Cytogenetic abnormalities in polycythemia vera: phenotypic correlates and prognostic relevance in 669 informative cases

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Abstract

The main objective of the current study was to provide a detailed account of the prognostic relevance of abnormal karyotype and associated specific cytogenetic abnormalities in polycythemia vera (PV). Six hundred and sixty-nine PV patients were informative, of whom 436 (65%) were evaluated within 1 year of diagnosis. Karyotype abnormalities were found in 67 (15%) patients, including isolated abnormalities of loss of Y chromosome (-Y; N=15; 3%), +9 (N=11; 3%), del(20q) (N=10; 2%), and +8 (N=4; 1%). Abnormal karyotype correlated with older age (P<0.01), lower platelet count (P<0.01), and grade ≥2 reticulin fibrosis (P<0.01). Specifically, del(20q) correlated with older age and grade ≥2 reticulin fibrosis, while +9 correlated with a higher incidence of a history of venous thrombosis. *SRSF2* and *IDH2* mutations clustered with normal karyotype. At a median follow-up of 7.4 years, 163 (37%) deaths, 50 (11%) cases of fibrotic transformation (post-PV MF) and 14 (3%) cases of leukemic transformation (LT) were documented. In univariate analysis, abnormal karyotype was associated with inferior overall survival (median 10.5 *vs*. 16.3 years; P<0.01); the statistical significance of this association was sustained in multivariable analysis (hazard ratio=2.0; P=0.02), along with associations with age ≥60 years (P<0.01), leukocytosis ≥15×10^9/L (P<0.01) and *SRSF2* mutation (P<0.01). Abnormal karyotype was also associated with post-PV MF (21% *vs*. 10%; P<0.01) and LT (7% vs. 2%; P<0.01); the statistical significance of this association was sustained in multivariable analysis for post-PV MF (hazard ratio=3.7; P<0.01), but not for LT (P=0.47). In regard to specific abnormalities, del(20q) was associated with progression to post-PV MF and ≥2 abnormalities with LT. The current study describes the spectrum of cytogenetic abnormalities in PV and their associated phenotypic and prognostic correlates.

Introduction

Driver mutations in *JAK2* (exon 12-15 including V617F) are present in virtually all patients with polycythemia vera (PV) and constitute a major diagnostic criterion.[1,2] Other gene mutations are detected in over 50% of PV patients and include *TET2* (22%), *ASXL1* (12%), *SRSF2* (3%), and *IDH2* (2%); the latter three mutations have been associated with inferior survival.[3] In the current molecular era, although cytogenetics has limited diagnostic utility in PV, it remains an essential component of clinical management for (i) prognostication, (ii) monitoring disease progression, and (iii) evaluation of anti-clonal activity of existing and investigational drug therapies.

In general, patients with PV have a life-expectancy of approximately 15 years; however, survival is age-dependent with a median survival of 37 years (age <40 years), 22 (age 41-60 years), and 10 years (age >60 years).[4,5] Moreover, in an international study including 1,545 patients with PV, survival was found to be inferior to that of the age- and gender-matched United States population and was adversely influenced by the presence of an abnormal karyotype (AK) at diagnosis (in 12%).[6] Additional risk factors for poorer survival included older age, leukocytosis, and venous thrombosis. A prognostic model based on these three variables delineated risk groups with median surviv-

als of 10.9-27.8 years.[6] The particular study also identified AK, along with older age, and leukocytosis ≥15×10^9/L as risk factors for leukemic transformation (LT).[6]

Current prognostication in PV relies on both clinical and genetic information and the Mutation-enhanced International Prognostic Scoring System (MIPSS-PV) considers AK as a risk variable, in addition to *SRSF2* mutations, age >67 years, leukocytosis ≥15×10^9/L, and a history of thrombosis.[7] As underlined above, the prognostic impact of AK in PV presents a compelling case for further investigation of cytogenetic profiles and their associated clinical and molecular phenotypes.. However, studies on the subject have thus far been constrained by the paucity of cytogenetic information from the time of PV diagnosis and across the disease course, along with inadequate follow-up duration. The current study offset this particular limitation and took advantage of a large cohort of patients with PV to describe the spectrum and prevalence of specific cytogenetic abnormalities during different phases of the disease including transformation to myelofibrosis (post-PV MF) and acute myeloid leukemia. We examined the clinical, molecular, and prognostic correlates of AK at the time of PV diagnosis, and also investigated the patterns of clonal evolution among those patients who underwent serial cytogenetic evaluations. Given the future prospect of disease-modifying therapies and genetics-based prognostication, it is important to re-assess the prognostic relevance of specific cytogenetic aberrations in PV, in the context of existing risk factors.

Methods

After institutional review board approval, cytogenetically annotated patients with PV who met the International Consensus Classification diagnostic criteria and was evaluated at the Mayo Clinic between January 1973 and September 2023 were retrospectively studied.[1] A subset of patients with grade ≥2 reticulin fibrosis (N=23) were included as PV cases in the absence of anemia, leukoerythroblastosis, worsening splenomegaly, or constitutional symptoms reminiscent of post-PV MF.[1] Cytogenetic analysis was performed on bone marrow aspirates and result reporting was in accordance with the International System for Human Cytogenomic Nomenclature (ISCN).[8] Every attempt was made to incorporate solely patients whose cytogenetic studies involved analysis of at least 20 metaphases. Chromosomal abnormalities were considered clonal if the same structural abnormality or extra chromosome appeared in at least two metaphases and monosomy in at least three metaphases. Recurrent cytogenetic abnormalities, namely loss of Y chromosome (-Y), trisomy 8 (+8), trisomy 9 (+9), and sole deletion of 20q (del20q), were categorized separately, while the remainder of the abnormalities were conventionally grouped as either 'single abnormality excluding -Y-, +8, +9, or del(20q)' or 'two or more abnormalities.' In order to examine clinical and laboratory correlations, as well as survival prognostication, we strived to capture exclusively informative cases in which cytogenetic analyses were performed either at the time of diagnosis or within 1 year thereafter. This approach was taken to eliminate the confounding influence of therapies and inadvertent inclusion of post-PV MF. Molecular correlations were computed in a subset of cases in which next-generation sequencing was performed.

Categorical variables were compared using the χ^2 test, while continuous variables were assessed through Wilcoxon/Kruskal-Wallis tests. Multivariable analyses were conducted using the Cox proportional hazards model. Cox regression analysis was applied to identify risk factors for overall survival, leukemia-free survival, and myelofibrosis-free survival. The overall survival analysis encompassed the period from the date of diagnosis to either the date of death or last contact, which was updated in December 2023. Myelofibrosis-free survival and leukemia-free survival were calculated from the time of diagnosis to the occurrence of the respective events after diagnosis. Time-to-event curves were generated using the Kaplan-Meier method and compared by the log-rank test. *P* values ≤0.05 were considered statistically significant. The JMP Pro 16.0.0 software package was used for all analyses.

Results

Prevalence of cytogenetic abnormalities at diagnosis and in different stages of the disease

Cytogenetic studies were performed in a total of 669 patients of whom 436 (65%) underwent cytogenetic evaluation at or within 1 year of diagnosis (baseline), 116 (17%) within 1-10 years, 47 (7%) after more than 10 years, and 70 (10%) at post-PV MF or LT. Table 1 illustrates the distribution of specific cytogenetic abnormalities across the disease course from baseline (within a year of diagnosis) and beyond the initial year following diagnosis. In 436 patients with cytogenetic information obtained within 1 year of diagnosis, karyotype was normal in 369 (85%), showed -Y in 15 (3%), and other abnormalities in 52 (12%). The most common abnormalities were +9 (N=11; 3%), del(20q) (N=10; 2%), +8 (N=4; 1%), other sole abnormalities (N=16; 4%), and ≥2 abnormalities (N=11; 3%) including complex karyotype (N=4; 1%). The frequency of AK was higher with longer disease duration, being 15% within 1 year of diagnosis, 28% in those diagnosed 1-10 years previously, 49% in those studied more than 10 years after diagnosis, and 50% at the time of post-PV MF or LT (*P*<0.01). Isolated del(20q), single abnormalities excluding del(20q), +8, +9, or -Y, and ≥2 abnormalities including complex karyotype were progressively acquired; respective frequencies within a year of diagnosis, over 10 years after diagnosis, and at

the time of post-PV MF or LT were 2%/21%/10% (20q-), 4%/17%/14% (single abnormality excluding del(20q), +8, +9, or -Y), 3%/11%/19% (≥2 abnormalities), and 1%/ 2%/10% (complex karyotype) (all *P* values <0.01). In addition, the frequency of isolated +8 was higher at fibrotic transformation than within a year of diagnosis (5% *vs*. 0.9%; *P*=0.04). Information on treatment initiated at the time of diagnosis was available for 134 of 436 patients with baseline cytogenetic assessment: the majority (N=106, 79%) of these patients were receiving aspirin, while cytoreductive therapy was initiated in 65 (49%) patients and consisted of hydroxyurea (N=50), pegylated interferon (N=13), or anagrelide (N=2). The use of cytoreductive therapy was not significantly different among patients with abnormal or normal karyotype (58% *vs*. 46%, *P*=0.55). Also, similar proportions of patients with abnormal and normal karyotype were receiving aspirin (73% *vs*. 76%, *P*=0.90).

Clonal evolution

Fifty-six patients had at least two cytogenetic studies performed at a median of 6.1 years (0.5-19.9 years), following the baseline cytogenetic evaluation. In addition, 11 patients had three, and four patients had four cytogenetic assessments during the chronic phase of PV. This cohort was used to identify patterns of clonal evolution, defined as the acquisition of cytogenetic abnormalities from a previously normal karyotype or the development of additional abnormalities in a previously AK. As shown in Table 2, cytogenetic clonal evolution was evident in both the chronic phase and at transformation (to post-PV MF and acute myeloid leukemia). At the time of the second or third biopsies in the absence of disease transformation, clonal evolution was documented in a total of 13 of 56 (23%) patients and three of 11 (27%) patients, respectively. On the other hand, cytogenetic studies performed at the time of post-PV MF and LT demonstrated clonal evolution in 14 of 31 (45%) and three of six (50%) patients, respectively. The frequency of AK became significantly higher from 15% at baseline to 30% at the time of second biopsy (*P*=0.02), 61% at post-PV MF (*P*<0.01) and 83% at LT (*P*<0.01). Compared to baseline, del(20q) (2% *vs*. 7% at the time of second biopsy, *P*=0.05), and ≥2 abnormalities (3% at baseline *vs*. 7%, *P*=0.07) were more likely to be acquired at the time of the second cytogenetic study, while frequencies of +8 (0.9% to 2%, *P*=0.55), and +9 (3% to 5%, *P*=0.25) were unchanged. Also, a higher frequency of del(20q) was identified at post-PV MF (23% *vs*. 2% at baseline, *P*<0.01) and ≥2 abnormalities at post-PV MF (26% *vs*. 3%, *P*<0.01) and LT (83% *vs*. 3%, *P*<0.01).

Table 1. Frequency of cytogenetic abnormalities among 669 informative patients with polycythemia vera, assessed at varying times from diagnosis.*

Variables	Within 1 year of diagnosis N=436	1-10 years after diagnosis N=116 *P*	>10 years after diagnosis N=47 *P*	At MF/AML transformation N=70 *P*	At MF transformation N=61 *P*	At AML transformation N=9 *P*
Normal karyotype, N (%)	369 (85)	83 (72) <0.01	24 (51) <0.01	35 (50) <0.01	32 (52) <0.01	3 (33) <0.01
Abnormal karyotype including -Y, N (%)	67 (15)	33 (28) <0.01	23 (49) <0.01	35 (50) <0.01	29 (48) <0.01	6 (67) <0.01
Abnormal karyotype excluding -Y, N (%)	52 (12)	31 (27) <0.01	23 (49) <0.01	34 (49) <0.01	29 (48) <0.01	5 (56) <0.01
Isolated -Y, N (%)	15 (3)	2 (2) 0.31	0 (0) 0.08	1 (1) 0.33	0 (0) 0.046	1 (11) 0.32
Isolated +9, N (%)	11 (3)	5 (4) 0.33	0 (0) 0.13	1 (1) 0.55	1 (2) 0.66	0 (0) 0.50
Isolated del (20q), N (%)	10 (2)	8 (7) 0.02	10 (21) <0.01	7 (10) <0.01	6 (10) <0.01	1 (11) 0.21
Isolated +8, N (%)	4 (0.9)	3 (3) 0.19	0 (0) 0.36	3 (4) 0.06	3 (5) 0.04	0 (0) 0.69
Single abnormality excluding -Y, +8, +9, or del(20q), N (%)	16 (4)	6 (5) 0.48	8 (17) <0.01	10 (14) <0.01	7 (11) 0.02	3 (33) <0.01
Two or more abnormalities, N (%)	11 (3)	9 (8) 0.01	5 (11) 0.01	13 (19) <0.01	12 (20) <0.01	1 (11) 0.23

*Patients included in each time frame are distinct and do not overlap. All *P* values are with reference to cytogenetics performed within 1 year of diagnosis; values <0.05 are considered statistically significant. MF: myelofibrosis; AML: acute myeloid leukemia.

Table 2. Incidence and pattern of cytogenetic clonal evolution among 436 patients with polycythemia vera stratified by disease subtype.

Variables	Before transformation				At transformation	
	Karyotype at baseline* N=436	Karyotype at time of second biopsy N=56 *P*	Karyotype at time of third biopsy N=11	Karyotype at time of fourth biopsy N=4	At MF transformation N=31 *P*	At AML transformation N=6 *P*
Karyotype, N (%)						
Normal karyotype	369 (85)	39 (70)	7 (64)	4 (100)	12 (39)	1 (17)
Abnormal karyotype	67 (15)	17 (30) 0.02	4 (36)	0 (0)	19 (61) <0.01	5 (83) <0.01
Isolated -Y	15 (3	2 (4) -	1 (9)	0 (0)	0 (0) -	0 (0) -
Isolated +9	11 (3)	3 (5) 0.25	0 (0)	0 (0)	0 (0) -	0 (0) -
Isolated del (20q)	10 (2)	4 (7) 0.049	0 (0)	0 (0)	7 (23) <0.01	0 (0) -
Isolated +8	4 (0.9)	1 (2) -	1 (9)	0 (0)	0 (0) -	0 (0) -
Single abnormality excluding -Y, +8, +9, or del(20q)	16 (4)	3 (5) -	0 (0)	0 (0)	4 (13) -	0 (0) -
Two or more abnormalities	11 (3)	4 (7) 0.07	2 (18)	0 (0)	8 (26) <0.01	5 (83) <0.01
Clonal evolution, N (%)	-	13 (23)	3 (27)	0 (0)	14 (45)	3 (50)

*Baseline; karyotype obtained within 1 year of diagnosis. All *P* values are with reference to karyotype obtained within 1 year of diagnosis. MF: myelofibrosis; AML: acute myeloid leukemia.

Phenotypic and molecular correlates (N=436)

Four hundred and thirty-six patients with PV (51% males; median age, 64 years) who underwent cytogenetic testing at or within 1 year of diagnosis were examined for phenotypic correlations (Tables 3 and 4). AK (N=67), compared to normal karyotype, correlated with older age (median 70 *vs*. 63 years, *P*<0.01), male gender (64% *vs*. 48%, *P*=0.02), lower platelet count at presentation (median 370 *vs*. 465×10^9/L, *P*<0.01) and grade ≥2 bone marrow fibrosis (15% *vs*. 4%, *P*<0.01). AK excluding loss of Y (N=52), compared to normal karyotype, also correlated with older age (median 69 *vs*. 63 years, *P*<0.01), lower platelet count (median 361 *vs*. 465×10^9/L, *P*<0.01) and grade ≥2 reticulin fibrosis (16% *vs*. 4%, *P*=0.01). By contrast, incidence rates of leukocytosis ≥15×10^9/L (21% *vs*. 21%, *P*=0.96), splenomegaly (34% *vs*. 28%, *P*=0.29), and history of thrombosis (30% *vs*. 34%, *P*=0.49) did not differ between patients harboring an abnormal or normal karyotype.

Among specific cytogenetic abnormalities, del(20q), compared to normal karyotype, was associated with older age (median 76 *vs*. 63 years, *P*<0.01) and grade ≥2 reticulin fibrosis (22% *vs*. 5%; *P*=0.03); +8 occurred exclusively in patients ≥60 years old (*P*=0.03); +9 (N=11) was associated with a higher incidence of prior venous thrombosis (45% *vs*. 17%, *P*=0.03). Single abnormalities (excluding del(20q), +8, +9, or -Y), were also associated with older age (median age 71 *vs*. 63 years, *P*=0.02). On the other hand, patients with ≥2 abnormalities, compared to those with a normal karyotype, were more likely to be males (73% *vs*. 48%, *P*=0.01), had a lower incidence of arterial thrombosis prior to/at diagnosis (0% *vs*. 22%, *P*=0.02), and had a lower platelet count at diagnosis (368 *vs*. 465×10^9/L, *P*=0.04).

One hundred and eighty-seven patients were examined for molecular correlations based on availability of mutational information (Tables 3 and 4). The most frequently mutated genes included *TET2* (N=32, 17%), *ASXL1* (N=14, 7%), *TP53* (N=8, 4%), *SRSF2* (N=7, 4%), *SH2B3* (N=6, 3%), *DNMT3A* (N=5, 3%), and *IDH2* (N=5, 3%). *SRSF2* and *IDH2* mutations were found to cluster with normal karyotype (5% *vs*. 0%; *P*=0.14 and 3% *vs*. 0%; *P*=0.16, in normal *vs*. AK [excluding -Y], respectively). As expected, *TET2* mutations were more likely to be present in patients with -Y (57% *vs*. 18%; *P*=0.02). By contrast, *TP53* and *ASXL1* mutations were uniformly distributed across normal karyotype and AK overall and when specific abnormalities were considered separately.

Table 3. Clinical and laboratory characteristics of 436 patients with polycythemia vera in whom cytogenetic studies were performed within 1 year of diagnosis.

Variables	All patients N=436	Normal karyotype N=369	Abnormal karyotype (including –Y) N=67 *P*	Abnormal karyotype (excluding –Y) N=52 *P*	-Y only N=15 *P*
Age at diagnosis in years, median (range)	64.1 (18.7-91.4)	62.7 (18.7-91.4)	70 (34.7-87.3) <0.01	68.9 (34.7-85.6) <0.01	73.2 (59.2-87.3) <0.01
Age ≥60 years, N (%)	258 (59)	205 (56)	53 (79) <0.01	39 (75) <0.01	14 (93) <0.01
Gender (male), N (%)	221 (51)	178 (48)	43 (64) 0.02	28 (54) 0.45	15 (100) <0.01
Hemoglobin, g/dL, median (range)	17.3 (14.1-24.1)	17.3 (14.1-24.1)	17.3 (15.6-22) 0.88	17.1 (15.6-22) 0.94	17.4 (16.6-19.9) 0.85
Platelet count $\times 10^9$/L, median (range)	442.5 (44-2747)	465 (48-2747)	370 (44-932) 0.02	361 (44-902) <0.01	526 (208-932) 0.36
Leukocytes $\times 10^9$/L, median (range)	10.9 (3.3-33.7)	11 (3.3-33.7)	10.6 (5.7-26) 0.97	10.3 (5.7-26) 0.90	11.3 (6.9-24) 0.88
Leukocytes ≥15$\times 10^9$/L, N (%)	90 (21)	76 (21)	14 (21) 0.96	11 (21) 0.93	3 (20) 0.96
Grade ≥2 reticulin fibrosis, N (%)	23/402 (6)	14/340 (4)	9/62 (15) <0.01	8/49 (16) 0.01	1/13 (8) 0.77
Palpable splenomegaly, N (%)	126 (29)	103 (28)	23 (34) 0.29	17 (33) 0.48	6 (40) 0.32
Any thrombosis at or prior to diagnosis, N (%)	146 (33)	126 (34)	20 (30) 0.49	15 (29) 0.44	5 (33) 0.95
Arterial thrombosis	92 (21)	82 (22)	10 (15) 0.16	7 (13) 0.13	3 (20) 0.83
Venous thrombosis	73 (17)	62 (17)	11 (16) 0.94	9 (17) 0.93	2 (13) 0.72
Any thrombosis after diagnosis, N (%)	103 (24)	88 (24)	15 (22) 0.79	11 (21) 0.66	4 (27) 0.80
Arterial thrombosis	60 (14)	48 (13)	12 (18) 0.30	9 (17) 0.41	3 (20) 0.46
Venous thrombosis	60 (14)	52 (14)	8 (12) 0.63	7 (13) 0.90	1 (7) 0.37
Patients evaluated for gene mutations, N	187	154	33	26	7
Mutations found, N (%)					
TET2	32 (17)	27 (18)	5 (15) 0.74	1 (4) 0.04	4 (57) 0.02
ASXL1	14 (7)	12 (8)	2 (6) 0.73	2 (8) 0.99	0 (0) 0.29
TP53	8 (4)	7 (5)	1 (3) 0.69	1 (4) 0.88	0 (0) 0.43
SRSF2	7 (4)	7 (5)	0 (0) 0.10	0 (0) 0.14	0 (0) 0.43
SH2B3	6 (3)	4 (3)	2 (6) 0.35	1 (4) 0.73	1 (14) 0.19
DNMT3A	5 (3)	4 (3)	1 (3) 0.89	0 (0) 0.26	1 (14) 0.19
IDH2	5 (3)	5 (3)	0 (0) 0.16	0 (0) 0.21	0 (0) 0.50
SF3B1	4 (2)	3 (2)	1 (3) 0.71	0 (0) 0.33	1 (14) 0.14
SETBP1	3 (2)	3 (2)	0 (0) 0.28	0 (0) 0.33	0 (0) 0.60

Continued on following page.

Variables	All patients N=436	Normal karyotype N=369	Abnormal karyotype (including –Y) N=67 *P*	Abnormal karyotype (excluding –Y) N=52 *P*	-Y only N=15 *P*
EZH2	3 (2)	2 (1)	1 (3) 0.51	1 (4) 0.41	0 (0) 0.67
IDH1	2 (1)	2 (1)	0 (0) 0.38	0 (0) 0.43	0 (0) 0.67
RUNX1	2 (1)	1 (0.6)	1 (3) 0.29	0 (0) 0.58	1 (14) 0.05
KIT	2 (1)	2 (1)	0 (0) 0.37	0 (0) 0.42	0 (0) 0.67
CBL	2 (1)	1 (0.6)	1 (3) 0.29	1 (4) 0.23	0 (0) 0.77
ZRSR2	2 (1)	1 (0.6)	1 (3) 0.29	0 (0) 0.58	1 (14) 0.05
NRAS	1 (0.5)	1 (0.6)	0 (0) 0.53	0 (0) 0.56	0 (0) 0.77
CEBPA	1 (0.5)	0 (0)	1 (3) 0.06	1 (4) 0.048	0 (0) -

All *P* values are for the comparison with a normal karyotype with values <0.05 considered statistically significant.

Prognostic impact of cytogenetic abnormalities (N=436)

At a median follow-up of 7.4 years (range, 0.04-42.4 years), a total of 163 (37%) patients have died, while 50 (11%) and 14 (3%) underwent fibrotic progression and LT, respectively. In addition, 60 arterial and venous thrombotic events each were recorded in a total of 103 (24%) patients. In univariate analysis for overall survival. AK, compared to normal karyotype, was associated with inferior survival (median overall survival; 10.5 *vs*. 16.3 years, *P*<0.01) (Figure 1A). In multivariate analysis, the adverse impact of AK remained statistically significant (*P*=0.02; hazard ratio [HR]=2.0, 95% confidence interval [95% CI]: 1.2-3.3), along with age ≥60 years (*P*<0.01; HR=5.5, 95% CI: 3.1-9.7), leukocyte count ≥15×10^9/L (*P*<0.01; HR=2.6, 95% CI: 1.5-4.3), and *SRSF2* mutations (*P*<0.01; HR=6.1, 95% CI: 2.3-16.1). In univariate analyses which considered specific cytogenetic abnormalities, survival was numerically shorter in the presence of del(20q) (11.3 *vs*. 14.9 months, *P*=0.08), and single abnormalities (excluding del (20q), +8, +9, or -Y) (11.1 vs. 14.8 months, *P*=0.15); however, these differences were no longer apparent on age-adjusted analysis.

Overall, the incidence of myelofibrosis was higher in patients with AK than in those with a normal karyotype (21% *vs*. 10%, *P*=0.14), particularly in patients harboring del(20q) (30%). In time-dependent analyses, AK was an independent predictor of myelofibrosis-free survival (*P*<0.01; HR=3.7, 95% CI: 1.8-7.8), along with *SRSF2* mutations (*P*<0.01; HR=18.4, 95% CI: 4.7-72.4) (Figure 1B). Similarly, the incidence of LT was higher in those with AK (7% vs. 2%; *P*=0.03) and was highest amongst those harboring ≥2 abnormalities (27%). Analysis of leukemia-free survival revealed AK to be a significant risk factor in univariate (*P*<0.01), but not multivariate analysis that included *SRSF2* mutation (*P*=0.47) (Figure 1C, Table 5). On the other hand, the presence of AK did not appear to have a significant impact on rates of either arterial thrombosis (18% *vs*. 13%; *P*=0.1) or venous thrombosis (12% *vs*. 14%; *P*=0.95), or on thrombosis-free survival (Figure 2). The borderline increased risk of arterial thrombosis in patients with AK was fully accounted for by their older age distribution.

Discussion

Cytogenetic studies provide a global overview of the entire genome at a low cost, are universally available and provide information that complements sequencing results.[9] In light of the advantages of conventional chromosome analysis, the current study, comprising the largest cohort of cytogenetically annotated patients with PV followed for up to five decades, provides one of the most comprehensive and mature accounts of cytogenetics in PV to date. Our study reveals that approximately 15% of PV patients display AK at or within 1 year of diagnosis and identifies several confirmatory as well as novel findings. Previous studies on clinical and molecular correlates in PV have consistently indicated that AK is an independent risk factor for inferior survival, with increased risk of fibrotic progression and LT.[6,10,11] Based on these findings, AK was integrated as a prognostic variable in the hybrid clinical plus genetic MIPSS-PV model.[7] Despite the acknowledged prognostic relevance of cytogenetics in PV, our understanding of the acquisition of specific abnormalities across disease phases remains limited. This can be attributed to the fact that cytogenetic testing is not routinely performed at the time

Table 4. Phenotypic and molecular correlates of specific cytogenetic profiles obtained within 1 year of the diagnosis of polycythemia vera.

Variables	Normal karyotype N=369	Isolated del(20q) N=10 *P*	Isolated +8 N=4 *P*	Isolated +9 N=11 *P*	Single abnormality excluding -Y, +8, +9, or del(20q) N=16 *P*	Two or more abnormalities N=11 *P*
Age at diagnosis in years, median (range)	62.7 (18.7-91.4)	759 (56.4-81.5) <0.01	71.1 (67.7-77.5) 0.09	61.7 (34.7-77.2) 0.87	70.7 (42.1-82.6) 0.02	68 (49.7-85.6) 0.23
Age ≥60 years, N (%)	205 (56)	8 (80) 0.11	4 (100) 0.03	6 (55) 0.95	13 (81) 0.03	8 (73) 0.25
Gender (male), N (%)	178 (48)	6 (60) 0.46	1 (25) 0.34	6 (55) 0.68	7 (44) 0.72	8 (73) 0.10
Hemoglobin, g/dL, median (range)	17.3 (14.1-24.1)	17.3 (15.6-21.4) 0.98	19.4 (16.5-20.4) 0.15	17.3 (15.7-20.1) 0.87	17.1 (16.1-22) 0.79	17 (16.1-20.4) 0.86
Platelet count $\times 10^9$/L, median (range)	465 (48-2,747)	336 (180-902) 0.21	321 (214-394) 0.07	364 (295-866) 0.15	397.5 (44-851) 0.18	368 (228-578) 0.04
Leukocytes $\times 10^9$/L, median (range)	11 (3.3-33.7)	10 (6.6-21) 0.96	10.2 (6.4-14.4) 0.50	12.4 (7.9-26) 0.28	10.3 (5.7-21) 0.91	9.8 (6.4-22.7) 0.44
Leukocytes ≥15$\times 10^9$/L, N (%)	76 (21)	3 (30) 0.49	0 (0) 0.18	2 (18) 0.84	5 (31) 0.33	1 (9) 0.31
Grade ≥2 reticulin fibrosis, N (%)	14/340 (4)	2/9 (22) 0.03	1/4 (25) 0.10	2/10 (20) 0.11	1/15 (7) 0.87	2/11 (18) 0.14
Palpable splenomegaly, N (%)	103 (28)	2 (20) 0.57	1 (25) 0.90	5 (45) 0.22	5 (31) 0.77	4 (36) 0.55
Any thrombosis at or prior to diagnosis, N (%)	126 (34)	4 (40) 0.70	2 (50) 0.52	5 (45) 0.45	3 (19) 0.18	1 (9) 0.05
Arterial thrombosis	82 (22)	3 (30) 0.58	1 (25) 0.90	1 (9) 0.25	2 (13) 0.32	0 (0) 0.02
Venous thrombosis	62 (17)	1 (10) 0.54	1 (25) 0.68	5 (45) 0.03	1 (6) 0.21	1 (9) 0.47
Any thrombosis after diagnosis, N (%)	88 (24)	3 (30) 0.66	0 (0) 0.14	2 (18) 0.65	4 (25) 0.92	2 (18) 0.65
Arterial thrombosis	48 (13)	2 (20) 0.54	0 (0) 0.29	2 (18) 0.63	4 (25) 0.21	1 (9) 0.69
Venous thrombosis	52 (14)	1 (10) 0.70	0 (0) 0.27	1 (9) 0.62	3 (19) 0.62	2 (18) 0.71
Patients evaluated for gene mutations, N	154	4	4	5	7	6
Mutations found, N (%)						
TET2	27 (18)	0 (0) 0.22	0 (0) 0.22	0 (0) 0.17	0 (0) 0.10	1 (17) 0.96
ASXL1	12 (8)	0 (0) 0.42	1 (25) 0.31	0 (0) 0.37	0 (0) 0.29	1 (17) 0.49
SRSF2	7 (5)	0 (0) 0.54	0 (0) 0.54	0 (0) 0.50	0 (0) 0.43	0 (0) 0.46
TP53	7 (5)	0 (0) 0.55	0 (0) 0.55	0 (0) 0.50	0 (0) 0.43	1 (17) 0.28
IDH2	5 (3)	0 (0) 0.61	0 (0) 0.61	0 (0) 0.57	0 (0) 0.50	0 (0) 0.53
SH2B3	4 (3)	1 (25) 0.10	0 (0) 0.65	0 (0) 0.61	0 (0) 0.55	0 (0) 0.58
DNMT3A	4 (3)	0 (0) 0.65	0 (0) 0.65	0 (0) 0.61	0 (0) 0.55	0 (0) 0.58
SF3B1	3 (2)	0 (0) 0.69	0 (0) 0.69	0 (0) 0.66	0 (0) 0.60	0 (0) 0.63

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Variables	Normal karyotype N=369	Isolated del(20q) N=10 *P*	Isolated +8 N=4 *P*	Isolated +9 N=11 *P*	Single abnormality excluding -Y, +8, +9, or del(20q) N=16 *P*	Two or more abnormalities N=11 *P*
SETBP1	3 (2)	0 (0) 0.69	0 (0) 0.69	0 (0) 0.66	0 (0) 0.60	0 (0) 0.63
EZH2	2 (1)	0 (0) 0.75	0 (0) 0.75	0 (0) 0.72	0 (0) 0.67	1 (17) 0.08
IDH1	2 (1)	0 (0) 0.75	0 (0) 0.75	0 (0) 0.72	0 (0) 0.67	0 (0) 0.69
KIT	2 (1)	0 (0) 0.75	0 (0) 0.75	0 (0) 0.72	0 (0) 0.65	0 (0) 0.69
NRAS	1 (0.6)	0 (0) 0.82	0 (0) 0.82	0 (0) 0.80	0 (0) 0.77	0 (0) 0.78
CBL	1 (0.6)	0 (0) 0.82	0 (0) 0.82	0 (0) 0.80	1 (14) 0.05	0 (0) 0.78

All *P* values are for the comparison with a normal karyotype with values <0.05 considered statistically significant.

of initial diagnosis of PV, and usually reserved for clinically suspected disease progression.[12] Furthermore, cytogenetic risk stratification in PV is not only challenged by the availability of limited baseline cytogenetic results, but also by the relatively low frequency of AK, and immature survival data. Foundational knowledge on the prognostic impact of AK in

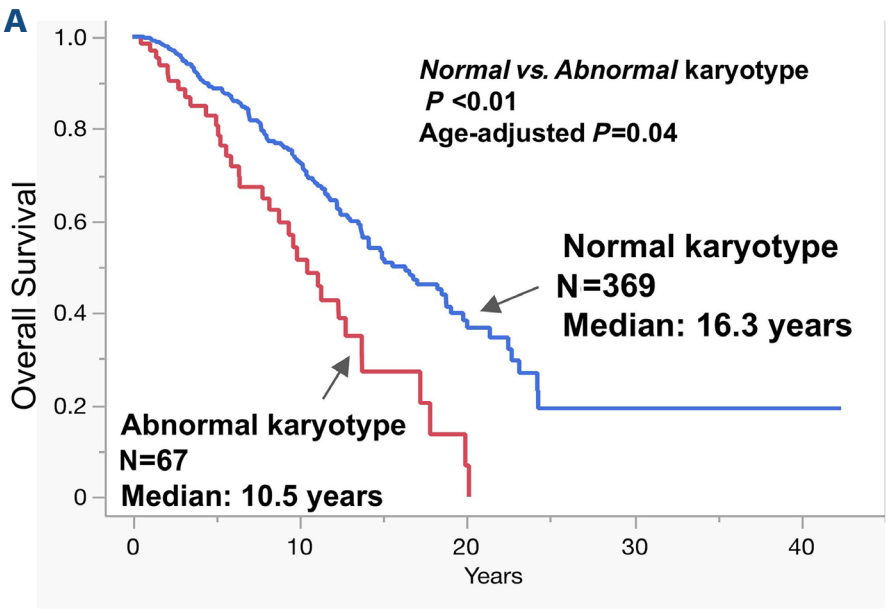

Figure 1. Abnormal karyotype and survival in polycythemia vera. (A) Overall survival in 436 patients with polycythemia vera, stratified by cytogenetics performed at or within 1 year of diagnosis. (B) Leukemia-free survival in 436 patients with polycythemia vera, stratified by cytogenetics performed at or within 1 year of diagnosis. (C) Myelofibrosis-free survival in 436 patients with polycythemia vera, stratified by cytogenetics performed at or within 1 year of diagnosis.

PV necessitates additional studies to identify specific cytogenetic aberrations and assess their prognostic interaction with *JAK2* V617F allele burden and other prognostically relevant mutations. In this regard, in our previous study, which investigated the prognostic significance of cytogenetic findings at diagnosis in 137 patients with PV with AK in 15%, we did not find a significant association between AK and *JAK2* allele burden or survival, likely due to the short follow-up duration of 38 months.[13] By contrast, a subsequent study which included 196 PV patients with AK in 19% (median follow-up 84 months) confirmed the adverse impact of AK on overall survival and leukemia-free survival.[10] Although the aforementioned study boasted a relatively larger sample size compared to preceding ones and featured a longer follow-up period, its scale remained modest, thereby limiting a thorough evaluation of individual cytogenetic abnormalities.[10] The current study builds upon our previous work and addresses prior limitations by using a large cohort of PV patients followed for up to five decades, enabling a robust analysis of individual abnormalities and at various phases of disease progression. Our study is unique in that it describes cytogenetics in PV as dynamic and correlated with the disease phase. We addressed this through two distinct approaches. Firstly, we compared the prevalence of cyto-

Table 5. Overall survival, leukemia-free survival and myelofibrosis-free survival prediction among 436 patients with polycythemia vera in whom cytogenetic studies were conducted within 1 year of diagnosis.

Variables	Overall survival			Leukemia-free survival			Myelofibrosis-free survival		
	Univariate *P*	Age-adjusted *P*	Multivariable *P* HR (95% CI)	Univariate *P*	Age-adjusted *P*	Multivariable *P* HR (95% CI)	Univariate *P*	Age-adjusted *P*	Multivariable *P* HR (95% CI)
Age at diagnosis, in years	<0.0001	-	-	0.37	-	-	0.34	-	-
Age ≥60 years	<0.0001	-	<0.0001 5.5 (3.1-9.7)	0.23	-	-	0.23	-	-
Gender	0.46	-	-	0.26	-	-	0.79	-	-
Leukocytes ≥15×10^9/L	0.0001	<0.0001	0.0007 2.6 (1.5-4.3)	0.50	-	-	0.20	-	-
Cardiovascular risk factors	<0.0001	0.22	-	0.07	-	-	0.91	-	-
Any thrombosis at or prior to diagnosis	0.04	0.12	-	0.95	-	-	0.06	-	-
Arterial thrombosis	0.001	0.13	-	0.86	-	-	0.10	-	-
Venous thrombosis	0.73	-	-	0.44	-	-	0.57	-	-
Abnormal karyotype including -Y (N=67)	0.0003	0.04	0.02 2.0 (1.2-3.3)	0.02	0.02	0.47 1.9 (0.4-9.0)	0.0004	0.0007	-
Abnormal karyotype excluding -Y (N=52)	0.002	0.04	-	0.007	0.009	-	0.0008	0.001	-
Isolated -Y (N=15)	0.07	-	-	0.43	-	-	0.34	-	-
Single abnormality excluding -Y, +8, +9, or del(20q) (N=16)	0.19	0.42	-	0.38	-	-	0.18	0.21	-
Isolated del(20q) (N=10)	0.08	0.44	-	0.44	-	-	0.07	0.07	-
Isolated +9 (N=11)	0.36	-	-	0.30	-	-	0.28	-	-
Two or more abnormalities (N=11)	0.25	-	-	0.001	0.002	-	0.08	0.09	-
Isolated+8 (N=4)	0.15	-	-	0.65	-	-	0.35	-	-
SRSF2 mutation (N=8)	<0.0001	0.001	0.002 6.1 (2.3-16.1)	0.004	0.005	0.004	0.003	0.005	0.001 18.4 (4.7-72.4)

HR: hazard ratio; 95% CI: 95% confidence interval.

genetic abnormalities at various timepoints after diagnosis including within a year of diagnosis, 1-10 years, beyond 10 years, and at post-PV MF and LT. Over the aforementioned timepoints, AK was increasingly prevalent, reaching ≥50% at disease transformation. Additionally, we identified the close association between AK and disease progression; specific abnormalities, such as isolated del(20q), single abnormalities (excluding del(20q), +8, +9, or -Y), and ≥2 abnormalities, were more likely at post-PV MF. Secondly, we confirmed the acquisition of karyotypic abnormalities among patients with subsequent bone marrow biopsies to illustrate clonal evolution both during the chronic phase and at transformation. The frequency of AK increased from baseline to the time of the second biopsy (without overt disease transformation), as well as during post-PV MF and LT, further emphasizing the instability and dynamic nature of chromosomal abnormalities throughout the course of the disease. These findings correspond to those of an MD Anderson study that documented cytogenetic clonal evolution to be a common phenomenon both in the chronic phase of PV and during disease transformation.[14] Similarly, a prior Mayo Clinic study documented new cytogenetic abnormalities in 25% of patients with PV and change in karyotype from "normal" to "abnormal", in the absence of overt disease transformation, showed a trend for adverse survival.[15] Collectively, these findings strengthen the argument for conducting karyotypic analysis at the time of diagnosis but also underscore the case for follow-up reassessment of karyotype to ensure continued surveillance. Salient findings include the association between AK at baseline and older age, lower platelet count and degree of bone marrow fibrosis.[16] With respect to specific abnormalities, we observed the following correlations: del(20q) (older age and grade ≥2 reticulin fibrosis); +9 (more frequent history of venous thrombosis); other sole abnormalities (older age); and ≥2 abnormalities (male gender, less frequent history of arterial thrombosis, lower platelet count). In regard to the prognostic interaction with mutations, *SRSF2* and *IDH2* mutations were shown to cluster with normal karyotype, while there was no significant disparity in frequency of other prognostically relevant mutations (*ASXL1* and *TP53*) between patients with a normal karyotype or AK, likely due to the small number of informative cases.

From a therapeutic standpoint, current goals of treatment in PV are mainly focused on reducing the risk of thrombotic complications and improving clinical symptoms.[2] Observations from the current study prompt further investigation into the efficacy of existing and investigational therapies in PV within the context of karyotype. In particular, the anti-clonal and disease-modifying potential of interferon has long been recognized in PV, with cases of complete or partial cytogenetic remission documented following treatment with interferon.[17-21] Recently, the PROUD-PV/CONTINUATION-PV phase III study of ropegylated interferon-α2b, compared to best available therapy,[22] showed superior molecular response with ropegylated interferon-α2b; 54.3% patients achieved a JAK2 V617F allele burden <10% after 5 years of treatment which was associated with a non-significant reduction in risk of disease progression.[23] It should be noted that the particular study did not provide any information on cytogenetics. Given the dynamic nature of cytogenetic abnormalities during the course of PV, one should consider addition of cytogenetic evaluation in response assessment, which currently incorporates clinical, hematologic and histological assessments as specified in the European LeukemiaNet response criteria.[24] Furthermore, *JAK2* V617F allele burden suppression to less than 1% by itself might not suffice as a marker of treatment response, since more than half of PV patients harbor additional non-*JAK2* mutations, which may persist despite reduction in *JAK2* levels. Regardless, additional prospective studies are warranted to examine the interaction between karyotype

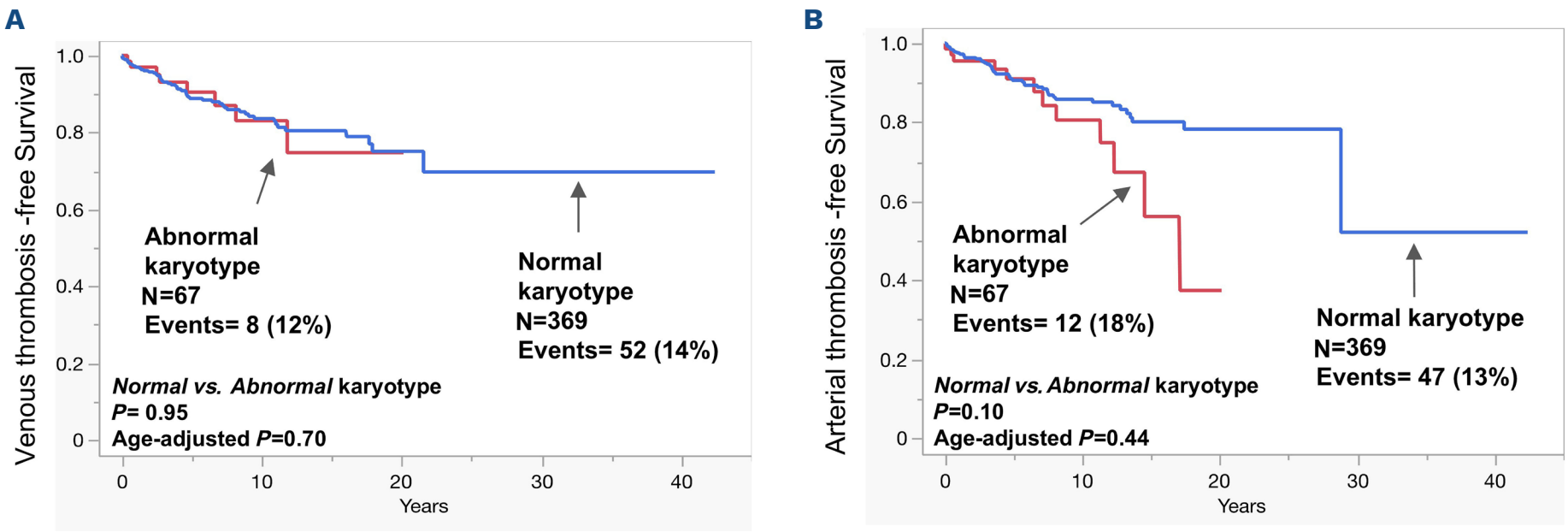

Figure 2. Abnormal karyotype and thrombosis in polycythemia vera. (A) Venous thrombosis-free survival in 436 patients with polycythemia vera, stratified by cytogenetics performed at or within 1 year of diagnosis. (B) Arterial thrombosis-free survival in 436 patients with polycythemia vera, stratified by cytogenetics performed at or within 1 year of diagnosis.

and *JAK2* V617F allele burden and other prognostically relevant mutations in order to confirm the anti-clonal and disease-modifying potential of long-acting interferon and other cytoreductive therapies.

In summary, the current study establishes that the presence of AK at the time of diagnosis of PV is an independent risk factor for survival and post-PV MF/LT. The increased risks of post-PV MF with del(20q) and LT with ≥2 abnormalities require confirmation in prospective studies. Furthermore, baseline cytogenetic evaluation should be complemented with serial cytogenetic and molecular monitoring, which holds promise not only for predicting disease progression but also monitoring response to potentially disease-modifying therapies.

Disclosures

NG is a member of advisory boards for DISC Medicine and Agios. The other authors have no conflicts of interest to disclose.

Contributions

MI, NG and AT designed the study, collected data, performed analyses and co-wrote the paper. MR, YJ and MA collected data. KR provided hematopathology expertise. CZM provided cytogenetics expertise. AP contributed patients.

Data-sharing statement

Requests for original data can be addressed to the corresponding author.

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