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Characteristics and clinical significance of cytogenetic abnormalities in polycythemia vera

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ABSTRACT

Up to 20% of patients with polycythemia vera have karyotypic abnormalities at the time of the initial diagnosis. However, the cytogenetic abnormalities in polycythemia vera have not been well characterized and their prognostic impact is largely unknown. In this study, we aimed to address these issues using a large cohort of polycythemia vera patients with cytogenetic information available. The study included 422 patients, 271 in polycythemic phase, 112 with post-polycythemic myelofibrosis, 11 in accelerated phase, and 28 in blast phase. Abnormal karyotypes were detected in 139 (33%) patients, ranging from 20% in those in the polycythemic phase to 90% among patients in accelerated/blast phase. Different phases harbored different abnormalities: isolated del(20q), +8 and +9 were the most common abnormalities in the polycythemic phase; del(20q) and +1q were the most common abnormalities in post-polycythemic myelofibrosis; and complex karyotypes were the most common karyotypes in accelerated and blast phases. Patients with an abnormal karyotype showed a higher frequency of disease progression, a shorter transformation-free survival and an inferior overall survival compared with patients with a normal karyotype in the same disease phase. Cytogenetics could be effectively stratified into three risk groups, low- (normal karyotype, sole +8, +9 and other single abnormality), intermediate- (sole del20q, +1q and other two abnormalities), and high-risk (complex karyotype) groups. We conclude that cytogenetic changes in polycythemia vera vary in different phases of disease, and carry different prognostic impacts.

Introduction

Polycythemia vera (PV) is a myeloproliferative neoplasm characterized by increased red blood cell production, a somatic gain-of-function mutation of *JAK2*, and panmyelosis in bone marrow (BM).[1,2] The natural course of PV usually includes three phases: the pre-polycythemic phase, polycythemic phase (PP), and post-polycythemic myelofibrosis (post-PV MF). The disease in a small subset of patients may transform into an accelerated phase (AP), with 10-19% blasts in the peripheral blood and/or BM, or a blast phase (BP) with ≥20% blasts in peripheral blood/BM.

Patients with PV generally have relatively long survival (median, 14-19 years). Potentially fatal complications include thrombosis, progression into myelofibrosis (post-PV MF) or transformation to BP.[3] The median survival for patients with post-PV MF is 5-6 years[4] and patients with blastic transformation often have a dismal prognosis with a median survival of <6 months.[5] The frequency of post-PV MF is 4.9-6% at 10 years and 6-14% at 15 years;[3,6] and the risk of BP is 2.3-14.4% at 10 years and 5.5-18.7% at 15 years.[3,7,8] Advanced age, leukocytosis, BM reticulin fibrosis, and splenomegaly have been reported to be risk factors for post-PV MF and BP;[7,9-12] while leukocytosis, advanced age, and history of thrombosis have been

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found to be independent risk factors for overall survival (OS).[3,7,11]

Cytogenetic abnormalities can be detected in 14-20% of patients at the time of the initial diagnosis of PV,[13-16] with del(20q), +8, +9 and +1q being the most commonly reported.[3,17,18] The low frequency of abnormal karyotypes has made prognostication of PV patients using cytogenetic data challenging and some studies have not shown a prognostic difference between patients with a normal or abnormal karyotype.[13] Recently other studies,[7,14,19] including one by the International Working Group for Myeloproliferative Neoplasms Research and Treatment (IWG-MRT),[7] have found that patients with an abnormal karyotype have a higher risk of disease progression and an inferior outcome. However, the prognostic impact of individual cytogenetic abnormalities was not further classified, and the three most common abnormalities, +8, +9, and del(20q) have not been shown conclusively to have prognostic value.[13,20]

Here we reviewed 422 patients with PV for whom we had detailed clinicopathological and cytogenetic information. We examined the characteristics of the abnormal karyotypes during different stages of PV; the correlation of acquisition of cytogenetic abnormalities (ACA) and disease progression; and the prognostic impact of different specific cytogenetic abnormalities during different stages of PV.

Methods

Patients

We searched the archives of The University of Texas MD Anderson Cancer Center (MDACC) for patients with PV who were diagnosed and/or managed at MDACC from January 2005 through June 2016. For patients whose initial diagnosis of PV was established at other hospitals, the pathological material was reviewed in our department to confirm the diagnosis. The clinical presentation, laboratory data, and pathological findings were collected at the time of diagnosis, and during the follow-up. Blasts were counted on peripheral blood smears based on a 200-cell differential count and on BM smears based on 500 cells. The degree of BM myelofibrosis was based on the European Consensus on grading of bone marrow fibrosis.[21] The diagnoses of PV and post-PV MF were based on the World Health Organization criteria;[1] AP was defined as ≥10% blasts and BP as ≥20% blasts in peripheral blood or BM or both. Disease progression (or transformation) was defined as disease that progressed from PP to post-PV MF, AP, or BP; or from post-PV MF to AP or BP. The study was approved by the institutional review board at MDACC.

Conventional cytogenetic analyses

Conventional chromosomal analyses were performed on G-banded metaphase cells prepared from unstimulated 24-h and 48-h BM aspirate cultures using standard techniques. The median number of metaphases analyzed was 20 (range, 12 to 30). The karyotypes were documented according to the International System for Human Cytogenetic Nomenclature (ISCN 2016).[22] In accordance with standard practice, if cytogenetic testing was performed within 4 months of initial diagnosis, it was considered to be "at initial diagnosis"; all other tests were considered as "beyond initial diagnosis". A complex karyotype was defined as three or more chromosomal abnormalities. Specific cytogenetic abnormalities identified in four or more patients were grouped separately and the rest were grouped as "other single" or "other double" abnormalities. ACA was defined as the acquisition of an abnormal clone(s) from a previously normal karyotype, or the acquisition of additional chromosomal abnormalities or abnormal clone(s) from a previously abnormal karyotype.

Statistical analyses

An unpaired *t*-test was used for numerical comparisons between groups. Chi-square and Fisher exact tests were applied for categorical variables. The date of diagnosis was calculated from the date that a BM was performed to establish the diagnosis. OS was estimated by the Kaplan–Meier method from the date of diagnosis until death from any cause (censored at last follow-up for patients who were alive). Transformation-free survival was estimated by the Kaplan-Meier method from the date of diagnosis to the date of disease progression to a higher stage or until death or the last follow-up. *P* values ≤0.05 were considered to be statistically significant.

Results

Clinical and pathological findings

A total of 477 patients were diagnosed and/or treated at our institute during the study period, 55 patients were excluded from this study because of lack of cytogenetic information at the time of diagnosis when the first BM evaluation was performed. Of the 422 patients included in the study, 114 patients had BM evaluation and cytogenetic analysis at the initial diagnosis, and the other 308 patients had BM evaluation at a median interval of 58 months after the initial diagnosis. Patients were diagnosed at a median age of 54 years (range, 11-84 years) and the male to female ratio was 227/195 (1.2:1) (Table 1). Prior to the first BM evaluation, 119 patients had not received any treatment, 76 had only been treated with phlebotomy, 10 had received aspirin only, 89 had received hydroxyurea only, 75 patients had been managed with two or three treatments including phlebotomy, hydroxyurea and/or aspirin and 53 patients were also treated with anagrelide (n=39), interferon (n=10), or imatinib (n=4). At the time of diagnosis, 271 (64%) patients were in PP, 112 (26.5%) in post-PV MF, 11 (3%) in AP, and 28 (6.6%) in BP. Since patients in AP shared very similar clinical features and disease course as patients in BP (*data not shown*), we combined these two groups of patients into one group (AP/BP) in this study.

Patients with a normal karyotype and those with an abnormal karyotype had a comparable age, a similar gender distribution and leukocyte counts at all PV stages. However, at the stage of PP, patients with an abnormal karyotype had a significantly lower hemoglobin level and platelet count, a higher frequency of splenomegaly, and a higher grade of BM myelofibrosis. In the stage of post-PV MF, patients with an abnormal karyotype had lower platelet counts and in the AP/BP, patients with normal and abnormal karyotypes showed similar clinical and pathological features (Table 1).

Cytogenetic features

The cytogenetic data are summarized in Table 2. The most common chromosomal abnormalities included del(20q) (n=31), +9 (n=10), and +8 (n=8) as a sole abnormality; +1q (n=15) as a component in double abnormalities, eight resulting from aberrations of +1, der(1;7)(q10;p10), and seven from other abnormalities. The most common chromosomal abnormalities detected

Table 1. Demographic, clinical and pathological features of the study patients.

Stages	Polycythemic phase (n=271)			Post-PV myelofibrosis (n=112)			Accelerated/blast phase (n=39)		
Karyotype	Normal (80%)	Abnormal karyotype (20%)	P	Normal (55%)	Abnormal karyotype (45%)	P	Normal (10%)	Abnormal karyotype (90%)	P
Gender (male/female)	119/98	26/28	0.4463	32/30	27/23	0.8504	2/2	21/14	1.0000
Age* (years)	54	54	0.4886	51	50	0.8608	65	54	0.1357
Splenomegaly (no/yes)	154/63	25/29	0.0012	15/47	10/40	0.6531	1/3	21/14	0.2998
Hemoglobin* (g/dL)	14.6	13.3	0.0124	10.0	9.7	0.2998	9.5	9.2	0.9618
Leukocytes* ($x10^9$/L)	11.3	12.6	0.7415	15.7	12.3	0.8764	9.9	9.4	0.3912
Platelets* ($x10^9$/L)	421.5	329	0.0025	323	212	0.0034	198	75	0.1315
Myelofibrosis									
• MF-0	64	10		0	0		0	5	
• MF-1	122	30		0	0		1	6	
• MF-2/3	23	13	0.0127	59	50		1	19	
Overall survival* (months)	137	116	0.0322	73	47	0.0460	46	9	0.1624

*Presented as median of the values.

in complex karyotypes were -5/del(5q) (n=18), -7/del(7q) (n=18), -17/del(17p)/add(17p) (n=14), and -18 (n=11). Of note, three cases had sole –Y, and no patient had del5q/-5 or del(7q)/-7 as a sole abnormality.

The distribution of chromosomal abnormalities varied in different stages of PV. In the PP, the most common cytogenetic abnormalities were sole del(20q), +8 or +9; in the phase of post-PV MF, the most common ones were sole del(20q) and +1q; and in the AP/BP, the most common one was a complex karyotype. The higher the disease stage, the higher the frequency of abnormal karyotypes: 20% in PP, 45% in post-PV MF, 90% in AP/BP. Additionally, the frequency of a complex karyotype increased as the disease stage advanced, 1.5% in PP; 10.7% in post-PV MF; 61.5% in AP/BP.

Among the 114 patients who had cytogenetic analyses performed at the time of initial diagnosis, 107 were in PP and seven in the post-PV MF phase. Among the 107 patients in PP, an abnormal karyotype was detected in 17 (15%) patients, a single abnormality, including +9 (n=6), +8 (n=3), del(20q) (n=3), -Y (n=1), and del(11q) (n=1) in 14, double abnormalities (1 with +1q) in two, and complex karyotype in one. Among the seven patients in post-PV MF, six had a normal karyotype and one (14.3%) had isolated add(21p).

Table 2. Cytogenetic abnormalities detected at the diagnosis (first bone marrow evaluation).

	Polycythemic phase (n=271)	Post-PV MF (n=112)	AP/BP phase (n=39)	Total (n=422)
Normal karyotype	217 (80%)	62 (55%)	4 (10%)	283 (67%)
Abnormal karyotype	54 (20%)	50 (45%)	35 (90%)	139(33%)
Single abnormalities	41 (76%)	29 (58%)	5 (14%)	75 (54%)
- del20q	18	12	1	31
- +9	10	0	0	10
- +8	6	1	1	8
- other single	7	16	3	26
Double abnormalities	9 (17%)	9 (18%)	6 (17%)	24 (17%)
- +1q	4	7	4	15
- other two	5	2	2	9
Complex	4 (7%)	12 (24%)	24 (69%)	40 (29%)
- del5q/-5	0	4	14	18
- del7q/-7	1	2	15	18
- del17p/-17/i(17q)	1	4	9	14

AP/BP: accelerated/blast phase; Post-PV MF: post-polycythemic myelofibrosis.

Clinical follow-up and disease progression

The median follow-up was 36 months (range, 0-168 months) from diagnosis (the first BM biopsy). Of the 372 patients for whom there was follow-up information, 66 were under observation or received phlebotomy only, 88 were treated with JAK2 inhibitors, 48 with interferon, 18 with imatinib, and 129 received single or combined therapies that included hydroxyurea, anagrelide, revlimid, azacitidine, or induction chemotherapies; ten patients also underwent allogeneic stem cell transplantation. Information on therapies was not available for 23 patients. At last follow-up, 123 (29%) patients had died, including 49 from PP, 44 from post-PV MF, and 30 from AP/BP. The median OS was 137, 60, and 9 months for patients in PP, post-PV MF, AP/BP stages, respectively; the OS was significantly different among patients in different stages (Figure 1).

Disease progression was assessed in patients who had two or more (up to 28) BM evaluations during the follow-up, and was evaluated in PP and post-PV MF stages separately.

As shown in Table 3, 146 (54%) patients in PP had follow-up BM evaluations, 45 (31%) patients showed disease progression, 31 patients progressed to post-PV MF and 14 progressed to AP/BP. Patients with an abnormal karyotype showed a higher risk of disease progression and a significantly shorter transformation-free survival compared with patients who had a normal karyotype (Figure 2A).

As also shown in Table 3, 76 (68%) patients in the post-PV MF phase had follow-up BM evaluations and 21 (28%) showed disease progression to AP/BP. Patients with an abnormal karyotype showed a significantly shorter transformation-free survival (Figure 2B), but a comparable risk of transformation, compared with patients who had a normal karyotype.

Of the 107 patients who had BM evaluation at the time of initial diagnosis and were in PP, 59 (55%) had at least one follow-up BM specimen for evaluation. Patients with an abnormal karyotype showed a significantly higher frequency of transformation (60% *versus* 10%, $P<0.0001$) and a shorter transformation-free survival (101 months *versus* undefined, $P=0.0004$) compared with patients who had a normal karyotype (Table 3).

We compared the disease progression among patients with different degrees of myelofibrosis (MF-0, MF-1, and MF-2/3) in PP. Among the 74 patients with MF-0, 32 patients had follow-up BM evaluations: 4/32 (12.5%) patients showed disease progression. Among the 152 patients with MF-1, 84 patients had follow-up BM evaluations: 26/84 (31%) patients showed disease progression. Among 36 patients with MF-2/3, 29 patients had follow-up BM evaluations: 15/29 (52%) patients showed disease progression. These data reveal that the higher the grade of MF, the higher the frequency of disease progression (P=0.0570 for MF-0 *versus* MF-1; $P=0.0090$ for the three groups).

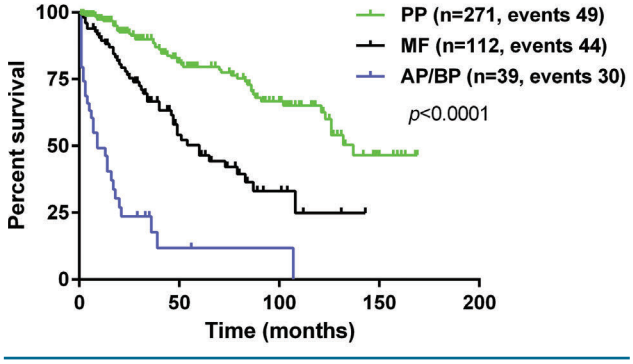

Figure 1. Overall survival of patients in different stages. Patients in a higher stage had a significantly inferior overall survival. AP/BP: accelerated/blast phase; MF: post-polycythemic myelofibrosis; PP: polycythemic phase.

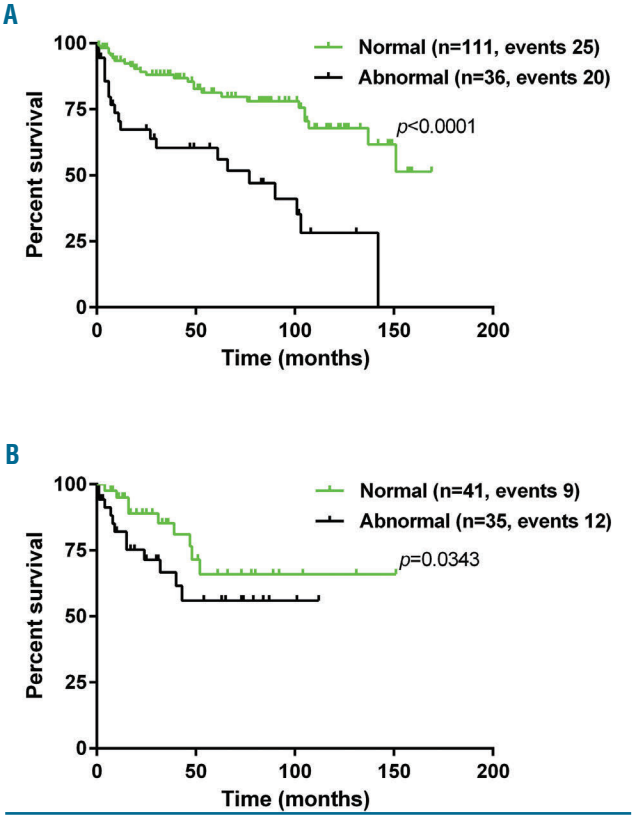

Figure 2. Transformation-free survival of patients with normal and abnormal karyotypes. Patients with an abnormal karyotype had a significantly shorter transformation-free survival. (A) Patients in polycythemic phase; (B) patients with post-polycythemic myelofibrosis.

Table 3. Disease progression of patients with normal and abnormal karyotypes.

Stages	Karyotype	Disease progression		Progressed to		TFS
		No	Yes	Post-PV MF	AP/BP	(median, months)
*Polycythemic phase (n=146)	Normal (n=111)	86 (77%)	25 (23%)	18 (16%)	7 (6%)	163
	Abnormal (n=36)	16 (44%)	20 (56%)	13 (34%)	7 (22%)	77
		$P=0.0003$		Total: 31%		$P<0.0001$
†*Polycythemic phase (n=59)	Normal (n=49)	44 (90%)	5 (10%)	2 (4%)	3 (6%)	Undefined
	Abnormal (n=10)	4 (40%)	6 (60%)	5 (50%)	1 (10%)	101
		$P<0.0001$		Total: 19%		$P=0.0004$
*Post-PV MF (n=76)	Normal (n=41)	32 (78%)	9 (22%)		9 (22%)	Undefined
	Abnormal (n=35)	23 (66%)	12 (34%)		12 (34%)	Undefined
		$P=0.3050$		Total: 28%		$P=0.0343$

*Only patients who had two or more bone marrow evaluations were included. †Patients had bone marrow evaluation and karyotyping analysis at initial diagnosis. AP/BP: accelerated/blast phase; post-PV MF: post-polycythemic myelofibrosis; TFS: transformation-free survival.

We also compared the OS among patients with MF-0, MF-1, and MF-2/3 in PP. The median OS for patients with MF-0, MF-1, and MF-2/3 were 169, 137, 126 months, respectively (P=0.0498 for MF-0 *versus* MF-1; P=0.0490 for the three groups).

Acquisition of cytogenetic abnormalities during the course of disease

Of the 383 patients in the PP and post-PV MF phase, 224 (136 in PP and 74 with post-PV MF) had at least two (and up to 25) cytogenetic analyses at different time points. After a median interval of 35 months (range, 1-163), 20 of 136 (14.7%) patients in PP had ACA, including 13 (of 110, 11.8%) with a normal karyotype and seven (of 32, 21.8%) with an abnormal karyotype (P=0.1580). Of the 74 patients with post-PV MF, 23 (31%) had ACA, including 10 (of 37, 27%) with a normal karyotype and 13 (of 37, 35%) with an abnormal karyotype (P=0.6160).

Of the 114 patients who had BM evaluation at the time of initial diagnosis, 59 (51.8%) had at least one follow-up cytogenetic analysis, and eight (13.6%) of them had ACA: six (12%) of 50 patients with a previously normal karyotype gained an abnormal clone, and two (22%) of nine patients with a previously abnormal karyotype showed clonal evolution.

The commonly acquired chromosomal abnormalities included del(7q)/-7 (n=8), +1q (n=7), del(5q)/-5 (n=6), del(20q) (n=5), del(17p)/-17 (n=4), and complex karyotypes (n=14); whereas +8 (n=2), and +9 (n=1) were infrequently acquired during the course of disease.

Correlation of abnormal karyotype, acquisition of cytogenetic abnormalities and disease progression

A total of 195 patients, 122 in PP and 68 in the phase of post-PV MF, had at least one follow-up analysis on both BM morphology and cytogenetics. Among the 122 patients (92 with normal and 30 with abnormal karyotype) in PP, 18 (14.8%) patients gained ACA, including 11 (12%) patients with normal karyotype and seven (23%) with abnormal karyotype (P=0.1439). A total of 39 (35%) patients, 13 (72%) with ACA and 26 (25%) without ACA (P=0.0002), showed disease progression. Among the 68 patients (35 with normal and 33 with abnormal karyotype) in the phase of post-PV MF, 21 (31%) patients gained ACA, including nine (26%) patients with normal karyotype and 12 (36%) with abnormal karyotype (P=0.4335). A total of 19 (28%) patients, ten (48%) with ACA and nine (19%) without ACA, showed disease progression (P=0.0213). The frequency of ACA was comparable among patients with a normal *versus* an abnormal karyotype, however, patients with ACA showed a significantly higher risk of disease progression compared to patients without ACA.

Prognostic significance of cytogenetic abnormalities

The median OS for patients with a normal *versus* an abnormal karyotype was 137 *versus* 116 months (P=0.0322) for patients in PP (Figure 3A); 73 *versus* 47 months (P=0.0460) for patients with post-PV MF (Figure 3B); and 46 *versus* 9 months (P=0.1624) for patients in AP/BP (Table 1).

We evaluated the prognostic impact of specific chromosomal abnormalities on patients' survival by stage (PP and post-PV MF, separately). As shown in Table 4 and in the *Online Supplementary Material*, in PP, patients with sole del(20q), double abnormalities and a complex karyotype had a significantly shorter OS than those with a normal karyotype, whereas there was not a significant difference in OS for patients with sole +8, +9, or other single abnormalities. For patients in the stage of post-PV MF, a complex karyotype correlated with a significantly inferior OS, while sole del(20q), other single abnormalities, or double abnormalities failed to show a significant effect.

Based on the results from the above analyses, we grouped the karyotypes into three risk groups: low-risk included a normal karyotype, sole +8, sole +9, and other single abnormalities; intermediate-risk included sole del(20q), double abnormalities (including +1q); and high-risk included complex karyotypes. As shown in Figure 4, patients with low-, intermediate -, and high-risk cytogenetics had significantly different OS, with a median OS of 169, 86, and 9 months in patients in PP (P<0.0001) (Figure 4A), and 83, 46, and 24 months in patients in the post-MF PV stage (P=0.0015) (Figure 4B).

Discussion

Historically, the diagnosis of PV has relied mainly on high hemoglobin level (>18.5 g/dL in men and >16.5 g/dL in women), presence of a *JAK2* mutation, and panmyelosis in the BM, and conventional cytogenetic testing is not routinely performed at the time of the initial diagnosis of PV, especially in the community hospital setting.

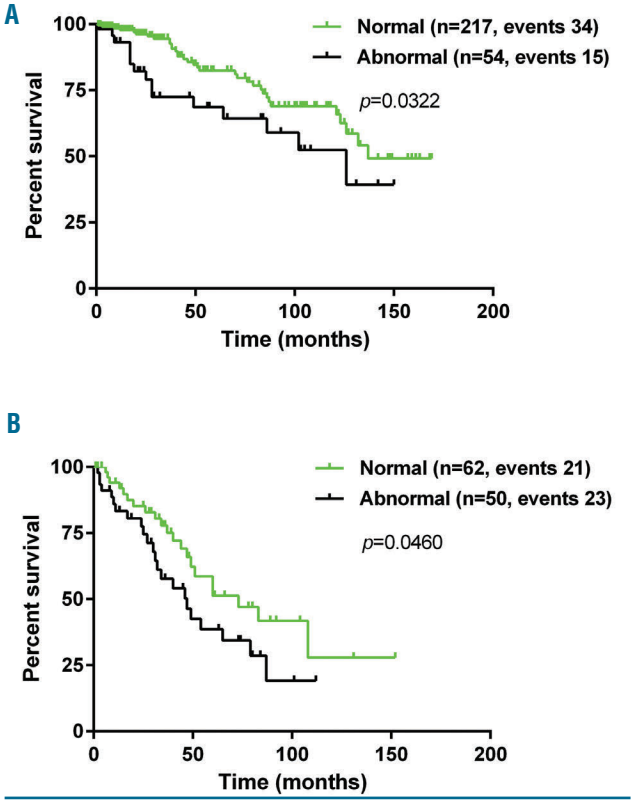

Figure 3. Overall survival of patients with normal and abnormal karyotypes. Patients with an abnormal karyotype had a significantly inferior overall survival. (A) Patients in polycythemic phase; (B) patients with post-polycythemic myelofibrosis.

Furthermore, cytogenetic testing may not be performed during follow-up, unless there is a suspicion of disease progression (e.g. substantial change in blood cell counts). In our own cohort of patients, karyotype information at the time of initial clinical diagnosis was not available for approximately two-thirds of patients, and about 40% of patients did not have follow-up cytogenetic analyses. These factors plus an overall low frequency of abnormal karyotypes detected in PP have greatly hindered the characterization and risk stratification of cytogenetics in patients with PV. Here we performed a retrospective study on 422 patients who had cytogenetic information at the time of diagnosis (with BM evaluation), and characterized the significance of cytogenetics at different stages of PV. We made a number of significant findings in this large cohort of patients, and the data provide clear guidelines for the application of cytogenetic information in PV patients.

We show, for the first time, that cytogenetic changes are dynamic in PV patients and correlate with the course of disease. The dynamic changes are first reflected in the frequency of abnormal karyotype, which increases with increasing disease stage (from 20% in the PP to 90% at BP). The dynamic changes are also shown in the distribution of specific cytogenetic abnormalities: +8 and +9 mainly in PP, +1q in the post-PV MF stage, del(5q)/-5, del(7q)/-7, del(17p)/-17/i(17q) and complex karyotypes in AP and BP. These dynamic changes in conjunction with the findings of ACA suggest that +8 and +9 are likely to be early genetic events during the pathogenesis of PV, while +1q, del(5q)/-5, del(7q)/-7 and complex karyotype are likely to be acquired during the course of disease and associated with advanced stage of disease. Similar findings have been suggested by others in earlier studies.[18,23]

We confirmed the prognostic relevance of abnormal karyotypes in PV,[7,14] but most importantly, we characterized for the first time the prognostic impact of specific recurrent cytogenetic abnormalities. Abnormalities of +8 or +9, predominantly detected in the PP, did not appear to have a significant prognostic effect, which is in line with the findings of a previous study.[13] Del(20q), the most common single abnormality in PV, showed a significant adverse effect in patients in the PP but not those with post-PV MF. Other single individual abnormalities when analyzed as a group did not show a significant effect on survival, either for patients in PP or the post-PV MF stage. It is noteworthy that these single individual abnormalities did not include -5/-5q or -7/-7q. The most common abnormality found in PV patients with double abnormalities was +1q, which showed a significant effect on OS in patients in PP, but not in those in the post-PV MF phase. A complex karyotype showed a significant adverse effect on OS in both the PP and post-PV MF stage, similar to what has been shown in acute myeloid leukemia (AML),[24] myelodysplastic syndromes (MDS),[25] and chronic myelomonocytic leukemia (CMML).[26]

Another clear conclusion drawn from this study was the association of acquisition of additional cytogenetic abnor-

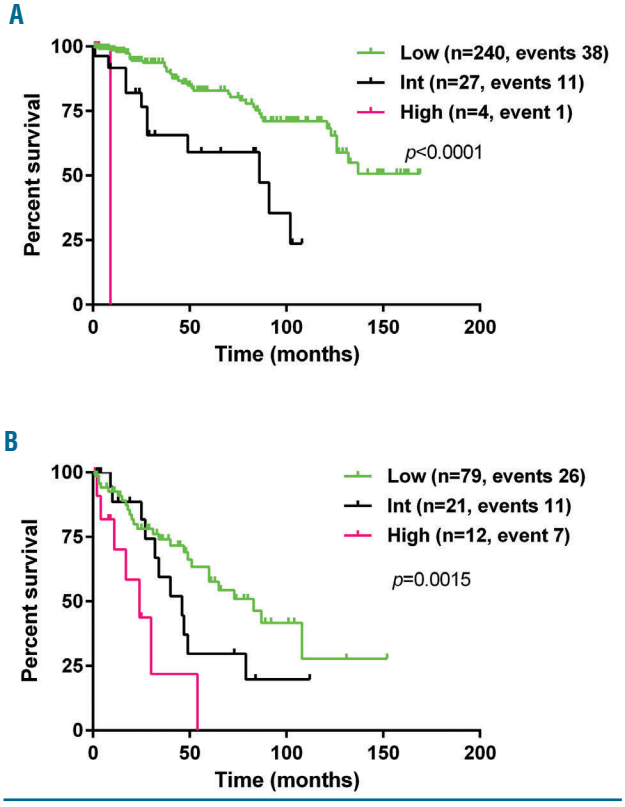

Figure 4. Overall survival of patients with low-, intermediate-, and high-risk cytogenetics. (A) Patients in polycythemic phase; (B) patients with post-polycythemic myelofibrosis.

Table 4. Impact of cytogenetic abnormalities on overall survival of patients in polycythemic phase and with post-polycythaemic myelofibrosis.

	Polycythemic phase (n=271)			Post-PV MF (n=112)		
	Cases N. (%)	Median OS (months)	P*	Cases N. (%)	Median OS (months)	P*
Normal karyotype	217 (80%)	137		62 (55%)	73	
Abnormal karyotype	54 (20%)	116	0.0322	50 (45%)	47	0.0460
Single abnormality						
- del20q	18	91	0.0011	12	34	0.2214
- +9	10	129	0.3742			
- +8	6	Undefined	0.3781	1		
- other single	7	Undefined	0.6102	16	87	0.9920
Double abnormalities	9	86	0.0483	9	41	0.1524
Complex	4	9	<0.0001	12	18	0.0017

*Compared to patients with a normal karyotype. Only groups with four or more patients were analyzed.

malities and disease progression. By studying patients who had both BM evaluation and chromosomal analysis during follow-up, we found that patients who started with an abnormal karyotype had a higher risk of disease progression and shorter transformation-free survival; ACA was associated with a higher risk of disease transformation. These results are in line with the prognostic impact of ACA in patients with MDS[27] and CMML.[28]

The prognostic effects of specific cytogenetic abnormalities are highly likely to be related to molecular changes that are induced by the corresponding chromosomal abnormalities. +8 is a common cytogenetic abnormality detected in various types of myeloid neoplasms and its prognostic effects are heterogeneous, but commonly assigned to the intermediate-risk group.[25,26,29] +9 is commonly detected in Philadelphia-negative myeloproliferative neoplasms but is uncommon in other types of myeloid neoplasms (e.g. AML, MDS), and it has been assigned to favorable risk in patients with primary myelofibrosis.[30] In our cohort, +8 and +9 were likely to be early genetic events and were not prognostically significant. +1q, commonly derived from aberrations of +1, and der(1;7)(q10;p10) (which also results in -7q), are relatively common abnormalities found in MDS and are often associated with low-risk MDS.[31,32] +1q results in the gain of cyclin-dependent kinases regulatory subunit 1B (*CKS1B*, located on 1q21), which can override the DNA damage response barrier, promote tumor development,[33] and is associated with an adverse prognosis in multiple myeloma.[34,35] In our cohort, +1q was detected mainly in post-PV MF, and was one of the most common acquired abnormalities during the course of disease. In multivariate analysis it showed a significant adverse effect on OS. Del(20q) is reported to be associated with a favorable prognosis in primary myelofibrosis and MDS;[25,30,36] however, in patients with *de novo* AML, del(20q) has been associated with a poor response to chemotherapy and is classified as intermediate-II risk.[29] In our cohort, del(20q) was the most common sole abnormality detected in both PP and post-PV MF and was significantly associated with a poorer OS in patients with PV in the PP. Interestingly, abnormalities involving chromosomes 5, 7 and/or 17 were rarely detected as a sole abnormality in PV, but were the most common abnormalities detected in complex karyotypes. These abnormalities were detected in about 44% (7/16) of patients in PP and with post-PV MF, and in 83% (20/24) of patients in AP and BP who had a complex karyotype. A complex karyotype has been associated with a higher risk of disease progression and inferior survival, as has also been shown in patients with AML,[24] MDS,[25] and CMML.[26] One of the commonly detected abnormalities in a complex karyotype was del(17p)/-17/i(17q), which results in deletion of *TP53*, a gene that plays a critical role in regulating cell-cycle arrest and apoptosis.[37] Loss of *TP53* is associated with a poorer prognosis in patients with MDS[38] and AML.[39]

Evaluation of myelofibrosis in patients in PP at initial diagnosis showed a slightly higher proportion of patients with a higher grade of myelofibrosis in our study compared with the proportion in the study conducted by Barbui *et al.*[40] The exact reason for this was not clear, but it might be partly due to differences in the referred populations of patients. Nonetheless, both our study and that by Barbui *et al.* showed that a higher degree of myelofibrosis was often associated with a higher risk of disease progression and a shorter OS. This supports the importance of evaluating BM fibrosis during the initial diagnosis.

In summary, we have reported the cytogenetic findings in a large series of patients with PV. The results show that the frequency of an abnormal karyotype and the distribution of cytogenetic abnormalities vary in different stages of PV. About 20% of patients may acquire cytogenetic abnormalities during the course of the disease, an event which is strongly associated with disease progression. Patients with an abnormal karyotype were at a higher risk of disease progression and shorter transformation-free survival and OS. Different cytogenetic abnormalities carried different prognoses and could be effectively stratified into three risk groups. These findings highlight the value of obtaining cytogenetic information in PV patients, which may be useful to guide clinical management and assess the prognosis of patients.

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