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# Refined cytogenetic-risk categorization for overall and leukemia-free survival in primary myelofibrosis: a single center study of 433 patients

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**We have previously identified sole +9, 13q- or 20q-, as 'favorable' and sole +8 or complex karyotype as 'unfavorable' cytogenetic abnormalities in primary myelofibrosis (PMF). In this study of 433 PMF patients, we describe additional sole abnormalities with favorable (chromosome 1 translocations/duplications) or unfavorable (−7/7q-) prognosis and also show that other sole or two abnormalities that do not include i(17q), −5/5q-, 12p-, inv(3) or 11q23 rearrangement are prognostically aligned with normal karyotype, which is prognostically favorable. These findings were incorporated into a refined two-tired cytogenetic-risk stratification: unfavorable and favorable karyotype. The respective 5-year survival rates were 8 and 51% (hazard ratio (HR): 3.1, 95% confidence interval (CI): 2.2–4.3; $P<0.0001$). Multivariable analysis confirmed the International Prognostic Scoring System (IPSS)-independent prognostic value of cytogenetic-risk categorization and also identified thrombocytopenia (platelets $<100\times10^9$/l) as another independent predictor of inferior survival ($P<0.0001$). A similar multivariable analysis showed that karyotype ($P=0.001$) and platelet count ($P=0.04$), but not IPSS ($P=0.27$), predicted leukemia-free survival; the 5-year leukemic transformation rates for unfavorable versus favorable karyotype were 46 and 7% (HR: 5.5, 95% CI: 2.5–12.0; $P<0.0001$). This study provides the rationale and necessary details for incorporating cytogenetic findings and platelet count in future prognostic models for PMF.**
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## Introduction

Current drug therapy for primary myelofibrosis (PMF) has not been shown to prolong survival. Therefore, an increasing number of patients are considering either allogeneic stem cell transplantation (allo-SCT) or participation in experimental drug treatment trials.[1–5] Allo-SCT is potentially curative in PMF, but its utility is limited by the relatively high incidence of treatment-related mortality and morbidity. The long-term toxicity profile of new drugs is unknown and they are not necessarily less risky than allo-SCT.[2] In general, it is reasonable to justify the risk of either allo-SCT or experimental drug therapy for PMF in the presence of a <5 years life expectancy or >20% 5-year risk of developing acute leukemia.[6] Accordingly, accurate prediction of both shortened survival and leukemic transformation is an essential component of the overall therapeutic decision-making process.[7]

The International Prognostic Scoring System (IPSS) for PMF is used to predict survival.[8] The IPSS uses five independent predictors of inferior survival: age >65 years, hemoglobin <10 g/100 ml, leukocyte count $>25\times10^9$/l, circulating blasts ⩾1% and constitutional symptoms.[8] The presence of 0, 1, 2 and ⩾3 adverse factors defines low, intermediate-1, intermediate-2 and high-risk disease with corresponding median survivals of approximately 11.3, 7.9, 4 and 2.3 years.[8] A dynamic prognostic model (DIPSS) that utilizes the same prognostic variables but can be applied at any time during the disease course was recently published.[9] DIPSS assigns two, instead of one, adverse points for hemoglobin <10 g/100 ml and risk categorization is accordingly modified: low (0 adverse points), intermediate-1 (1 or 2 points), intermediate-2 (3 or 4 points) and high (5 or 6 points). The corresponding median survivals were not reached, 14.2, 4 and 1.5 years. Neither IPSS nor DIPSS considers cytogenetic findings in its prognostic model.

Approximately, one-third of patients with PMF present with abnormal karyotype, whose prognostic value was revisited in three recent studies, each comprising 202,[10] 200[11] and 131[12] patients evaluated at or within 1 year of diagnosis. All three studies confirmed the previously reported[13] favorable prognostic impact of sole 20q- or sole 13q-. In addition, two of the three studies identified sole +9 as another favorable cytogenetic marker.[11,12] The list of unfavorable cytogenetic abnormalities from these and other related studies includes complex karyotype (⩾3 abnormalities), sole +8 and an abnormal karyotype that includes abnormalities of chromosomes 5, 7, 17 or 12p-.[10–14] The purpose of this study, which includes more than twice the number of patients included in previous studies,[10–12] was to identify additional prognostically relevant cytogenetic abnormalities in PMF and refine cytogenetic-risk categorization for overall and leukemia-free survival.

## Materials and methods

This study was approved by the Mayo Clinic Institutional Review Board. Clinical and laboratory data were collected from consecutive patients with PMF seen at our institution and in whom cytogenetic information at or within 1 year of diagnosis was available. The diagnoses of PMF and leukemic transformation were according to the 2001 World Health Organization criteria.[15] Bone marrow chromosome and *JAK2*V617F mutation analysis were performed according to previously published methods.[16,17] Cytogenetic results were interpreted and reported according to the International System for Human Cytogenetic Nomenclature; abnormal karyotype was defined by

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the presence of at least two metaphases with structural abnormalities or monosomy or three metaphases with polysomy, regardless of number of metaphases examined.[18] In order to be consistent with methodology used in recent studies,[11,12] the presence of <20 evaluable metaphases did not disqualify patients from study inclusion as long as ⩾10 metaphases were examined in those patients with 'normal' reports.

In order to examine the prognostic impact of specific cytogenetic abnormalities, initial cytogenetic group assignment required the presence of at least five informative cases.[11] Sole cytogenetic abnormalities occurring in less than five patients were grouped together and classified into two separate operational subgroups: 'other high-risk' and 'other indeterminate-risk' sole abnormalities. Assignment to the 'other high-risk' category was based on previously published observations on the prognostic impact of a particular cytogenetic abnormality in PMF and the sole abnormalities in this regard included −5/5q-, i(17q), 12p-, 11q23 rearrangement and inv(3).[10–14] All other sole abnormalities that did not meet the threshold criteria of five informative cases were grouped together as 'indeterminate-risk sole abnormalities'. Patients with two cytogenetic abnormalities were also classified into two 'high-risk' and 'indeterminate-risk' subgroups based on the presence or absence of an unfavorable abnormality including +8, −7/7q-, −5/5q-, i(17q), 12p-, inv(3) or 11q23 rearrangement.

All statistical analyses considered parameters at diagnosis and before the initiation of specific therapy. Differences in the distribution of continuous variables between categories were analyzed by either Mann–Whitney (for comparison of two groups) or Kruskal–Wallis (comparison of three or more groups) test. Patient groups with nominal variables were compared by $\chi^2$ test. Overall survival analysis was considered from the date of diagnosis to date of death (uncensored) or last contact (censored). Leukemia-free survival was calculated from the date of diagnosis to date of leukemic transformation (uncensored) or last contact/date of death (censored). Additional analyses that censored patients at time of allo-SCT were performed for both overall and leukemia-free survival in order to avoid possible confounding of survival effect from the particular treatment modality. Overall and leukemia-free survival curves were prepared by the Kaplan–Meier method and compared by the log-rank test. Cox proportional hazard regression model was used for multivariable analysis. *P*-values <0.05 were considered significant. The Stat View (SAS Institute, Cary, NC, USA) statistical package was used for all calculations.

## Results

A total of 433 patients with PMF, which included the 200 patients from our previous report,[11] constituted this study population; presenting clinical and laboratory features are outlined in Table 1. Dates of diagnosis spanned from January 1978 to November 2009. Median age at diagnosis was 65 years (range 14–92) and 268 (62%) were males. The IPSS-risk distributions could be accurately assigned for 384 patients: low in 46 (12%) patients, intermediate-1 in 97 (25%), intermediate-2 in 93 (24%) and high in 148 (39%). Twenty-six percent of the patients presented with thrombocytopenia (platelets $<100\times10^9$/l) and 17% with leukopenia (leukocytes $<4\times10^9$/l). *JAK2*V617F mutation analysis was performed in 174 patients and mutational frequency was 60%.

At the time of this report, 269 (62%) patients had died and median follow-up of patients who were alive was approximately 4 years. During this period, 34 (8%) cases of leukemic transformation were documented. As expected, the IPSS[13] effectively delineated patient groups with different prognosis (Figure 1); median survivals in low, intermediate-1, intermediate-2 and high IPSS-risk categories were 15, 7, 3.6 and 2.2 years, respectively ($P<0.0001$). The corresponding 5-year survival rates were 85, 65, 40 and 19% with hazard ratios (HRs) (95% confidence interval (CI)) of 7.7 (4.4–13.6; $P<0.0001$), 4.0 (2.2–7.1; $P<0.001$), 2.0 (1.1–3.6; $P=0.02$) for high, intermediate-2 and intermediate-1-risk groups, respectively.

Treatment received during disease course was markedly heterogeneous and mostly pursued for palliative purposes; 57 (14%) cases of splenectomy and 17 (4%) of allo-SCT were documented, whereas the spectrum of drugs used included hydroxyurea, androgens, danazol, erythropoiesis stimulating agents, prednisone, thalidomide, lenalidomide, interferon, anagrelide, busulfan and experimental agents. As most deaths occurred elsewhere, cause of death was not accurately documented in most instances, but when known, included leukemic transformation ($n=28$), infection ($n=22$), 'progressive disease' ($n=10$), intracerebellar/intracranial bleed ($n=5$), heart failure ($n=5$), non-infectious respiratory failure ($n=5$), other malignancies ($n=5$), myocardial infarction or cardiac arrest ($n=4$), stroke ($n=3$), gastrointestinal bleeding ($n=3$), intra-abdominal bleeding ($n=3$), hepatic failure ($n=3$), complications of allo-SCT ($n=2$), pancreatitis ($n=1$), disseminated intravascular coagulopathy ($n=1$) and motor vehicle accident ($n=1$).

### *Cytogenetic details at presentation*

Cytogenetic findings were normal in 275 (64%) patients. Among the 158 (36%) patients with abnormal karyotype, 109 (69% of abnormal cases) represented sole abnormalities, 23 (15%) two abnormalities and 26 (17%) three or more (that is complex) abnormalities. The most frequent sole abnormality was 20q- constituting 28% ($n=30$) of all sole abnormalities, 19% of all abnormal cases and 7% of the entire study cohort (Table 1). Other sole abnormalities that met the threshold criteria of five informative cases, for consideration as a separate cytogenetic category, included +8 ($n=14$), 13q- ($n=10$), chromosome 1 translocation/duplication ($n=12$), +9 ($n=9$) and −7/7q- ($n=5$).

Twenty-nine patients had sole abnormalities that were each seen in less than five patients; these cases were classified into two separate groups for further analysis, as elaborated in Materials and methods: 'other high-risk sole abnormalities' ($n=6$) and 'other indeterminate-risk sole abnormalities' ($n=23$). The former included i(17q), −5/5q-, 12p-, 11q23 rearrangement or inv(3) and the latter as outlined in the footnote of Table 1. Twenty-three patients had two abnormalities that did ($n=8$) or did not ($n=15$) include known or putative unfavorable abnormalities including +8, −7/7q-, i(17q), −5/5q-, 12p-, 11q23 rearrangement or inv(3). In other words, as was previously outlined in the context of 'other' sole abnormalities, patients with two cytogenetic abnormalities were sub-classified into two separate groups for further analysis.

### *Comparison of clinical characteristics among cytogenetic categories*

Based on the above, 12 cytogenetic categories were initially considered and compared for their presenting clinical and laboratory features (Table 1). Significant differences were noted in regards to platelet count considered as a continuous variable

**Table 1** Cytogenetic categories in 433 patients with primary myelofibrosis and their presenting clinical characteristics

| *Variables* | *All patients (n = 433)* | *Normal karyotype (n = 275)* | *Sole 20q- (n = 30)* | *Sole 13q- (n = 10)* | *Sole +9 (n = 9)* | *Sole Chrom. 1 trans/dup. (n = 12)*[a] | *Other indeterminate risk sole abns. (n = 23)*[b] | *Two abns. excluding unfavorable (n = 15)*[c] | *Two abns. including unfavorable (n = 8)*[c] | *Complex karyotype (n = 26)* | *Sole+8 (n = 14)* | *Sole −7/7q- (n = 5)* | *Other high-risk sole abns. (n = 6)*[d] | P *value* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Age in years, median (range) | 65 (14–92) | 65 (14–87) | 71 (44–83) | 57 (40–85) | 65 (46–77) | 60 (54–73) | 64 (42–84) | 60 (39–76) | 72 (62–78) | 64 (15–83) | 69 (39–79) | 64 (57–84) | 62 (59–92) | 0.08 |
| Age >65 years, *n* (%) | 206 (48) | 131 (48) | 18 (60) | 3 (30) | 4 (44) | 4 (33) | 11 (48) | 3 (20) | 7 (88) | 13 (50) | 8 (57) | 2 (40) | 2 (33) | 0.18 |
| Males, *n* (%) | 268 (62) | 174 (63) | 22 (73) | 5 (50) | 5 (56) | 6 (50) | 16 (70) | 12 (80) | 1 (13) | 15 (58) | 7 (50) | 1 (20) | 4 (67) | 0.06 |
| Hemoglobin, g/100 ml median (range) | 10.2 (5–16.1) | 10.3 (5–16.1) | 10.3 (7.1–15) | 10.9 (9.2–13) | 10.8 (7.8–14) | 10.4 (7.9–14.5) | 9.4 (7.7–14.2) | 10.3 (7.7–15.4) | 9.9 (7.4–11.3) | 9.1 (6.3–12.4) | 10.4 (7.1–13) | 10 (7.5–10.4) | 9.8 (8.6–12.9) | 0.18 |
| Leukocytes $\times 10^9$/l median (range) | 8.3 (0.9–113) | 8.7 (0.9–113) | 5.5 (1.4–57) | 7.8 (5–45) | 9.9 (2.9–30) | 9.4 (2.4–22.8) | 8.7 (1.5–44.8) | 12.5 (1.4–99.1) | 3.9 (2.1–14.6) | 5 (1.53–50) | 11.15 (1.8–27) | 6.3 (4.8–69.2) | 13.05 (2.8–44.1) | 0.07 |
| Platelets $\times 10^9$/l median (range) | 229 (6–1765) | 252 (11–1765) | 146 (12–459) | 336 (13–453) | 176.5 (38–419) | 253 (88–558) | 335 (32–966) | 182 (19–1304) | 133 (19–968) | 65 (6–598) | 152 (17–684) | 77 (62–259) | 156 (33–404) | <0.0001 |
| *IPSS risk (%) n evaluable = 384* | | | | | | | | | | | | | | 0.26 |
| Low | 12 | 14 | 8 | 20 | 14 | 9 | 10 | 21 | 0 | 5 | 0 | 0 | 0 | |
| Intermediate-1 | 25 | 26 | 28 | 20 | 43 | 46 | 15 | 21 | 12 | 9 | 36 | 25 | 60 | |
| Intermediate-2 | 24 | 23 | 28 | 50 | 14 | 9 | 30 | 43 | 12 | 27 | 21 | 0 | 0 | |
| High | 39 | 37 | 36 | 10 | 29 | 36 | 45 | 15 | 76 | 59 | 43 | 75 | 40 | |
| Constitutional symptoms, *n* (%), *n* evaluable = 418 | 150 (36) | 88 (33) | 9 (31) | 2 (20) | 9 (56) | 3 (27) | 12 (52) | 5 (33) | 5 (63) | 12 (50) | 4 (29) | 3 (60) | 2 (40) | 0.29 |
| Circulating blasts ⩾1%, *n* (%), *n* evaluable = 386 | 211 (55) | 134 (54) | 10 (42) | 4 (40) | 2 (29) | 8 (73) | 9 (45) | 5 (36) | 6 (75) | 17 (74) | 10 (71) | 1 (33) | 5 (100) | 0.05 |
| Hemoglobin <10 g/100 ml, *n* (%) | 222 (52) | 135 (49) | 18 (62) | 4 (40) | 3 (38) | 4 (33) | 14 (61) | 6 (40) | 4 (50) | 18 (72) | 8 (57) | 5 (100) | 3 (50) | 0.17 |
| Leukocytes $>25 \times 10^9$/l, *n* (%) | 58 (14) | 38 (14) | 1 (4) | 2 (20) | 1 (13) | 0 | 5 (22) | 3 (20) | 0 | 4 (16) | 1 (7) | 2 (40) | 1 (17) | 0.4 |
| Platelets $<100 \times 10^9$/l, *n* (%) | 110 (26) | 57 (21) | 11 (38) | 0 | 2 (25) | 2 (17) | 4 (17) | 4 (27) | 2 (25) | 16 (64) | 6 (43) | 3 (60) | 3 (50) | 0.0001 |
| Leukocytes $<4 \times 10^9$/l, *n* (%) | 71 (17) | 29 (11) | 12 (41) | 0 | 2 (25) | 3 (25) | 4 (17) | 2 (13) | 4 (50) | 9 (36) | 4 (29) | 0 | 2 (33) | <0.0001 |
| Splenectomy, *n* (%) | 57 (14) | 35 (13) | 4 (14) | 1 (10) | 2 (25) | 2 (18) | 3 (13) | 3 (13) | 1 (13) | 2 (9) | 2 (14) | 1 (20) | 2 (40) | 0.9 |
| *JAK2*V617F *n* tested (% positive) | 174 (60) | 114 (54) | 14 (71) | 6 (33) | 5 (100) | 6 (67) | 9 (78) | 6 (83) | 1(100) | 5 (60) | 6 (67) | 1 (100) | 1 (100) | 0.35 |
| SCT, *n* (%) | 17 (4) | 11 (4) | 1 (3) | 0 | 0 | 1 (8) | 0 | 0 | 0 | 2 (8) | 1 (7) | 1 (20) | 0 | 0.68 |
| Deaths, *n* (%) | 269 (62) | 165 (60) | 17 (57) | 3 (30) | 5 (56) | 6 (50) | 15 (65) | 9 (60) | 5 (63) | 24 (92) | 12 (86) | 4 (80) | 4 (67) | 0.04 |
| AML, *n* (%) | 34 (8) | 16 (6) | 1(3) | 1 (10) | 0 | 2 (17) | 3 (13) | 1 (7) | 0 | 6 (23) | 1 (7) | 1 (20) | 2 (33) | 0.04 |

Abbreviations: abns., abnormalities; AML, acute myeloid leukemia; Chrom., chromosome; IPSS, International Prognostic Scoring System;[8] SCT, stem cell transplantation; trans/dup., translocation or duplication.

[a]dup(1)(q21q32), add(1q), +der(1;7)(q10;p10), der(6)t(1;6)(q21;p21.3), +der(1;9)(p10;q10), +der(1;9)(q10;p10), t(1;13)(p12;q12), t(1;7)(q10;p10), trp(1)(p22p34.3), der(10)t(1;10)(q12;q26), t(1;12)(p32;q15), add(1q).

[b]Other indeterminate-risk sole abnormalities: del(1)(p34.1p36.1), del(2)(q11.2q13.3), t(2;12) (p13;q14), del(4)(q21.3q25), t(5;17)(q22;q21), +6, add(6)(p23), t(7;12)(q11.2;q15), t(7;12)(q22;q24.1), t(7;12)(q22;q24.3), t(8;12)(q24.1;q15), del(8)(q21.2), del(9)(q22q32), +der(11)t(11;?), tdic(11;22)(q13;11.2), del(12)(q13q22), t(13;15)(q12;q15), add(13)(p13), ins(13;13)(q1?4;q12q14), XXXX, XXYY, +14, +19, +21, +F (+F represents either +19 or +20).

[c]Unfavorable abnormalities: +8, −7/7q-, i(17q), −5/5q-, 12p-, inv(3) or 11q23 rearrangement.

[d]Other high-risk sole abnormalities: i(17q), −5/5q-, 12p-, inv(3) or 11q23 rearrangement.

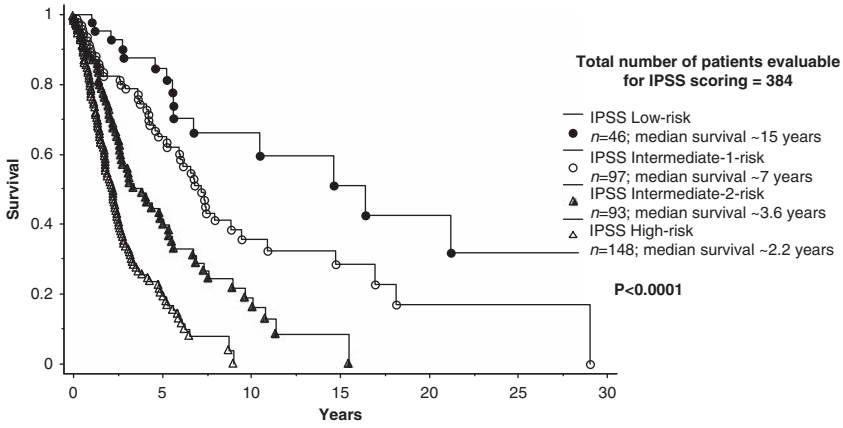


**Figure 1** Survival data of patients with primary myelofibrosis stratified by the International Prognostic Scoring System.

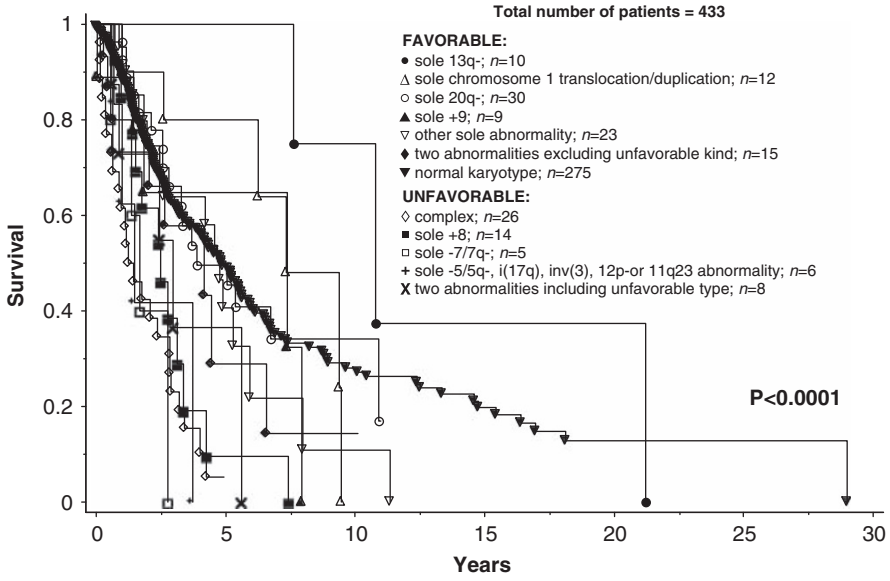


**Figure 2** Survival data of patients with primary myelofibrosis stratified by specific cytogenetic categories.

($P<0.0001$), thrombocytopenia (platelet count $<100\times10^9$/l; $P=0.0001$) and leukopenia (leukocyte count $<4\times10^9$/l; $P<0.0001$) (Table 1). These differences were also evident when analysis was restricted to patients with favorable karyotype; the incidences of leukopenia and thrombocytopenia were highest in patients with sole 20q- (41 and 38%, respectively) and lowest in those with sole 13q- (0 and 0%, respectively) (Table 1).

### *Establishment of a two-tired cytogenetic-risk stratification*

In an effort to identify cytogenetic categories of similar prognosis, each one of the above-discussed 12 cytogenetic categories was separately compared with both normal and complex karyotype.

The survival of patients with sole 13q-, sole 20q-, sole +9, sole chromosome 1 translocation/duplication, 'other sole abnormalities of indeterminate-risk' or 'two abnormalities *excluding* an unfavorable type' was similar to that of patients with normal karyotype (*P*-value of 0.07, 0.96, 0.91, 0.46, 0.3 and 0.3, respectively; Figure 2) and significantly superior to that of patients with complex karyotype (*P*-value of <0.0001, <0.0001, 0.01, 0.0001, 0.0002 and 0.02, respectively; Figure 2). These cytogenetic subcategories were, therefore, grouped together with normal karyotype and assigned a 'favorable karyotype' prognostic category (Table 2). Of note, the borderline *P*-value of 0.07 for survival comparison of normal karyotype and 13q- was in favor of the latter.

The survival of patients with sole +8, sole −7/7q-, 'other high-risk sole abnormalities' or 'two abnormalities *including* an unfavorable type' was similar to that of patients with complex karyotype (*P*-value of 0.30, 0.84, 0.74 and 0.14, respectively; Figure 2). These cytogenetic categories were accordingly grouped together with complex karyotype and assigned an 'unfavorable karyotype' prognostic category (Table 2).

Table 2 compares the clinical presentation of the above-defined 'favorable' and 'unfavorable' cytogenetic-risk groups. Unfavorable karyotype was associated with thrombocytopenia (platelet count $<100\times10^9$/l; $P<0.0001$), leukopenia (leukocyte count $<4\times10^9$/l; $P=0.0004$), circulating blasts $\geqslant1\%$ ($P=0.003$), lower hemoglobin level ($P=0.003$) and high-risk IPSS score ($P=0.01$). These findings support the need for multivariable analysis in deciphering the IPSS-independent prognostic value of cytogenetic-risk categorization. Males were over-represented in the favorable karyotype group, but the explanation for this observation is not immediately clear (64%; $P=0.01$).

**Table 2** Cytogenetic-risk groups of 433 patients with primary myelofibrosis

| *Variables* | *All patients (n = 433)* | *Favorable karyotype (n = 374)*[a] | *Unfavorable karyotype (n = 59)*[b] | P-*value* |
|---|---|---|---|---|
| Age (years), median (range) | 65 (14–92) | 65 (14–87) | 66 (15–92) | 0.23 |
| Age > 65 years, *n* (%) | 206 (48) | 174 (47) | 32 (54) | 0.27 |
| Males (%) | 268 (62) | 240 (64) | 28 (47) | 0.01 |
| Hemoglobin, g/100 ml, median (range) | 10.2 (5–16.1) | 10.3 (5–16.1) | 9.8 (6.3–12.9) | 0.003 |
| Leukocyte count $\times 10^9$/l, median (range) | 8.3 (0.9–113.2) | 8.3 (0.9–113.2) | 6.3 (1.5–69.2) | 0.08 |
| Platelet count $\times 10^9$/l, median (range) | 229 (6–1765) | 246 (11–1765) | 99 (6–968) | <0.0001 |
| *IPSS risk group (%) n evaluable = 384* | | | | 0.01 |
| Low | 12 | 13 | 2 | |
| Intermediate-1 | 25 | 26 | 23 | |
| Intermediate-2 | 24 | 25 | 19 | |
| High | 39 | 36 | 56 | |
| Constitutional symptoms, *n* (%), *n* evaluable = 418 | 150 (36) | 124 (34) | 26 (46) | 0.07 |
| Circulating blasts ⩾1%, *n* (%), *n* evaluable = 386 | 211 (55) | 172 (52) | 39 (74) | 0.003 |
| Hemoglobin <10 g/100 ml, *n* (%) | 222 (52) | 184 (50) | 38 (66) | 0.02 |
| Leukocytes $>25 \times 10^9$/l, *n* (%) | 58 (14) | 50 (13) | 8 (14) | 0.94 |
| Platelets $<100 \times 10^9$/l, *n* (%) | 110 (26) | 80 (22) | 30 (52) | <0.0001 |
| Leukocytes $<4 \times 10^9$/l, *n* (%) | 71 (17) | 52 (14) | 19 (33) | 0.0004 |
| Splenectomy, *n* (%) | 57 (14) | 49 (14) | 8 (15) | 0.85 |
| *JAK2*V617F status *n* tested (% positive) | 174 (60) | 160 (59) | 14 (71) | 0.37 |
| Transplanted, *n* (%) | 17 (4) | 13 (4) | 4 (7) | 0.2 |
| Deaths, *n* (%) | 269 (62) | 220 (59) | 49 (83) | 0.0004 |
| Leukemic transformations, *n* (%) | 34 (8) | 24 (6) | 10 (17) | 0.005 |

Abbreviation: IPSS, International Prognostic Scoring System.[8]
[a]Favorable karyotype: normal karyotype or sole or two abnormalities that do not include the above-listed unfavorable cytogenetic abnormalities.
[b]Unfavorable karyotype: complex karyotype or sole or two abnormalities that include +8, −7/7q-, i(17q), −5/5q-, 12p-, inv(3) or 11q23 rearrangement.

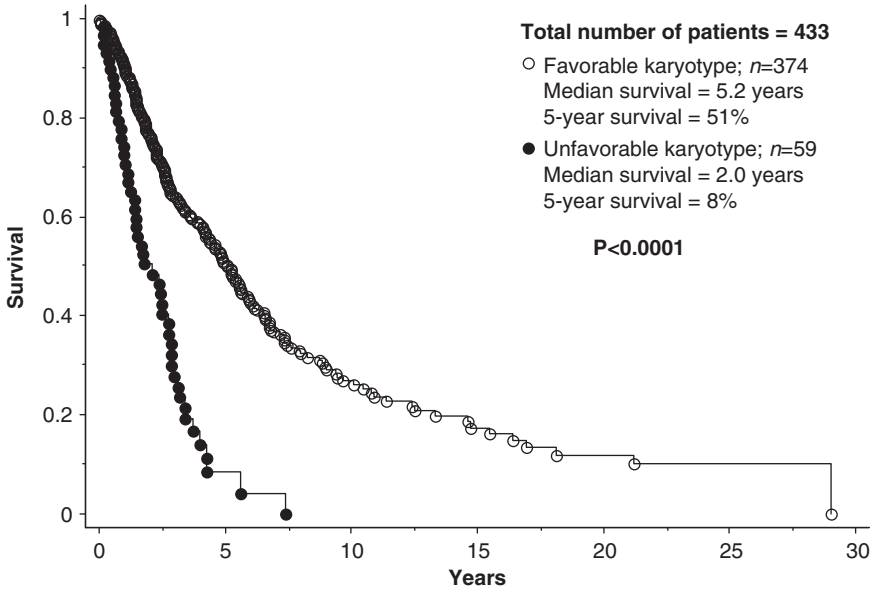


**Figure 3** Survival data of patients with primary myelofibrosis stratified by two-tired cytogenetic-risk categorization: unfavorable (complex karyotype or sole or two abnormalities that include +8, −7/7q-, i(17q), inv(3), −5/5q-, 12p- or 11q23 rearrangement) and favorable (all other scenarios including normal karyotype).

### *Comparison of overall and leukemia-free survival between favorable and unfavorable karyotype*

Median survivals of patients with favorable and unfavorable karyotype were 5.2 and 2.0 years, respectively ($P<0.0001$; Figure 3). The corresponding 5-year survival rates were 51 and 8% (HR: 3.1, 95% CI: 2.2–4.3; $P<0.0001$). Multivariable analysis, which included variables that were found to be significant in univariate analysis (Table 2), confirmed the IPSS-independent prognostic value of cytogenetic-risk categorization ($P<0.0001$; HR: 2.1, 95% CI: 1.5–3.1) and also identified thrombocytopenia (platelet count $<100 \times 10^9$/l) as another independent predictor of inferior survival ($P<0.0001$; HR: 1.9, 95% CI: 1.4–2.6); the HRs (95% CI) for IPSS high-risk, intermediate-2 and intermediate-1-risk groups were 6.4 (3.6–11.3), 3.7 (2.0–6.7) and 1.9 (1.1–3.4), respectively ($P<0.0001$). A similar multivariable analysis showed that cytogenetic-risk profile ($P=0.001$; HR: 4.1, 95% CI: 1.7–9.6) and platelet count ($P=0.04$; HR: 2.3, 95% CI: 1.0–5.0), but not

IPSS ($P=0.27$), predicted leukemia-free survival; the 5-year leukemic transformation rates for unfavorable and favorable karyotype were 46 and 7%, respectively (HR: 5.5, 95% CI: 2.5–12.0; $P<0.0001$). These results, in terms of both overall and leukemia-free survival analysis, did not change when patients receiving allo-SCT were censored at the time of their transplant.

## Discussion

Recent studies have been mostly consistent in their report on the prognostic value of cytogenetic findings in PMF.[10–12] There is now a general agreement on the favorable prognostic impact of normal karyotype and sole abnormalities of 20q-, 13q- and +9. All other abnormalities were usually lumped together and prognostically considered as being unfavorable.[10,19] It is reasonable to assume that this latter group includes prognostically diverse cytogenetic abnormalities and their identification as such should enable further refinement of cytogenetic-risk categorization in PMF. It is also important to determine the prognostic impact of both cytogenetic-risk categorization and IPSS on leukemia-free survival.

In our previous report of 200 patients with PMF,[11] there were adequate numbers of informative cases that enabled us to individually associate normal karyotype ($n=117$) or sole abnormalities of 20q- ($n=21$), 13q- ($n=8$) or +9 ($n=6$) with favorable survival and complex karyotype ($n=13$) or sole +8 ($n=7$) with unfavorable survival. This is now confirmed by this study of 433 PMF patients, which features a higher number of cases in each of these cytogenetic categories: normal karyotype ($n=275$), sole 20q- ($n=30$), sole 13q- ($n=10$), sole +9 ($n=9$), sole +8 ($n=14$) and complex karyotype ($n=26$). What is new in this study was the fact that we had sufficient numbers of patients with sole abnormalities of chromosome 1 including translocation/duplication ($n=12$) and −7/7q- ($n=5$) to examine their individual impact on survival and label the former as being prognostically favorable and the latter unfavorable. Of note, this particular observation is inconsistent with a previously published study from Japan, which had suggested a similar survival between patients with −7/7q- and normal karyotype.[10]

Also new in this study was our successful strategy to distinguish two prognostically different groups of patients with other sole abnormalities that did not meet the threshold number (that is $\geqslant 5$ informative cases) for individual assessment of prognostic impact. As outlined in Materials and methods, these were operationally categorized into 'other high-risk' and 'other indeterminate-risk' subgroups of sole abnormalities based on putative prognostic relevance derived from previously published observations.[10–14] The former included sole abnormalities of i(17q), −5/5q-, 12p-, 11q23 rearrangement or inv(3) and we show that the survival of patients with any one of these abnormalities was significantly inferior to that of patients with normal karyotype and similar to that of patients with complex karyotype, sole +8 or sole −7/7q-. In contrast, the survival of patients with other sole abnormalities that did not include +8, −7/7q-, i(17q), −5/5q-, 12p-, 11q23 rearrangement or inv(3) was similar to that of patients with normal karyotype and significantly superior to that of patients with complex karyotype. The same was true for patients with two abnormalities whose survival was aligned with either complex or normal karyotype depending on the presence or absence of the above-mentioned unfavorable cytogenetic abnormalities. A previous study had similarly suggested a negative prognostic impact for abnormalities of chromosomes 5, 7 and 17, but the particular observation was confounded by the inclusion of complex karyotype and cases with two abnormalities in the analysis.[12]

Based on the above, we were able to devise a two-tired cytogenetic-risk stratification with highly significant differences in overall and leukemia-free survival (Figures 3 and 4): unfavorable (complex karyotype or sole or two abnormalities that include +8, −7/7q-, i(17q), inv(3), −5/5q-, 12p- or 11q23 rearrangement) and favorable (all other cytogenetic findings including normal karyotype). The prognostic relevance of karyotype was independent of other previously established predictors of overall (IPSS and thrombocytopenia)[8,20] and leukemia-free (thrombocytopenia)[21,22] survival in PMF. In the original IPSS report,[8] cytogenetic information was available in 409 patients and the presence of 'abnormal' karyotype was significantly associated with inferior survival.[8] Additional cytogenetic details were not available to delineate the

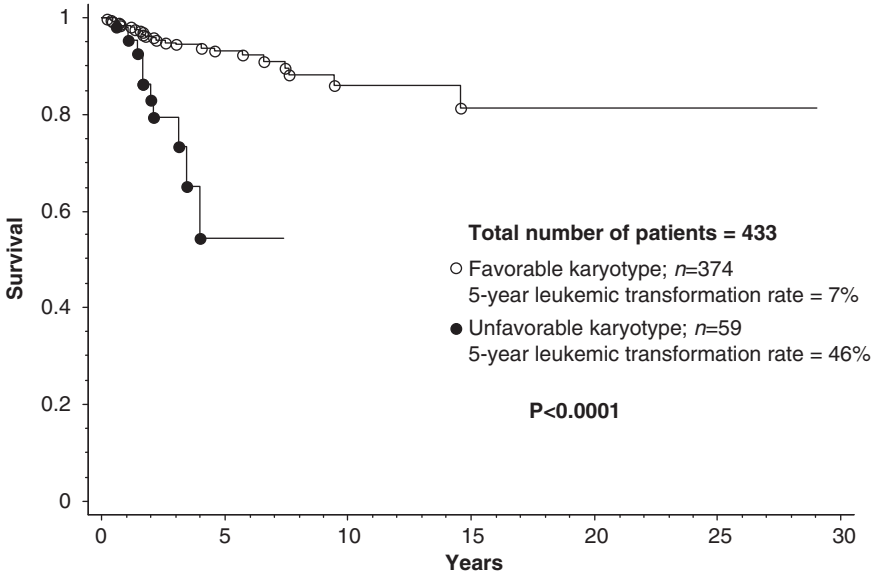


**Figure 4** Leukemia-free survival data of patients with primary myelofibrosis stratified by two-tired cytogenetic-risk categorization: unfavorable (complex karyotype or sole or two abnormalities that include +8, −7/7q-, i(17q), inv(3), −5/5q-, 12p- or 11q23 rearrangement) and favorable (all other scenarios including normal karyotype).

prognostic impact of specific cytogenetic categories and leukemia-free survival analysis was not performed. In this study, IPSS was not found to be predictive of leukemic transformation. We instead found a platelet count of $<100\times10^9$/l to be a powerful and independent adverse factor for both overall and leukemia-free survival. These observations strongly favor the inclusion
of both cytogenetic information and presence or absence of thrombocytopenia in future prognostic models for PMF. On the other hand, additional studies are needed to validate recently described molecular profiles of potential prognostic importance before their formal consideration.[17,23–27]

Finally, it was interesting to note a highly significant association between sole 20q- and both leukopenia and thrombocytopenia. Such an association was not evident in the other favorable cytogenetic categories and suggests a 20q- haploinsufficient gene effect, which might also explain a previously reported association between MDS with sole 20q- and thrombocytopenia.[28]

## Conflict of interest

The authors declare no conflict of interest.

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