

## Chronologic Sequence in Appearance of Clinical and Laboratory Findings Characteristic of Chronic Myelocytic Leukemia

By Nanao Kamada and Haruto Uchino

Clinical and laboratory findings of chronic myelocytic leukemia (CML) were analyzed by means of two different methods; first, retrospective analysis of hematologic data from 16 patients whose laboratory examinations had been performed twice a year for 5–10 yr prior to the development of CML and whose diagnosis was made in a very early stage of the disease, and, second, analysis of hematologic data at the time of diagnosis from 102 Ph<sup>1</sup>-positive CML patients. The same results were obtained with each method. A possible chronologic sequence in the appearance of abnormalities was the demonstration of the Ph<sup>1</sup> chromosome in the bone marrow (leukocyte count of less than  $10 \times 10^9$ /liter), basophilia, thrombocytosis and low neutrophil alkaline phosphatase activity

( $10 \times 10^9$ /liter), increase of immature granulocytes ( $20 \times 10^9$ /liter), increase of vitamin B<sub>12</sub> in the serum ( $25 \times 10^9$ /liter), splenomegaly ( $52 \times 10^9$ /liter), and subjective symptoms (higher than  $70 \times 10^9$ /liter). The curve showing proliferation of Ph<sup>1</sup>-positive cells was calculated to be  $\log y = 0.053x + 10.023$  ( $x$ , months from time when the leukocyte count was  $10 \times 10^9$ /liter;  $y$ , leukocyte count of  $\times 10^9$ /liter). From the formula, the elapsed time from outbreak of a single cell with a Ph<sup>1</sup> chromosome in the body to a leukemic cell mass of  $10 \times 10^9$ /liter was estimated to be 6.3 yr. The hypothesis that malignant transformation of Ph<sup>1</sup>-positive cells could develop in the early proliferative stage may contribute to elucidating the pathophysiology of blastic crisis of CML.

**P**HILADELPHIA-POSITIVE chronic myelocytic leukemia (CML) with variant translocations other than the standard one (9;22) has been reported;<sup>1,9</sup> and the frequency of these cases seems to be about 10%, among Ph<sup>1</sup>-positive cases. However, the clinical features and prognoses of these patients do not differ from those of standard Ph<sup>1</sup>-positive cases.<sup>10</sup> Apart from the Ph<sup>1</sup> chromosome, the clinical signs of CML are less variable than in other forms of leukemia, characterized by insidious onset, progressive splenomegaly, low activity of neutrophil alkaline phosphatase (AP), leukocytosis of greater than  $10^9$ /liter with grossly elevated platelet, neutrophil, and (usually) basophil counts, and immature granulocytes in the peripheral blood. The bone marrow is hypercellular with an increased number of immature granulocytes and megakaryocytes.

There is a paucity of reports concerning the development of clinical signs of CML, especially on the chronological sequence of appearance. By the end of 1975, a total of 76 survivors exposed within a radius of 4 km to the atomic bomb in Hiroshima, Japan in 1945 had been diagnosed as CML, including 27 of our own cases during the 14-yr period 1962–1975. The establishment of a

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medical and hematologic survey program for atomic bomb survivors under the sponsorship of the Japanese Government in 1962 and the development of a technique for detecting the Ph<sup>1</sup> chromosome in bone marrow cells enabled us to detect the disease in the preclinical stage and also made it possible to analyze the past hemograms retrospectively to 1962.

The purposes of the present study were to determine the chronological appearance of clinical features characteristic of CML so as to evaluate the progression of CML and also to estimate the time from appearance of a single cell with a Ph<sup>1</sup> chromosome in the body to a state of  $10 \times 10^9$ /liter of leukemic cells in the peripheral blood.

#### MATERIALS AND METHODS

From April 1962 to December 1975, a total of 102 patients with or without a history of atomic bomb exposure were diagnosed as CML at the Atomic Bomb Survivors Health Control Clinic (ABSHCC) and/or Hiroshima University Hospital by laboratory examinations such as leukocyte differential, neutrophil alkaline phosphatase staining, and chromosome study of bone marrow cells. Chromosome preparations were made using a modification of the direct bone marrow technique of Tjio and Whang.<sup>11</sup> Medical records kept at ABSHCC, where routine examinations of the blood, urine, liver function, and blood pressure were performed twice a year on about 80,000 atomic bomb survivors, were used in the analyses. Such data as leukocyte differential, neutrophil AP score, serum iron, and vitamin B<sub>12</sub> prior to CML development were also analyzed retrospectively for each patient. Neutrophil AP scores for normal adults ranged from 170 to 320 (rate 65%, 98%).

#### RESULTS

Of the 102 CML patients, 41 were radiation-related cases: 27 were directly exposed to atomic bomb radiation, 19 of whom were exposed within 2 km at the time of bombing; 8 were exposed between 2.1 and 4.0 km. Of the remaining 14 cases, 10 had entered the city within 3 days following the atomic bomb detonation, and 4 were offspring of atomic bomb survivors. Sixty-one were non-exposed patients. Twenty patients, including 16 atomic bomb survivors who were serially examined, were diagnosed in the early phase of the disease with leukocyte counts of less than  $50 \times 10^9$ /liter without hepatosplenomegaly, without any subjective symptoms, and with the Ph<sup>1</sup> chromosome in most of the bone marrow cells in metaphase. The frequency of Ph<sup>1</sup>-positive cells in the bone marrow in metaphase was 100% in 17 patients whose leukocyte counts at the

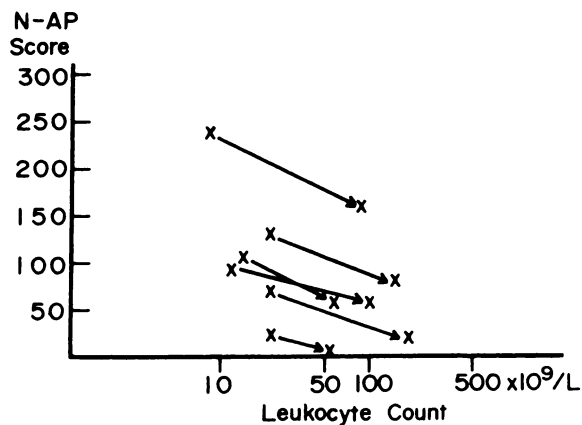


Fig. 1. Neutrophil AP score and leukocyte count during the untreated period (6-18 mo). Each arrow indicates an individual case.

time of bone marrow aspiration ranged from 8.5 to  $42 \times 10^9$ /liter. In only three patients were Ph<sup>1</sup>-negative cells found, being 2.6%, 6.5%, and 14.4%, and the leukocyte counts at the time of bone marrow aspiration were 12.4, 20, and  $13.3 \times 10^9$ /liter, respectively. Neutrophil AP in these patients demonstrated a relatively low activity even though the leukocyte count was less than  $20 \times 10^9$ /liter at the time of diagnosis. A further decrease in neutrophil AP score was observed with an increase in leukocyte count (Fig. 1).

An absolute increase of basophils was found in the early stage of the disease. Figure 2A shows the absolute basophil counts per liter of 102 CML patients at the time of diagnosis. An obvious relationship between absolute number of basophils and leukocyte count could be expressed by the formula  $y = 35.17x^{0.592}$ . The leukocyte count at the y intercept was calculated as  $8.9 \times 10^9$ /liter. The line indicates that basophils had already increased in the peripheral blood two to five times greater than the normal range, even when the leukocyte count was within  $20 \times 10^9$ /liter. The platelet count did not show any definite relationship to the leukocyte count. However, a relatively higher platelet count was observed in cases found in the early phase of the disease (Fig. 2B).

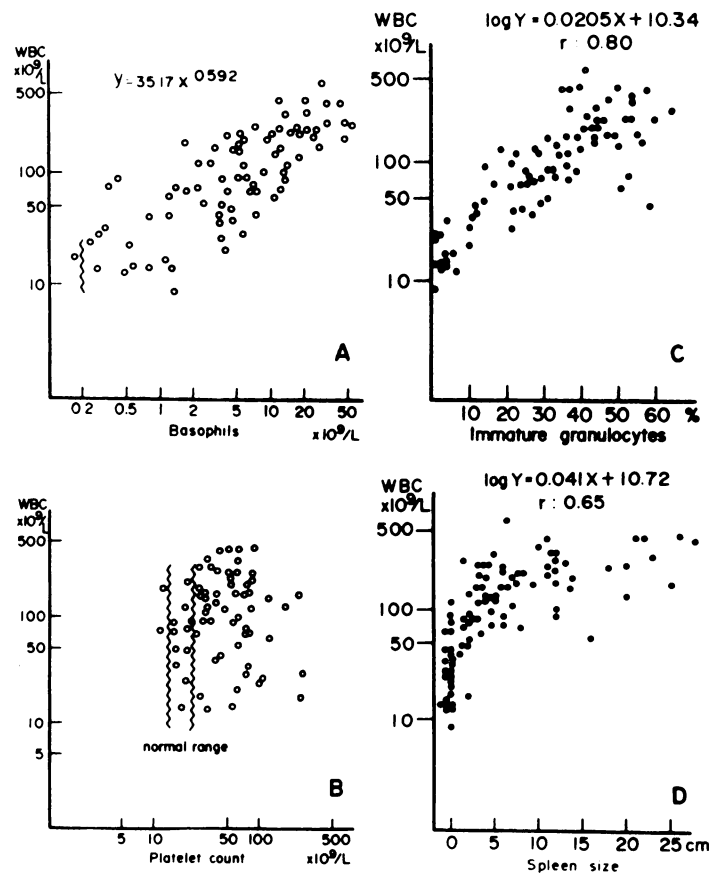


Fig. 2. Clinical and laboratory data at time of diagnosis of 102 CML patients. (A) Absolute number of basophils; (B) platelet count; (C) percentage of immature granulocytes; (D) spleen size.

The percentage of immature granulocytes at the time of diagnosis is shown in Fig. 2C. The regression line of immature granulocytes was calculated as  $\log y = 0.025x + 10.34$ , with coefficient  $r = 0.80$  ( $x$ , percentage of immature granulocytes;  $y$ , leukocyte count  $\times 10^9/\text{liter}$ ). From this formula, the leukocyte count at the  $y$  intercept was calculated as  $21.8 \times 10^9/\text{liter}$ . The regression formula of spleen size recorded in cm beneath the costal margin of patients at the time of diagnosis was given by  $\log y = 0.041x + 10.72$  (Fig. 2D). This observation indicated that the spleen became palpable when the leukocyte count was about  $52 \times 10^9/\text{liter}$  in the peripheral blood and that once the spleen became palpable spleen size markedly increased with minimal elevation of the leukocyte count. In four cases serum vitamin  $B_{12}$  was serially measured during the early phase of the disease. A high serum vitamin  $B_{12}$ , such as  $8000 \mu\text{g}/\text{liter}$ , was observed in two patients whose leukocyte counts of  $25 \times 10^9/\text{liter}$  were two to three times higher than the normal value. The leukocyte count when the line crossed the  $y$  axis gave a starting point for each abnormal finding in the peripheral blood. Basophils, immature granulocytes, and spleen size began to increase at leukocyte counts of around 8.9, 21.8, and  $52 \times 10^9/\text{liter}$ , respectively.

A possible chronological sequence in the appearance of abnormalities characteristic of CML is summarized in Fig. 3. The  $\text{Ph}^1$  chromosome was confirmed to be present in the very early phase of the disease (leukocyte count less than  $10 \times 10^9/\text{liter}$ ). Thereafter, basophilia, a relatively high platelet count, and low neutrophil AP activity became apparent (at the  $10 \times 10^9/\text{liter}$  level). Immature granulocytes (more than 5%) appeared in the peripheral blood at a leukocyte level of  $20 \times 10^9/\text{liter}$ . Serum vitamin  $B_{12}$  increased at a leukocyte count of about  $25 \times 10^9/\text{liter}$ . The spleen became palpable when the leukocyte count reached about  $52 \times 10^9/\text{liter}$ . Lastly, subjective symptoms, such as easy fatigability or gastrointestinal disturbance, developed.

The increase in leukocyte count during the early phase of CML was calculated from ten patients who received hematologic examinations at different times in the course of the disease during the untreated period (Table 1). The regression formula was  $\log y = 0.053x + 10.023$ , with coefficient  $r = 0.79$  ( $x$ , months from time when the leukocyte count was  $10 \times 10^9/\text{liter}$ ;  $y$ , leukocyte

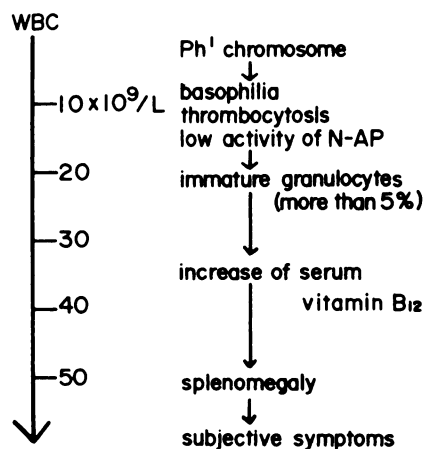


Fig. 3. Chronological sequence in appearance of abnormalities characteristic of CML.

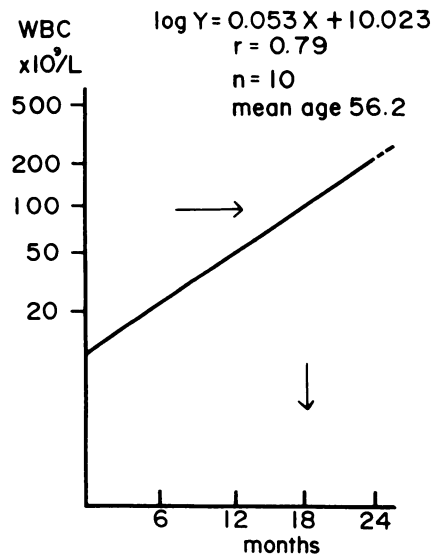
**Table 1. Increase of White Blood Cells in the Early Stage of CML**

Case	WBC ( $\times 10^9$ /liter)		
	At Time of Diagnosis or Examination Prior to Diagnosis	At Time of Treatment	Interval (mo)
1	9.7	50	7
2	9.7	34.7	9
3	8.7	90	11
4	6.4	45.5	12
5	13.8	48	12
6	8.8	45.3	13
7	11.7	93.0	15
8	8.8	40.2	15
9	10.6	55.8	18
10	8.9	51	24

count  $\times 10^9$ /liter). According to this formula the elapsed time from leukocyte count of  $10 \times 10^9$  to  $100 \times 10^9$ /liter was 19 mo (Fig. 4).

**DISCUSSION**

The present study was considered to be significant and unique in that it was carried out on 102 patients in whom the presence of Ph<sup>1</sup> chromosome in the bone marrow was confirmed and that 20 of the 102 patients were diagnosed in the early phase of the disease. These distinctive features permitted calculation with confidence of the regression lines of basophils, immature granulocytes, splenomegaly, and elapsed time of the disease. These calculations led to a possible chronological sequence in which the abnormalities of CML appeared, i.e., presence of Ph<sup>1</sup> chromosome in the bone marrow, basophilia, thrombocytosis and low neutrophil AP activity, appearance of immature granulocytes in the peripheral blood, increase of vitamin B<sub>12</sub> in serum, splenomegaly, and finally subjective symptoms.



**Fig. 4. Rising curve of leukocyte count in the early phase of CML. Elapsed time from WBC of  $10 \times 10^9$  to  $100 \times 10^9$ /liter was calculated from the formula as 19 mo.**

Relatively few observations have been reported on the early phase of CML. Wintrobe and Hasenbush<sup>12</sup> described five cases of preclinical CML characterized by unexplained leukocytosis (11, 20, 26, 28, and  $77 \times 10^9$ /liter), relatively small percentages of immature granulocytes, and relatively long periods from leukocytosis to development of splenomegaly. Weitz<sup>13</sup> reported a case of CML in an x-ray technician who received frequent hematologic examinations for 29 mo. He observed an increase in basophils and eosinophils in the early phase of the disease, with a leukocyte count of less than  $10 \times 10^9$ /liter, and pointed out that the increase of these cells might be the earliest sign of the disease. An observation of the same kind was made in atomic bomb survivors by Molony and Lange.<sup>14</sup> They reported three patients with CML who were diagnosed on routine medical examination and noted basophilia in two of their cases. In the present study basophilia was confirmed in the early phase of the disease in 15 of 16 patients who had been examined 5–10 yr prior to the clinical onset of CML.

There are some reports supporting the hypothesis that the Ph<sup>1</sup> chromosome is a marker of the preleukemic state and not a leukemic process itself.<sup>15,16</sup> It may be assumed that Ph<sup>1</sup>-positive cells must be influenced by host conditions and might disappear spontaneously during the very early phase of the disease, as in other malignant tumors.<sup>17</sup> However, it seems that in most of the CML cases Ph<sup>1</sup>-positive cells are really present in the bone marrow when the leukocyte count in the peripheral blood is  $5 \times 10^9$ /liter. A single-cell origin of Ph<sup>1</sup>-positive cells has been clearly demonstrated by glucose-6-phosphate dehydrogenase (G-6-PD) enzyme,<sup>18</sup> 46/47 mosaicism,<sup>19</sup> and behavior of fluorescein,<sup>20</sup> and it is apparent that one Ph<sup>1</sup>-positive cell proliferates gradually up to the  $10^{11}$ /liter level in the body under some favorable proliferative condition.

The time needed for a large amount of cell mass ( $10^{11}$ /liter) of Ph<sup>1</sup>-positive cells to accumulate was first calculated by Skipper<sup>21</sup> as about 3.2 yr, based on cell generation time. In the present study the proliferative curve of Ph<sup>1</sup>-positive cells was calculated as  $\log y = 0.053x + 10.023$ , with the leukocyte count increasing from  $10 \times 10^9$ /liter. If the Ph<sup>1</sup>-positive cells increased exponentially during the course of the disease up to the level of  $100 \times 10^9$  cells (Fig. 4), the time when the first Ph<sup>1</sup>-positive cell appeared in the body (when  $y = 1$ ,  $x = 75.9$ ) could be calculated from the formula. The value, 75.9 mo (6.3 yr), is much longer than that given by Skipper but shorter than the 9.3 yr calculated from the cell generation time based on 100 days doubling time of Ph<sup>1</sup>-positive cells.<sup>22</sup> Furthermore, a proliferation period of 6.3 yr seems reasonable when compared with the latent period of CML in atomic bomb survivors<sup>23</sup> (8 yr; a peak incidence of CML was observed in 1953) and of polycythemia vera treated with <sup>32</sup>P and/or x-rays (leukemia usually develops 5–10 yr after treatment).<sup>24</sup>

The development of CML can be divided into three stages: proliferative stage of 6.3 yr, preclinical stage of 19 mo, and advanced stage of 3 yr of mean survival time from diagnosis. When a patient is diagnosed as having CML at a leukocyte count of  $100 \times 10^9$ /liter in the peripheral blood, a mutation resulting in a Ph<sup>1</sup> chromosome must have occurred about 8 yr before. During the proliferative stage, spontaneous regression of Ph<sup>1</sup>-positive cells may occur. Some Ph<sup>1</sup>-positive cells may have undergone further malignant transformation resulting

in blastic crisis, which has been reported as acute lymphocytic (or myelocytic) leukemia with Ph<sup>1</sup> chromosome<sup>25-29</sup> or CML without chronic phase.<sup>30-32</sup> In 1967 Zuelzer<sup>33</sup> suggested that if the Ph<sup>1</sup> chromosome itself were predisposed to the development of acute leukemia, transformation to acute leukemia could occur any time after mutation. If it occurred early in the disease, the resulting leukemia might resemble acute leukemia; if it occurred later, it might be considered to be blastic crisis. Chronological representation of the development of CML facilitates an easier understanding of blastic crisis of the disease and also suggests that some important problems are involved in relation to early transformation and cytologic and cytogenetic features of these blast cells. Most cases of CML terminate in blastic crisis, and the time from diagnosis until blastic transformation differs among individual patients. Further study on cell characterization of blast cells in the chronic phase by means of cell surface markers, colony-forming activity, cytogenetic analysis, and search for factor(s) that suppress maturation of leukemic cells may provide aids in predicting the time of blastic transformation.

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