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LETTER

Chronic myeloproliferative neoplasms

Cytogenetic clonal evolution in myeloproliferative neoplasms: contexts and prognostic impact among 648 patients with serial bone marrow biopsies

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To the Editor,

Karyotype is diagnostically and prognostically essential in patients with myeloproliferative neoplasms (MPN); cytogenetic abnormalities occur in approximately a third of patients with primary myelofibrosis (PMF) [1] and 5–20% of those with essential thrombocythemia (ET) [2] or polycythemia vera (PV) [3]. "Abnormal" karyotype in PV [3] and "unfavorable" or "very high risk (VHR)" karyotype in PMF [1] have been associated with inferior survival. In PV, median survivals were estimated at 9 years for patients with abnormal karyotype vs. 14 years for those with normal karyotype [3]. In PMF, median survival estimates were 4.4 years for "favorable" karyotype (i.e. normal karyotype or sole abnormalities of 13q−, + 9, 20q−, chromosome 1 translocation/duplication or sex chromosome abnormality including −Y), 1.2 years for VHR karyotype (i.e. single or multiple abnormalities of −7, i(17q), inv(3)/3q21, 12p−/12p11.2, 11q−/11q23, or other autosomal trisomies not including +8/+9) and 2.9 years for "unfavorable" karyotype (i.e. all other abnormalities) [1]. It is currently not known if acquisition of cytogenetic abnormalities during the clinical course of MPN carries similar prognostic relevance. The main objective of the current retrospective study was to examine the incidence and pattern of changes in karyotype of patients with MPN and their impact on survival.

After approval from the Mayo Clinic institutional review board, study patients were recruited from institutional databases, based on documentation of at least two serial bone marrow (BM) biopsies. Diagnoses were according to the 2016 World Health Organization criteria [4]. Cytogenetic analysis and reporting was done according to the International System for Human Cytogenetic Nomenclature (ISCN) criteria [5]. Cytogenetic analysis in all instances was performed on fresh BM aspirates, placed in hypotonic trypsin–colcemid solution and processed according to standard techniques for chromosome analysis using Giemsa/ Trypsin/Leishman (GTL) banding with trypsin and Leishman stain [6]. Thrombolytic agents were added to clotted BM specimens in order to improve success rates [7]. Chromosomal abnormalities were considered clonal if the same structural abnormality or extra chromosome appears in at least 2 and monosomy in at least 3 metaphases; [5] a normal karyotype required at least 10 metaphases evaluated and absence of any clonal abnormalities, including loss of Y. In addition to documenting the presence or absence of changes in karyotype, a notation was made regarding disease phase, at the time of the repeat biopsy, in order to allow accurate interpretation of the data and evaluation of survival impact. Statistical analyses considered clinical and laboratory data collected at the time of collection of BM for cytogenetic studies. Survival was calculated from the time point of documented change in karyotype. JMP® Pro 13.0.0 software from SAS Institute, Cary, NC, USA, was used for all statistical calculations.

At least two serial BM biopsies were documented in 648 patients with MPN (median age 60 years; 61%

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males), including 153 with ET (median age 57 years; 48% males), 103 with PV (median age 56 years; 59% males) and 392 with PMF (median age 64 years; 66% males). Supplementary Table 1 lists the baseline clinical and laboratory characteristics of the study patients stratified by MPN subtype; as expected, patients with PMF were older ($p < 0.001$) and were more likely to display adverse disease features including anemia ($p < 0.001$), thrombocytopenia ($p < 0.001$), marked leukocytosis ($p < 0.001$) and circulating blasts ($p < 0.001$) while patients with ET were more likely to be females ($p < 0.001$). Risk distribution according to conventional risk models included, for PMF, 6% high risk, 41% intermediate-2 risk, 36% intermediate-1 risk and 17% low risk [8]; for ET, 21% high risk, 47% intermediate risk and 32% low risk [9]; and for PV, 27% high risk, 32% intermediate risk and 41% low risk (Supplementary Table 1) [10]. Driver mutation distribution was also as expected and is outlined in Supplementary Table 1. Median follow-up for all study patients was 6.4 years (range 0–36.7) and 4.8 years (range 0–30.8) for PMF, 10.6 years (range 0–35) for ET and 10.5 years (range 0–36.7) for PV; during this time, a total of 373 (57%) deaths, 85 (13%) leukemic transformations and 74 (11%) fibrotic progressions were documented; the corresponding figures for PMF, ET and PV are outlined in Supplementary Table 1 and included 70% deaths in PMF, 44% in ET and 34% in PV.

Baseline karyotype, which represented first episode cytogenetic study, was performed at the time of initial diagnosis (or within 1 year of diagnosis) in 572 (88%) cases, including 96% in ET, 92% in PV and 84% in PMF; the corresponding figures for cytoreductive treatment-naïve patients at time of baseline karyotype was 92% for ET, 92% for PV and 92% for PMF. Baseline karyotype was abnormal in 188 (29%) patients, including 14 (9%) with ET, 14 (14%) with PV and 156 (40%) with PMF (Table 1); in PMF, the abnormal karyotype included 5% VHR, 17% unfavorable and 18% favorable. All 648 study patients had undergone at least two serially documented BM biopsies; 227 patients had three, 108 four, 48 five, 19 six, and 4 seven repeat BM biopsies during their clinical course (Table 1). Considering sample size adequacy as well as the fact that most of the acquired events occurred during the first repeat BM biopsy (Table 1), the particular time point was chosen for subsequent analysis of impact on survival. Median time between initial (i.e. baseline) and the first repeat BM biopsy was 35 months; this interval was similar ($p = 0.32$) between patients with (39 months) and without (33 months) clonal evolution. Unfortunately, accurate treatment data during this period were not captured. However, the median time to clonal evolution was significantly shorter for PMF (25 months), compared to PV (91 months) and ET (143 months) ($p < 0.001$). At the time of the first repeat BM biopsy, cytogenetic clonal evolution was documented in a total of 152 patients: 14 (9%) patients with ET, 27 (26%) with PV and 111 (28%) with PMF; indications for the repeat biopsy for these patients included suspicion of disease progression in 52 (34%) patients, clinical trial participation in 46 (30%), disease monitoring without evidence of progression in 39 (26%), confirm diagnosis in 10 (7%) and pre-transplant evaluation in 5 (3%). Cytogenetic clonal evolution was associated with clinically overt progression to blast or fibrotic phase disease in 64% of the cases with ET, 34% of those with PV and 11% of patients with PMF. Supplementary Table 2 lists the specific cytogenetic abnormalities acquired during the first repeat BM biopsy, confirming their more aggressive nature, compared to baseline. A change in karyotype from "normal" to "abnormal", in the absence of overt disease transformation into blast or fibrotic phase disease, showed a trend for adverse survival in both ET (HR 2.8; 95% CI 0.7–7.9) and PV (HR 1.9, 95% CI 0.7–4.8) (Fig. 1). In PMF, emergence of VHR abnormalities significantly affected survival (HR 3.4, 95% CI 1.7–5.9) with a similar trend for emergence of unfavorable (HR 1.4, 95% CI 0.9–2.1), but not favorable ($p = 0.53$), abnormalities (Fig. 1).

Supplementary Table 3 considers only those patients with normal karyotype at baseline ($n = 464$), including 139 with ET, 89 with PV and 236 with PMF. Overall, subsequent BM biopsies during the clinical course of the disease revealed new cytogenetic abnormalities in 19 (14%) patients with ET, 22 (25%) with PV and 75 (32%) with PMF (Supplementary Table 3). Changes in karyotype were mostly seen during the first repeat BM biopsy and were often associated with disease transformation into blast or fibrotic phase disease (Supplementary Table 3). Similar patterns of changes were demonstrated in 184 patients who started with an abnormal karyotype at baseline (Supplementary Table 4).

The current study documents that cytogenetic clonal evolution, in the absence of an accompanying leukemic or fibrotic progression, is relatively infrequent in ET and more frequent in PV and PMF; the study also suggests prognostic relevance for such events. However, before endorsing periodic assessment of karyotype in patients with MPN, our observations need to be validated by prospective studies that account for indication bias; the latter point is most important considering the fact that a substantial number of patients in the current study who displayed clonal evolution during a repeat BM biopsy were suspected of progressive disease (see above). In other words, accurate interpretation on the prognostic relevance of acquired cytogenetic abnormalities in MPN requires accounting for disease progression, which is difficult to ascertain short of a prospective study design; also, such studies should be accompanied by Next generation sequencing (NGS), in

Table 1 Incidence and pattern of cytogenetic clonal evolution among 648 patients with myeloproliferative neoplasms stratified by disease subtype

	All patients ($n = 648$)	Essential thrombocythemia ($n = 153$)	Essential thrombocythemia clonal evolution; *n* (%) *Disease phase at time of clonal evolution*	Polycythemia vera ($n = 103$)	Polycythemia vera clonal evolution; *n* (%) *Disease phase at time of clonal evolution*	Primary myelofibrosis ($n = 392$)	Primary myelofibrosis clonal evolution; *n* (%) *Disease phase at time of clonal evolution*
Median age in years (range)	60 (18–85)	57 (18–85)		56 (18–84)		64 (20–83)	
Males; *n* (%)	393 (61%)	74 (48%)		61 (59%)		258 (66%)	
Disease risk at baseline		IPSET[a] Low; *n* (%) = 49 (32) Intermediate; *n* (%) = 72 (47) High; *n* (%) = 32 (21)		IWG-MRT[a] Low; *n* (%) = 42 (41) Intermediate; *n* (%) = 33 (32) High; *n* (%) = 28 (27)		DIPSS[a] Low; *n* (%) = 67 (17) Intermediate-1; *n* (%) = 140 (36) Intermediate-2; *n* (%) = 163 (41) High; *n* (%) = 22 (6)	
Karyotype at baseline; *n* (%)	$N = 648$ Normal; 460 (71) Abnormal; 188 (29)	$N = 153$ Normal; 139 (91) Abnormal; 14 (9)		$N = 103$ Normal; 89 (86) Abnormal; 14 (14)		$N = 392$ Normal; 236 (60) Abnormal; 156 (40) Favorable; 72 (18)[a] Unfavorable; 63 (17) Very high risk; 21 (5)	
Karyotype at time of second biopsy; *n* (%)	$N = 648$ Normal; 390 (60) Abnormal; 258 (40)	$N = 153$ Normal; 125 (82) Abnormal; 28 (18)	Clonal evolution; 14 (9) *Chronic phase; 5 (36)* *Fibrotic phase; 6 (43)* *Blast phase; 3 (21)*	$N = 103$ Normal; 70 (68) Abnormal; 33 (32)	Clonal evolution; 27 (26) *Chronic phase; 18 (67)* *Fibrotic phase; 5 (19)* *Blast phase; 4 (15)*	$N = 392$ Normal; 195 (50) Abnormal; 197 (50) Favorable; 66 (18)[a] Unfavorable; 96 (23) Very high risk; 35 (9)	Clonal evolution; 111 (28) *Chronic phase; 99 (89)* *Blast phase; 12 (11)*
Karyotype at time of third biopsy; *n* (%)	$N = 227$ Normal; 110 (48) Abnormal; 117 (52)	$N = 37$ Normal; 23 (62) Abnormal; 14 (38)	Clonal evolution; 7 (19) *Chronic phase; 5 (71)* *Fibrotic phase; 2 (29)*	$N = 27$ Normal; 15 (57) Abnormal; 12 (44)	Clonal evolution; 2 (7) *Chronic phase; 2 (100)*	$N = 163$ Normal; 72 (44) Abnormal; 91 (56) Favorable; 30 (18)[a] Unfavorable; 40 (25) Very high risk; 21 (13)	Clonal evolution; 35 (21) *Chronic phase; 33 (94)* *Blast phase; 2 (6)*

Table 1 (continued)

	All patients ($n = 648$)	Essential thrombocythemia ($n = 153$)	Essential thrombocythemia clonal evolution; *n* (%) *Disease phase at time of clonal evolution*	Polycythemia vera ($n = 103$)	Polycythemia vera clonal evolution; *n* (%) *Disease phase at time of clonal evolution*	Primary myelofibrosis ($n = 392$)	Primary myelofibrosis clonal evolution; *n* (%) *Disease phase at time of clonal evolution*
Karyotype at time of fourth biopsy; *n* (%)	$N = 108$ Normal; 43 (40) Abnormal; 65 (60)	$N = 16$ Normal; 7 (44) Abnormal; 9 (56)	Clonal evolution; 1 (6) *Chronic phase; 1 (100)*	$N = 10$ Normal; 5 (50) Abnormal; 5 (50)	Clonal evolution; 0	$N = 82$ Normal; 31 (38) Abnormal; 51 (62) Favorable; 14 (16)[a] Unfavorable; 21 (36) Very high risk; 16 (20)	Clonal evolution; 12 (15) *Chronic phase; 12 (100)*
Karyotype at time of fifth biopsy; *n* (%)	$N = 48$ Normal; 14 (29) Abnormal; 34 (71)	$N = 10$ Normal; 2 (20) Abnormal; 8 (80)	Clonal evolution; 0	$N = 8$ Normal; 5 (63) Abnormal; 3 (37)	Clonal evolution; 0	$N = 30$ Normal; 7 (23) Abnormal; 23 (77) Favorable; 7 (23)[a] Unfavorable; 13 (44) Very high risk; 3 (10)	Clonal evolution; 2 (7) *Chronic phase; 2 (100)*
Karyotype at time of sixth biopsy; *n* (%)	$N = 19$ Normal; 5 (26) Abnormal; 14 (74)	$N = 3$ Normal; 2 (67) Abnormal; 1 (33)	Clonal evolution; 0	$N = 3$ Normal; 2 (67) Abnormal; 1 (33)	Clonal evolution; 0	$N = 13$ Normal; 1 (8) Abnormal; 12 (92) Favorable; 4 (31)[a] Unfavorable; 6 (46) Very high risk; 2 (15)	Clonal evolution; 2 (15) *Chronic phase; 2 (100)*
Karyotype at time of seventh biopsy; *n* (%)	$N = 4$ Normal; 0 Abnormal; 4 (100%)	$N = 1$ Normal; 0 Abnormal; 1 (100%)	Clonal evolution; 0	$N = 0$	Clonal evolution; 0	$N = 3$ Normal; 0 Abnormal; 3 (100) Favorable; 1 (33)[a] Unfavorable; 1 (33) Very high risk; 1 (33)	Clonal evolution; 0

IPSET International Prognostic Score for Essential Thrombocythemia, *IWG-MRT* International Working Group-Myeloproliferative Neoplasm Research and Treatment, *DIPSS* Dynamic International Prognostic Scoring System (see text for references)

[a]Favorable karyotype for the purposes of Table 1 did not include normal karyotype, which is annotated separately

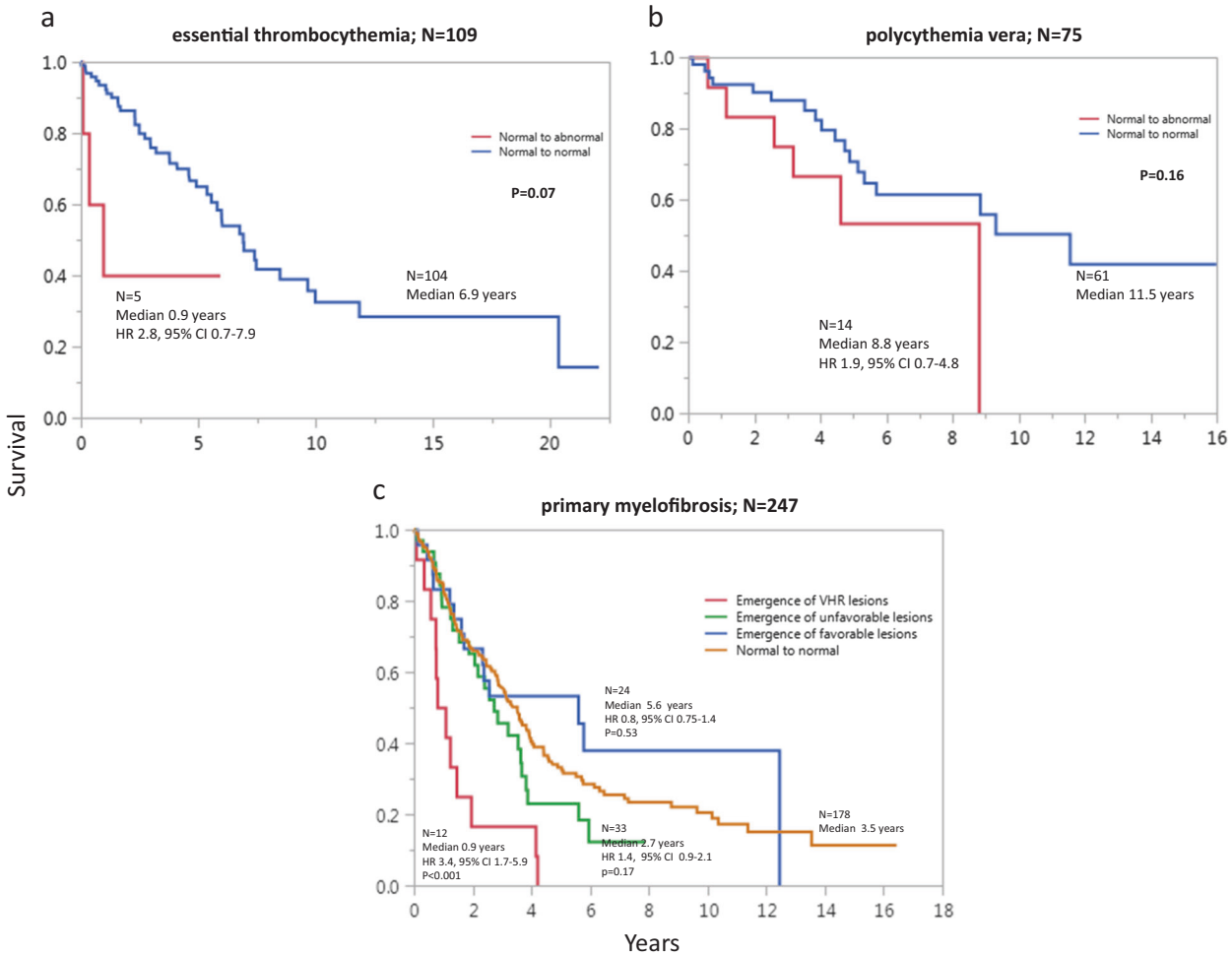

Fig. 1 Survival data among 431 patients with myeloproliferative neoplasms, including 109 with essential thrombocythemia (**a**), 75 with polycythemia vera (**b**) and 247 with primary myelofibrosis (**c**). All 431 patients underwent at least two serially documented bone marrow biopsies. Patients with changes in karyotype that was associated with overt evidence of disease transformation into blast or fibrotic phase disease were excluded from analysis

order to clarify the prognostic interaction between karyotype and mutations.

Author contributions All authors have reviewed the manuscript and gave their approval. AT designed the study, contributed patients, helped with abstract patient information, performed statistical analysis and wrote the paper; MN was primarily involved in data abstraction and preparation of tables; AP and NG contributed patients and helped with abstract patient information; RPK was in charge of cytogenetic information; CAH was in charge of information on pathology; DP and RV helped with original abstraction of clinical and laboratory information from patient histories.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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