

Chromosomal Aberrations in Polycythemia Vera

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THE RELATIVELY HIGH RISK of developing leukemia in the course of polycythemia vera (PV),¹ and the presence of both specific and non-specific chromosomal aberrations in patients with myeloproliferative disorders,²⁻⁵ led us to undertake a prospective follow-up study of patients with PV. In this study, an attempt is made to correlate the presence of various types of chromosomal abnormalities in polycythemia with the subsequent development of leukemia, and to look for predictive indicators.

The present report is a preliminary cross-sectional look at the first peripheral blood cytogenetic analysis by diagnostic category and type of treatment.

PATIENTS AND METHOD

The study group is comprised of patients with any form of polycythemia seen at two major hospitals since January 1966. The non-PV groups and regular blood donors serve as controls.

In each case, bone marrow and peripheral blood chromosomal analyses are performed on first referral to the hospital ward or clinic, and are repeated once a year. Between these examinations, the patient is followed by his personal physicians, but additional peripheral blood cytogenetical examinations are performed if clinically indicated. Current analysis is limited to the first peripheral blood examination of patients treated by P³² and/or phlebotomy, and to those with no previous therapy. Bone marrow findings will be reported later, since at this point the number of successful bone marrow analyses does not enable meaningful evaluation.

Peripheral blood leukocytes were cultured in T.C. 199 medium with Phytohemagglutinin to induce mitosis.⁶ Cells were harvested 72-96 hours from onset of culture, after exposure to colchicin for four hours. Chromosome counts and microscopic analysis were performed on 40 randomly selected scorable cells per person, on the average. Only examinations with a minimum of 10 scorable cells were included.

RESULTS

A variety of nonspecific chromosomal aberrations, including hyper- and hypodiploidy, breaks, fragments, and apparent abnormal chromosomes, were noted in PV patients with no previous radiation treatment (Fig. 1). Similar abnormalities were present in benign erythrocytosis without radioactive treatment. In both groups there was a striking lack of pattern in the chromosomal aberrations, not only when comparing cells of different patients, but, excluding one or two cases, when comparing cells of the same individuals as well. It is of interest that the aberrations in P³² treated patients (Figs. 2-3) did not

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First submitted November 22, 1968; accepted for publication August 14, 1969.

Supported in part by USPHS Research Agreement BDPEC-CD-IS-17.

differ in nature from those observed in the untreated group, except for the considerably more frequent occurrence of dicentric chromosomes.

The percentage of aneuploid cells among cells counted, by diagnostic group, was computed for each individual separately (Fig. 4), and for each diagnostic group as a whole (Table 1). The frequency of aneuploidy is significantly higher in benign erythrocytosis, and in the treated and untreated PV groups as compared with blood donors, relative polycythemia, and secondary polycythemia [p (F arcsine transformation) < 0.001]. There is no statistical difference among the former three groups, although cases with higher values appear more frequently among the P^{32} treated PV patients. The excess of



Fig. 1.—Karyotype of patient with polycythemia vera before radiophosphorus therapy (note trisomy in group E; four accessory acentric chromosomes).



Fig. 2.—Chromosomal aberrations in patient with polycythemia vera, after radio-phosphorus therapy (note double monosomy in Group B, large submetacentric accessory chromosome and two rings).

aneuploidy consists of both hypo- and hyperdiploid cells, though hypodiploid cells appear more frequently than hyperdiploid ones. In addition, the range of aneuploidy is wider in benign erythrocytosis and in the two PV groups, with about 3.5 per cent of the cells deviating by at least two chromosomes from the normal count, as compared with less than 1 per cent in the other three diagnostic groups.

Polyploid cells appear more frequently in P^{32} treated PV patients, as compared to nontreated PV, benign erythrocytosis, and relative polycythemia.

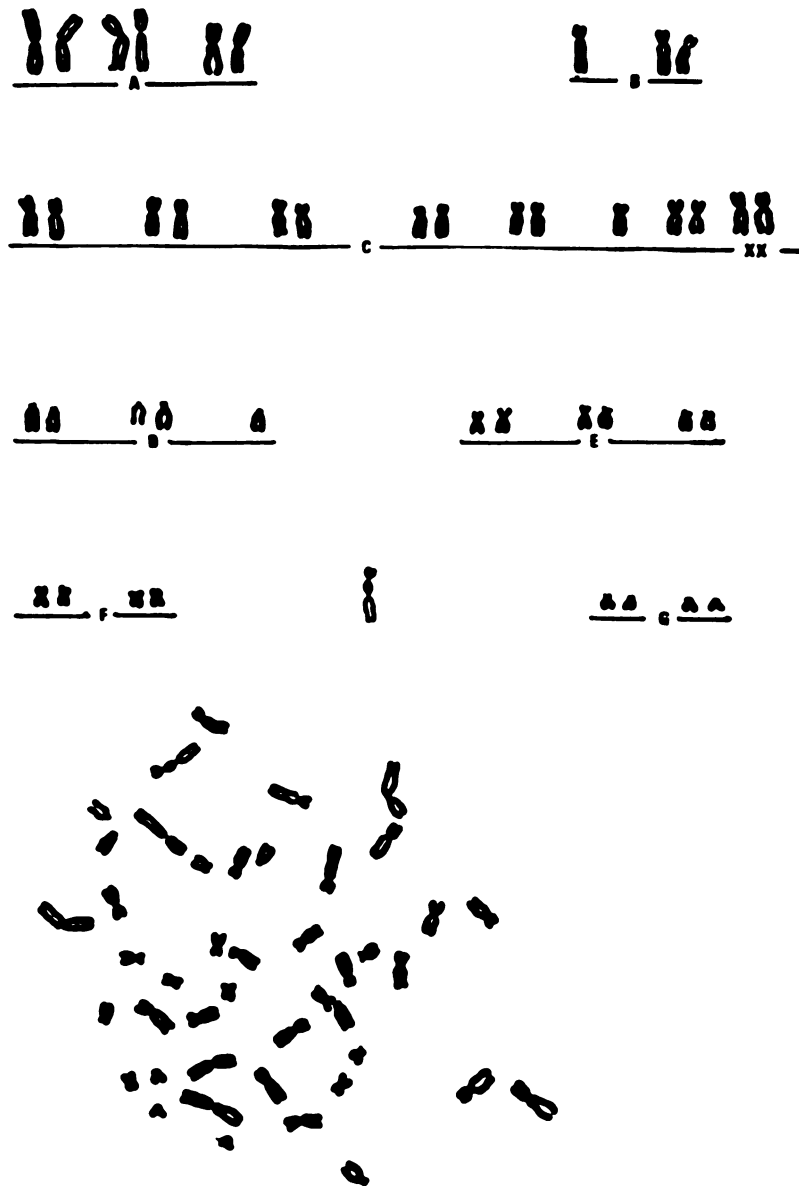


Fig. 3.—Patient with polycythemia vera, after radiophosphorus therapy (note monosomy in groups B, C and D, and a dicentric chromosome).

This frequency is also unaccountably high in secondary polycythemia. However, the overall low number of polyploid cells does not enable a meaningful statistical analysis.

Chromosome count as such, would be inadequate to reveal all aberrations in the karyotype in this system, due to the frequent occurrence of chromosomal rearrangement, such as a monosomy in one or more chromosome groups ac-

Table 1.—Aneuploidy by Diagnostic Group and Treatment

Diagnostic Group	Treatment Group	Number of Patients	Number of Cells Counted †	Per cent Diploid Cells (46)	Per cent Hypodiploid Cells (<46)	Per cent Hyperdiploid Cells (>46)	Per cent Cells with 46 ± 1 Chromosomes	Polyploid Ratio ‡
Blood Donors	N.R.*	7	143	96.5	2.8	0.7	100.0	No polyploids
Relative Polycythemia	N.R.	5	197	94.4	4.1	1.5	99.5	1:99.5
Secondary Polycythemia	N.R.	5	158	95.6	2.5	1.9	99.4	1:32.6
Benign Erythrocytosis	N.R.	10	419	89.0	8.6	2.4	96.9	1:105.8
Polycythemia Vera	N.R.	11	400	88.7	8.0	3.3	96.5	1:101.0
Polycythemia Vera	P32	17	847	85.7	9.9	4.4	96.3	1:36.3
Total		55	2164					

* No radiation.

† Excluding polyploid cells.

‡ Polyploid cells/cells counted.

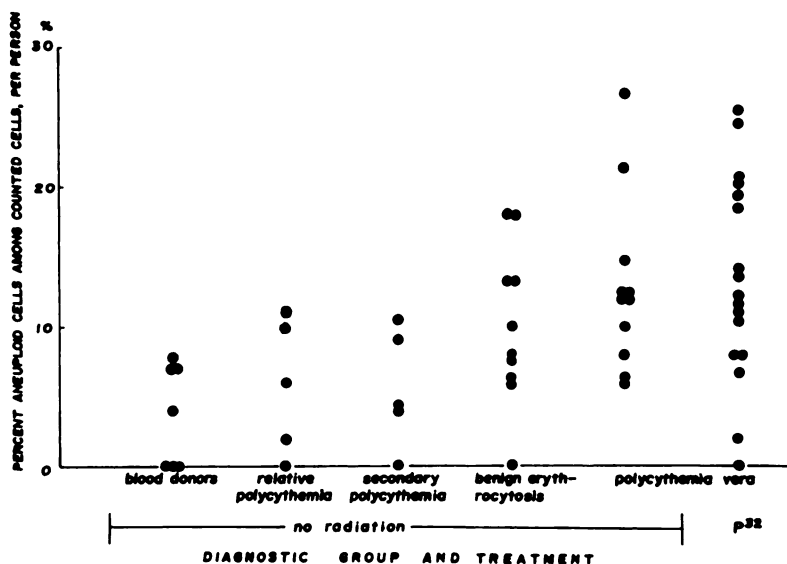


Fig. 4.—Per cent aneuploid cells per person by diagnostic category and type of treatment.

accompanied by trisomy in another group, and/or the occurrence of apparent abnormal chromosomes. To assess the amount of such changes, detailed karyotype analysis of randomly selected cells would be needed, rather than the routine selective method used (karyotyping, for each patient, a number of cells which displayed aberrations, as well as some which appeared normal under the microscope). Consequently, an indirect method was devised (Fig. 5) to evaluate the degree of such change, taking into account only cells presenting with chromosomal rearrangement or simple aneuploidy. For each

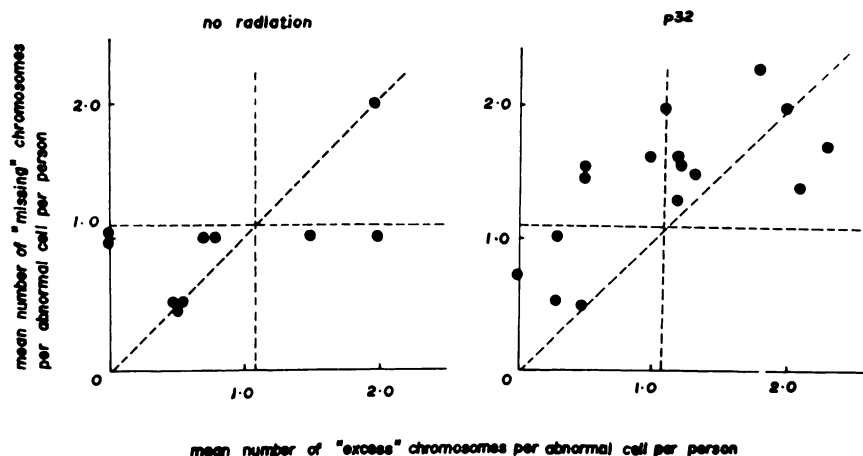


Fig. 5.—Comparison of mean no. of "excess" and of "missing" chromosomes per abnormal cell, among polycythemia vera patients with and without P³² treatment.

person, the mean number of "missing" chromosomes (i.e., absence of one member or both of a pair of chromosomes) per cell with such abnormal karyotype pattern, was plotted against the mean number of "additional" chromosomes (i.e., trisomies or apparent abnormal chromosomes which could not be placed in any group). Since the percentage of aneuploid cells in the treated and untreated PV groups showed only moderate differences (Fig. 4), and the method of selection of cells for karyotype analysis was similar in both PV groups, this computation seems adequate for comparative purposes.

The results, based on 66 such cells from 16 P^{32} treated PV patients (out of 113 cells with detailed karyotype analysis), and on 19 such cells from 10 untreated PV patients (out of 45 cells with such analysis), are presented in Fig. 5. The data suggest that in P^{32} treated PV patients the abnormal karyotypes present with relatively heavier structural changes than in the nontreated group [p (Fisher's exact χ^2 test) < 0.05]. Also, consistent with the excess of hypodiploid over hyperdiploid cells in the cell population (Table 1), there are relatively more "missing" than "additional" chromosomes in both groups. (This imbalance is somewhat exaggerated because cells with diploid count were less-frequently selected for analysis than aneuploid cells—about 10 per cent compared to 32 per cent, respectively. However, the bias was present to the same extent in both PV groups, so that it could not affect the relative degree of change per abnormal cell.)

Dicentric chromosomes constituted, as mentioned above, the one aberration which was typical to the radiation treated patients. No correlation was found between percentage of dicentric chromosomes to total dose of P^{32} given throughout the course of the disease. This is not unexpected, since these represent unstable changes. On the other hand, when correlated with the

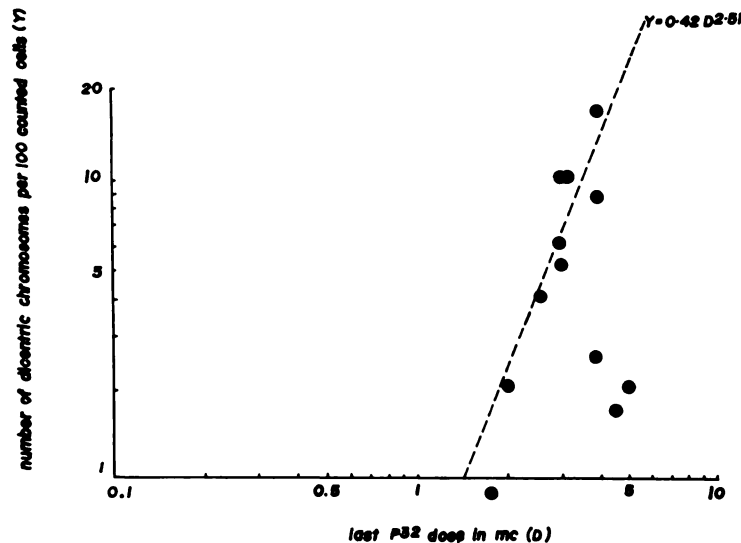


Fig. 6.—Regression of number of dicentric chromosomes per 100 counted cells on last P^{32} dose among polycythemia vera patients.

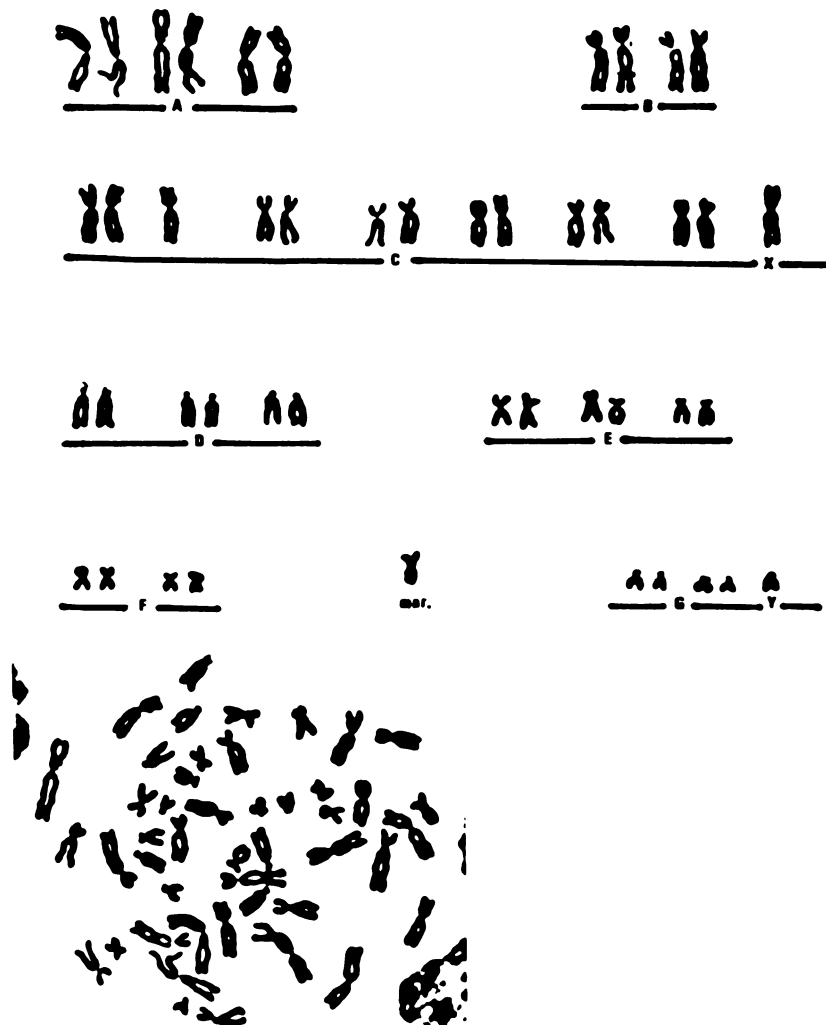


Fig. 7.—Representative bone marrow karyotype of patient Z.K. (see text). Note monosomy in group C and marker chromosome.

last P^{32} dose, an exponential rise in the frequency of dicentric chromosomes with increasing dose is demonstrated. When the data are plotted on a log-log scale (Fig. 6), a linear regression line can be fitted.

Three patients deviated significantly from the rest of the group, having a low percentage of dicentric cells despite a high P^{32} dose. It is of interest that one of these received the last P^{32} treatment six years prior to the cytogenetic examination, while all other patients received the treatment within the preceding nine months. The other two patients differ from the rest of the group from the clinical standpoint. One of these converted into myeloid metaplasia within a short time following this examination, and the other (Z.K.) has had persistent leukocytosis for the past four years, accompanied by an extreme

PERIPHERAL BLOOD			BONE MARROW		
I.E.					
S.G.					
Z.K.					
	21	22	Y	21	22

Fig. 8.—Presence of a Ph^1 , or Ph^1 -like chromosome in two patients with polycythemia vera and one with erythrocytosis.

binding capacity of vitamin B_{12} and a Ph^1 -like chromosome both in the peripheral blood and in the bone marrow, but with no young forms in the peripheral blood, and with a high score of leukocyte alkaline phosphatase activity. A marker chromosome was consistently noted in the bone marrow (Fig. 7).

A Philadelphia-like chromosome was found in the peripheral blood cultures in one additional PV patient and in one patient with benign erythrocytosis in the bone marrow only (Fig. 8). All three were post-radiophosphorous treatment.

COMMENT

Aneuploidy in untreated patients with PV has been reported previously,^{5,7} as were nonspecific chromosomal changes of both stable and unstable types in persons exposed to radiophosphorus.⁸ The latter are similar to those observed among individuals subjected to irradiation, such as in patients with ankylosing spondylitis,⁹ or the survivors of the atomic bombing in Hiroshima and Nagasaki.¹⁰ On the other hand, the relatively high percentage of aberrations in nonradioactively treated patients with benign erythrocytosis is puzzling. It would be of interest to find out to what extent this could be attributed to low-dosage radiation in the course of blood volume examinations or to diagnostic x-rays. Another unexpected finding is that, with the exception of the relatively high frequency of dicentric chromosomes, only moderate

quantitative differences can be noted between radioactively treated PV patients and those receiving no irradiation. These observations are, however, based primarily upon cultures of lymphocytes with Phytohemagglutinin. The extent to which the changes observed here are representative of other cell lines will be assessed when direct bone marrow analysis is completed.

The presence of a Philadelphia chromosome has been reported so far only in one P^{32} treated PV patient who developed chronic myeloid leukemia subsequently.¹¹ Another report of this aberration in two brothers with PV, neither of whom had findings pointing toward leukemia,¹² has in the meantime been refuted.¹³ Our findings of three patients with a Ph^1 -like chromosome, and particularly the one with both persistent leukocytosis and a Ph^1 -like chromosome in the bone marrow and in the peripheral blood, raises the general question of whether apparently random chromosomal changes attributed to radiation and/or other causes could predispose to leukemia. Would a deletion occurring in, say, chromosomes 6 or 15 lead to a more benign disease pattern in the future, while if the hit occurred in G21 it might lead to chronic myeloid, or perhaps even to acute leukemia? Furthermore, does the hit in chromosome 21 have to be in a specific locus on this chromosome in order to initiate a pathological process leading to the development of a full-blown picture of chronic myeloid leukemia? Otherwise, when a different site on G21 is damaged, a Philadelphia-like chromosome with no subsequent leukemia might result. Further follow-up of the patients might hopefully shed some more light on this problem.

SUMMARY

A number of chromosomal aberrations, occurring in polycythemia vera patients are presented, with an emphasis on their nonspecific nature. Among radiophosphorus-treated patients the frequency of these aberrations was moderately higher. The one finding typical to P^{32} treated patients was the presence of dicentric chromosomes, and a dose response curve to the last P^{32} dose was demonstrated. A Philadelphia-like chromosome was observed in two patients with polycythemia vera and in one with benign erythrocytosis, all post P^{32} therapy.

A question is raised whether it is possible that the future course of the polycythemic patient is dependent upon the type and location of chromosomal damage, and in turn, on the establishment of a clone of cells with a selective developmental advantage.

ACKNOWLEDGMENTS

The aid of Mr. S. G. Levin and Mr. A. Hibshoosh in statistical analysis is appreciated.

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