>
<td></td>
<td>remission 6500</td>
<td>3200</td>
</tr>
</tbody>
</table>

Total 157.5 110.5 8.75 38.35 177 28
Table 1.—Group B
Reticulin Increased Less Than 35 Per Cent of the Time (11 Patients)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Duration Followed (months)</th>
<th>Months Complete Remission</th>
<th>Months Partial Remission</th>
<th>Months Relapse</th>
<th>Treatment prior to Admission</th>
<th>Reticulin on Admission</th>
<th>Greatest Amount Reticulin</th>
<th>Months Reticulin Increased</th>
<th>Months Reticulin Decreased in Remission</th>
<th>Number of Marrow Cytology Examined</th>
<th>Number of Dry Taps</th>
<th>Time in Dry Tap Examination (months)</th>
<th>Bone Pain</th>
<th>Bone Infection</th>
<th>Status as of December 31, 1962</th>
<th>Highest White Blood Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>R. A.</td>
<td>2</td>
<td>12½</td>
<td>1</td>
<td>6½</td>
<td>5</td>
<td>¼ mo.</td>
<td>1+</td>
<td>3+</td>
<td>2½</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>22</td>
<td>9</td>
</tr>
<tr>
<td>M. B.</td>
<td>11</td>
<td>10</td>
<td>6½</td>
<td>1</td>
<td>2½</td>
<td>—</td>
<td>1+</td>
<td>2+</td>
<td>½</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>J. F.</td>
<td>4</td>
<td>11</td>
<td>0</td>
<td>2</td>
<td>6</td>
<td>—</td>
<td>1+</td>
<td>3+</td>
<td>2½</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>13</td>
<td>4</td>
</tr>
<tr>
<td>N. I.</td>
<td>4</td>
<td>7½</td>
<td>6½</td>
<td>—</td>
<td>½</td>
<td>—</td>
<td>2+</td>
<td>3+</td>
<td>½</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>M. J.</td>
<td>3</td>
<td>13</td>
<td>1½</td>
<td>2½</td>
<td>9½</td>
<td>2 mo.</td>
<td>1+</td>
<td>3+</td>
<td>3½</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>21</td>
<td>8</td>
</tr>
<tr>
<td>G. K.</td>
<td>13</td>
<td>1½</td>
<td>3½</td>
<td>1</td>
<td>3½</td>
<td>—</td>
<td>1+</td>
<td>3+</td>
<td>1½</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>L. L.</td>
<td>1½</td>
<td>11½</td>
<td>7</td>
<td>½</td>
<td>4½</td>
<td>2</td>
<td>2+</td>
<td>3+</td>
<td>5½</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>14</td>
<td>6</td>
</tr>
<tr>
<td>V. P.</td>
<td>2</td>
<td>11</td>
<td>9½</td>
<td>1½</td>
<td>1½</td>
<td>—</td>
<td>3+</td>
<td>3+</td>
<td>½</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>S. R.</td>
<td>2</td>
<td>10</td>
<td>5½</td>
<td>3½</td>
<td>1½</td>
<td>9 mo.</td>
<td>2+</td>
<td>3+</td>
<td>1½</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>D. W.</td>
<td>6</td>
<td>10</td>
<td>2½</td>
<td>½</td>
<td>7</td>
<td>10 mo.</td>
<td>N</td>
<td>2+</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>13</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>11½</strong></td>
<td><strong>43½</strong></td>
<td><strong>23½</strong></td>
<td><strong>45½</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><strong>150</strong></td>
<td><strong>58</strong></td>
</tr>
</tbody>
</table>

Table 1.—Group C
Reticulin Increased More Than 80 Per Cent of the Time (10 Patients)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Duration Followed (months)</th>
<th>Months Complete Remission</th>
<th>Months Partial Remission</th>
<th>Months Relapse</th>
<th>Treatment prior to Admission</th>
<th>Reticulin on Admission</th>
<th>Greatest Amount Reticulin</th>
<th>Months Reticulin Increased</th>
<th>Months Reticulin Decreased in Remission</th>
<th>Number of Marrow Cytology Examined</th>
<th>Number of Dry Taps</th>
<th>Time in Dry Tap Examination (months)</th>
<th>Bone Pain</th>
<th>Bone Infection</th>
<th>Status as of December 31, 1962</th>
<th>Highest White Blood Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. D.</td>
<td>44</td>
<td>2½</td>
<td>0</td>
<td>0</td>
<td>2½</td>
<td>3½ mo.</td>
<td>3+</td>
<td>4+</td>
<td>2½</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>9+</td>
<td>9</td>
</tr>
<tr>
<td>D. L.</td>
<td>15</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>½ mo.</td>
<td>1+</td>
<td>4+</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>25</td>
<td>24</td>
</tr>
<tr>
<td>F. L.</td>
<td>59</td>
<td>3½</td>
<td>0</td>
<td>1½</td>
<td>1½</td>
<td>2 mo.</td>
<td>1+</td>
<td>3+</td>
<td>3½</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>D. S.</td>
<td>5</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>11</td>
<td>2 mo.</td>
<td>3+</td>
<td>3+</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>J. S.</td>
<td>14</td>
<td>4</td>
<td>0</td>
<td>¾</td>
<td>3½</td>
<td>½ mo.</td>
<td>2+</td>
<td>4+</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>A. S.</td>
<td>38</td>
<td>11</td>
<td>0</td>
<td>7½</td>
<td>3½</td>
<td>—</td>
<td>4+</td>
<td>4+</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>R. S.</td>
<td>8</td>
<td>7</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>—</td>
<td>4+</td>
<td>4+</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>P. V.</td>
<td>29</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>¾ mo.</td>
<td>3+</td>
<td>4+</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>A. O.</td>
<td>52</td>
<td>1</td>
<td>0</td>
<td>1½</td>
<td>½</td>
<td>4½ mo.</td>
<td>3+</td>
<td>3+</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>C. K.</td>
<td>5/12</td>
<td>½</td>
<td>0</td>
<td>½</td>
<td>1½</td>
<td>1 mo.</td>
<td>3+</td>
<td>3+</td>
<td>½</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>53.25</strong></td>
<td><strong>3</strong></td>
<td><strong>11.25</strong></td>
<td><strong>39</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><strong>119</strong></td>
<td><strong>109</strong></td>
</tr>
</tbody>
</table>

From www.bloodjournal.org by guest on October 3, 2017. For personal use only.
Fig. 7.—Patient J. S. with acute lymphocytic leukemia. Two weeks’ duration of symptoms. Admission marrow. H & E stain. X500.

Fig. 8.—Same area as fig. 7. Silver stain reveals marked increase in reticulin (3+) unsuspected from H & E section. X500.

Fig. 9.—Same patient as figs. 7 and 8, 4 months later. Vim-Silverman biopsy. Note infarcted area (lack of staining of nuclei) in right half of section. H & E stain. X220.

Fig. 10.—Same area, reticulin stain. X220. Note uniform distribution of reticulin through infarcted and noninfarcted area.
patients had severe pain without bone infarction. As a general rule, severe bone pain indicated the occurrence of bone infarction.

Determinations of serum calcium, phosphorus, uric acid, and alkaline phosphatase were obtained in the majority of cases. However, no correlations could be made between these measurements and the occurrence of increased reticulin or bone infarction.

To confirm our suspicion that there was a greater incidence of bone infar-
Figs. 13–16.—See legends, facing page.
tion in acute lymphocytic leukemia than in acute myelogenous leukemia, bones from all patients with acute leukemia who died at the Clinical Center between June 1, 1961 and October 1, 1962 were examined. None of 33 patients with acute myelogenous leukemia, five of 35 patients with acute lymphocytic leukemia, and one of three with acute leukemia, type unclassified, had bone infarction.

**DISCUSSION**

The surprising frequency of reticulin fibrosis in patients with acute leukemia raised the question whether it might reflect a sampling error, especially since Vim-Silverman needle biopsies frequently included part of the bony cortex. It was considered possible that reticulin might be massed in this area. However, multiple Vim-Silverman specimens and larger bone sections taken at autopsy clearly indicated that the distribution of reticulin was usually uniform and the Vim-Silverman needle biopsies representative.

Increased reticulin was often present quite early in acute lymphocytic leukemia. Among the 40 patients with acute lymphocytic leukemia, 30 had less than a month's history when admitted. Of these, nine already had a definite increase in reticulin. Conversely, of the total of 13 patients who had increased reticulin on admission, 11 had a history of 2 months or less. Only eight of the 27 patients who did not have increased reticulin on admission later developed significant reticulin fibrosis, which was usually reversible after short duration. These findings suggest that duration of disease is only a minor factor in reticulin fibrosis. The appearance of reticulin fibrosis before the institution of chemotherapy and the similarity of treatment in all patients make it appear unlikely that chemotherapy contributes to reticulin fibrosis.

The pathogenesis of reticulin fibrosis is not known. Possibly some patients respond to marrow invasion in this particular fashion, or certain leukemias may in some manner evoke this response because of rapid growth or some other characteristic. The process may be induced by the products of cellular death. Possibly, in the cases resistant to therapy, there is continued death of leukemic cells under the influence of therapy.

Reticulin fibrosis may denote a particular subgroup of patients with acute lymphocytic leukemia. The response to therapy of the patients with persistent reticulin fibrosis (Group C) was clearly poorer than those (Group A) without it. Their course was complicated by prolonged periods of severe leukopenia. They tended to be older and comprised the majority of cases of "non-responders" described by Freireich. Patients who had reticulin fibrosis for short periods (Group B) had a response intermediate between Groups A and C.

---

Fig. 13.—Patient F. L. with acute lymphocytic leukemia in whom infarction had been demonstrated 3 months earlier. This autopsy specimen depicts diffuse leukemic infiltration with fresh infarction in lower half of section. H & E stain. X100.

Fig. 14.—Same area. Reticulin (3+) pre-existing in freshly infarcted area. H & E stain. X100.

Fig. 15.—Same patient. Leukemic infiltration of wall of periosteal blood vessels. H & E stain. X100.

Fig. 16.—Same patient. Leukemic infiltration of entire vessel wall, raising intima and partially occluding vessel. H & E stain. X280.
However, since the average age of Group C is much higher than that of Group A, the poorer prognosis might be etiologically related more to age than to reticulin fibrosis.

The occurrence of dry taps has been attributed to faulty technic, fibrosis, and aplasia of a marrow packed with abnormal cells. We have not found marrows packed with abnormal cells difficult to aspirate unless there was also reticulin fibrosis. However, in nearly every instance when the marrow reticulin was increased, regardless of the degree of cellularity, the physician experienced difficulty in obtaining marrow by aspiration. Conversely, in the cases in which marrow was persistently difficult to aspirate, reticulin was always found to be increased.

Although failure to obtain marrow by aspiration in cases of acute lymphocytic leukemia could usually be ascribed to the presence of increased reticulin, such fibrosis could not account for single dry taps obtained a few weeks after initiation of chemotherapy. These dry taps occurred between two successful marrow aspirations, a prior one with frankly leukemic marrow, and a subsequent one of normal composition. Such marrows did not contain increased amounts of reticulin, but did have abundant, amorphous eosinophilic material separating scattered normal or abnormal cells. This material effaced the normal architecture. It probably represented the result of rapid dissolution of leukemic cells and was difficult to recognize as marrow in aspiration specimens. These marrows were unlike hypocellular marrows of aplastic anemia which have numerous fat cells and focal islands of hematopoietic cells in which aspirations usually were successful.

That reticulin fibrosis might be the major cause of dry taps in conditions other than leukemia cannot be stated. The rarity of aspiration failures during leukemic remissions, when the marrows were structurally normal, confirmed the impression that in experienced hands dry taps are seldom due to technical difficulties and that specific causes should be sought in such instances.

Ischemic bone infarction has previously been demonstrated at autopsy of patients with acute leukemia. Infarction was thought to occur in the terminal weeks of life. We have found that it may also occur early in the course of the disease and that there may be repeated bouts of ischemic infarction. Infarction occurred only when the marrow was packed with leukemic cells. In several patients the leukemic cells invaded the walls and occluded the lumina of periosteal blood vessels. In others the tumor may have outgrown its blood supply. Even when it was extensive and widespread, bone infarction usually failed to produce specific radiographic changes. However, aseptic necrosis of the femoral head simulating Legg-Perthes disease was observed in one patient and in others there was nonspecific mottling of the bone.

Infarction heralded a difficult period in the patient’s management; only one patient subsequently had a complete remission and patients generally had a prolonged period of pancytopenia following episodes of infarction.

Bone infarction was noted only in patients with acute lymphocytic leukemia. It must be quite uncommon in myelogenous leukemia. In our series of sequential marrow biopsies, it was seen in six of 40 patients with acute lymphocytic leukemia and in none of 20 with acute myelogenous leukemia. A study
of a series of autopsy specimens of bones from patients with acute leukemia showed an incidence of six of 35 with acute lymphocytic leukemia and none of 33 cases of acute myelogenous leukemia.

The correlation of bone infarction with acute lymphocytic leukemia is in keeping with the observations of Carver, who found gross bone lesions in six of 86 patients with acute and chronic lymphocytic leukemia and in only one of 83 with myelogenous leukemia. In contrast, Hashimoto reported the occurrence of bone necrosis in nine of 58 patients with acute myelogenous leukemia. However, in his series of 90 patients with leukemia of all types, 58 were diagnosed as acute myelogenous leukemia and only eight as lymphocytic leukemia. Our experience as well as that of others is that acute lymphocytic leukemia is much more frequent than reported in this series. It is likely, therefore, that the diagnostic criteria used by Hashimoto in the classification of his leukemias differs from that in general use in the U.S.A.

All patients with bone infarction also had persistently increased reticulin (Group C). The presence of 3 or 4+ reticulin throughout the areas of infarction clearly indicated that reticulin formation was not a part of the peripheral organization of the infarct, but had preceded it. Interestingly, infarction of tumor and bone is also common in primary reticulum cell sarcoma of bone. The significance of the coexistence of reticulin fibrosis and infarction is unknown.

Bone pain is known to be frequent in patients with leukemia, but it is severe in only a minority of patients, and is then often associated with infarction. Severe pain was noted in four of 30 patients under 15 years of age and in five of 10 patients 15 years or older. Six of the nine had infarction.

Bone infarction, and with it severe pain, occurred relatively more frequently in adults with acute lymphocytic leukemia than in children. No explanation is apparent from our data. We feel that bone infarction should be included in the list of causes of bone pain in acute lymphocytic leukemia and that it is the major cause of severe bone pain.

A group of cases having some features in common with our cases of acute lymphocytic leukemia with persistent reticulin fibrosis (Group C) have been reported as leukemic reticuloendotheliosis or histiocytic leukemia. Leukemic reticuloendotheliosis is said to occur at any age (5 months to 76 years), but predominantly in adults. Most patients have leukopenia and are resistant to therapy. Marrow aspiration attempts frequently result in "dry taps." The leukemic cells are peroxidase-negative and generally non-phagocytic. At autopsy the distribution of leukemic infiltration is identical with that of acute lymphocytic leukemia. The features which have been claimed to differentiate leukemic reticuloendotheliosis from acute lymphocytic leukemia are larger cells; more abundant cytoplasm with irregular borders; small, blunt pseudopodia; an eosinophilic reticulin network; prominent sinusoids in marrow and spleen with reticuloendothelial cells projecting into the lumen. We have not encountered in our material any cases that could be differentiated by these features from acute lymphocytic leukemia.

The occasional occurrence of early fibrosis described here in acute leukemia may be common to other myeloproliferative disorders. In particular, Dameshek
and Gunz\textsuperscript{21} have described cases of myeloid metaplasia with early connective tissue fibrosis of the marrow under the term “acute myelofibrosis.” Possibly, a study of reticulin formation in cases of myeloid metaplasia may be of prognostic significance as it appears to be in acute lymphocytic leukemia.

**Summary**

Reticulin fibrosis was found in 21 of 40 patients with acute lymphocytic leukemia when marrows were studied sequentially by Vim-Silverman needle biopsies. Reticulin fibrosis frequently occurred early in the development of the disease. Mild degrees were completely reversible with remission, severe degrees usually persisted, even through remissions. Fibrosis appeared to develop during relapse. Duration of the disease in itself had little influence on the degree of reticulin fibrosis, and collagen fibrosis seldom followed reticulin fibrosis even after many months’ duration.

The prognosis of patients with reticulin fibrosis of their marrows was definitely poorer than for the group without increased reticulin. Reticulin fibrosis virtually always prevented successful marrow biopsies by the standard technic of needle aspiration.

Bone necrosis occurred in 11 of 75 patients with acute lymphocytic leukemia, but in none of 53 patients with acute myelogenous leukemia studied by Vim-Silverman needle biopsies during life or at autopsy. Bone necrosis was the major cause of severe bone pain and it was always associated with reticulin fibrosis of the marrow. Bone infarcts were not associated with short survival in all cases, but in general the prognosis of patients with bone necrosis was even poorer than that of patients with reticulin fibrosis but without demonstrable infarction.

**Summario in Interlingua**

Fibrosis a reticulina esseva constatate in 21 de 40 patientes con acute leucemia lymphocytic quando le medullas esseva studiate sequentialmente per biopsia a agulía Vim-Silverman. Fibrosis a reticulina occurreva frequentemente in un stadio precoce del disveloppamento del morbo. Leve grados se reverteva completamente in le remission; grados sever persisteva usualmente, semper in remission. Fibrosis pareva disveloppar se in recidivas. Le duration del morbo per se habeva pauc influentia super le grado de fibrosis a reticulina, e illo esseva rar que fibrosis a collageno sequeva fibrosis a reticulina, semper post menses de presentia del secunde.

Le prognose pro patientes con fibrosis a reticulina in lor medullas esseva definitivemente minus favorable que pro le grupo de patientes sin augmentos de reticulina. Le presentia de fibrosis a reticulina preveniva quasi sin excepcion le successo de biopsias per le technica standard de aspiration a agulía.

Necrosis ossee occurreva in 11 de 75 patientes con acute leucemia lymphocytic sed in nulle del 53 patientes con acute leucemia myelogene qui esseva studiate per medio de agulías Vim-Silverman o necropticamente. Necrosis ossee esseva le causa major de sever dolores de osso, e illo esseva semper
associate con fibrosis a reticulina in le medulla. Infarcimento de osso non eseva necessarimente associate con breve superviventia, sed—a generalmente parlar—le prognose de patientes con necrosis ossee eseva ancora minus favorabile que in le caso de patientes con fibrosis a reticulina e sin demon-strabile infarcimento.

ACKNOWLEDGMENTS

We thank Dr. L. B. Thomas, Head, Surgical Pathology and Post-mortem Service, Pathologic Anatomy Branch, Clinical Center for the use of the facilities and postmortem material of his Service; Dr. Patrick Reames, Diagnostic X-Ray Department, Clinical Center for reviewing the radiographic films; Miss Mary Catherine Bowling and Miss Vivian J. Franco for skilled technical assistance.

REFERENCES

17. Silverman, F. N.: The skeletal lesions in leukemia: Clinical and roentgenographic observations in 103 infants and children with a review of the

Donald W. Kundel, M.D., Resident, Clinical Pathology Department, Clinical Center, National Institutes of Health, Bethesda, Md.

George Brecher, M.D., Chief, Section on Hematology, Clinical Pathology Department, Clinical Center, National Institutes of Health, Bethesda, Md.

Gerald P. Bodey, M.D., Clinical Associate, Medicine Branch, National Cancer Institute, National Institutes of Health, Bethesda, Md.

Geoffrey M. Brittin, M.D., Resident, Clinical Pathology Department, Clinical Center, National Institutes of Health, Bethesda, Md.
Reticulin Fibrosis and Bone Infarction in Acute Leukemia. Implications for Prognosis

DONALD W. KUNDEL, GEORGE BRECHER, GERALD P. BODEY and GEOFFREY M. BRITTIN