Myeloid neoplasms with isolated del(5q) and *JAK2* V617F mutation: a "grey zone" combination of myelodysplastic and myeloproliferative features?

Myelodysplastic syndrome (MDS) with isolated del(5q) [MDS del(5q)] is a World Health Organization (WHO)-recognized MDS entity. It is characterized by an interstitial deletion on chromosome 5q occurring either in isolation or with one additional cytogenetic abnormality, other than monosomy 7 or del(7q). Its presentation includes anemia, usually macrocytic, normal to elevated platelet count and normal white blood count. Bone marrow (BM) is normocellular to mildly hypercellular, often with erythroid hypoplasia; the presence of non-lobated megakaryocytes is characteristic.[1] MDS del(5q) is considered a "low-grade" MDS; however, transformation to acute myeloid leukemia does occur and is frequently associated with gain of a *TP53* mutation.[2-4] The occurrence of other mutations has not been extensively studied.

A small proportion of MDS del(5q) shows concomitant *JAK2* V617F mutation.[5-7] Due to the limited data available, it is unclear whether *JAK2* V617F affects the phenotypic manifestations and/or prognosis. To clarify this issue, we retrospectively reviewed the files of the Division of Hematopathology at New York Presbyterian/Weill Cornell Medicine (NY, USA).

A total of 47 cases of MDS del(5q) were identified between 2001 and 2018. Material was reviewed to verify that the diagnoses met the criteria of the Revised 4th Edition of the WHO Classification. Six cases (12.7%) had the *JAK2* V617F mutation. A summary of the main clinical and pathological data at diagnosis and information on

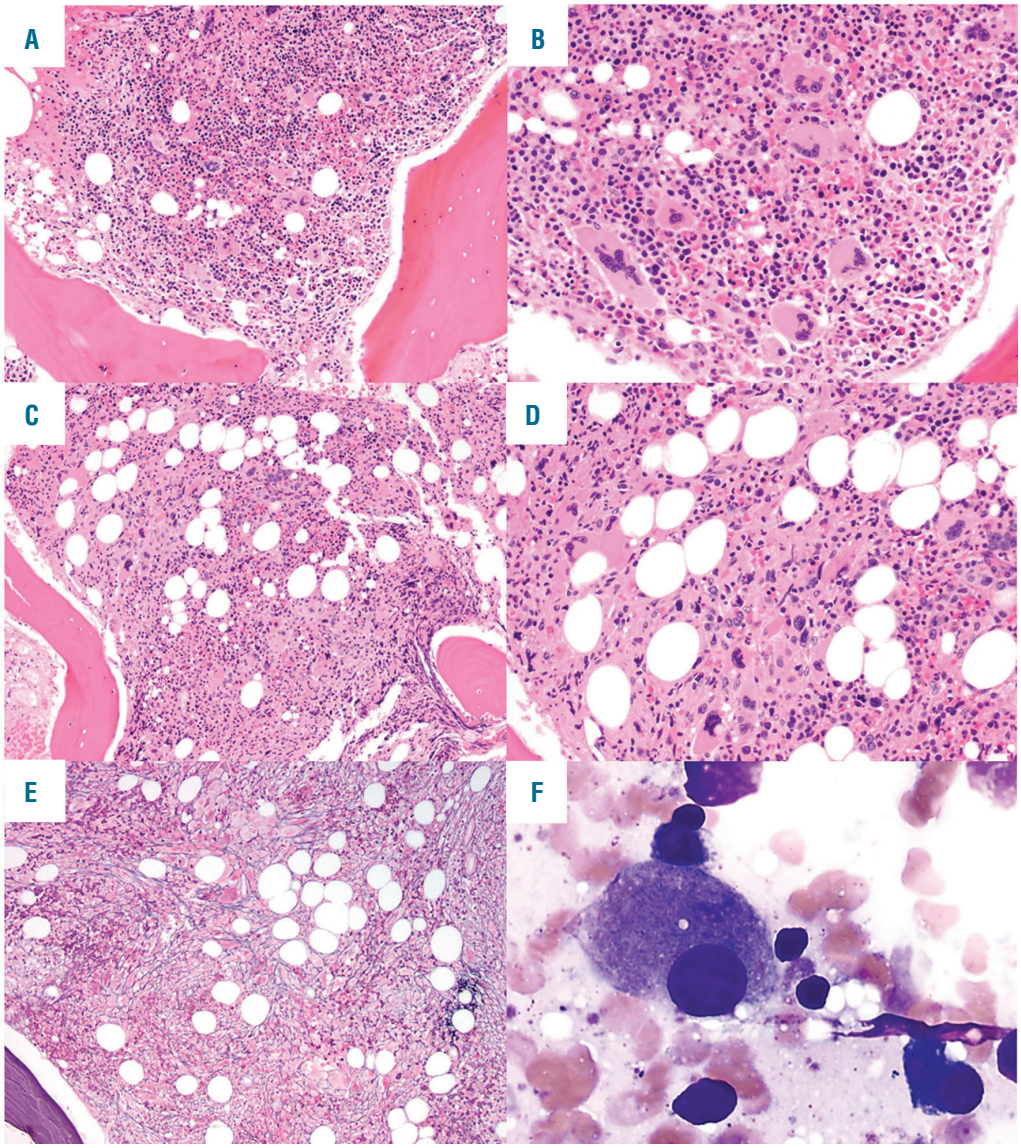

Figure 1. Microscopic findings of myeloid neoplasms with isolated del(5q) and concurrent *JAK2* mutation. (A, B) Hypercellular bone marrow with increased megakaryopoiesis, including many large hyperlobulated forms and fewer small hypolobated forms (A: 10x, B: 20x). (C, D) Hypercellular bone marrow with increased megakaryopoiesis, including pleomorphic large hyperchromatic forms and small monolobated forms with frequent megakaryocytic clustering (C: 10x, D: 20x). (E) Reticulin stain highlighting a marked increase in coarse and intersecting reticulin fibers (10x). (F) Bone marrow aspirate with a small megakaryocyte with non-lobated rounded nucleus, typical of myelodysplastic syndrome with isolated del(5q).

Table 1. Summary of main clinical and pathological data at diagnosis and follow-up of six patients with myeloid neoplasms with isolated del(5q) and concurrent *JAK2* V617F mutation.

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Clinical data						
Age at diagnosis (years)	50	73	84	78	75	81
Sex	female	female	female	female	male	female
Hemoglobin (g/dL)	12.8	11.1	8.4	12.7	11.8	14.6
MCV (fL)	89	107.8	86	89	94	77
WBC (x10^9/L)	10.7	4.1	10	7.8	13.9	13.1
Platelets (x10^9/L)	900	355	188	523	763	418
LDH (IU/L)	na	153	na	na	765	553
Spleen (cm)	normal	normal	14	na	13.8	16
Bone marrow findings						
BM cellularity	↑	n	na	↑	↑	↑
M:E ratio	normal	normal	na	↑	normal	normal
Mega number	↑	↑	na	↑	↑	↑
Mega small and hypolobated	yes	yes	na	yes	yes	yes
Mega large and hyperlobated	yes	no	na	yes	yes	yes
Dyserythropoiesis	no	no	na	no	no	no
Dysgranulopoiesis	no	no	no	na	no	no
Blasts (BMA)	1	1	1	1	3	4
Fibrosis*	MF2	na	na	MF2	MF1	MF1
Subsequent BM	Blasts 2%; MF2	Blasts 3%; MF2	Blasts 1%; MF3	Blasts 1%; MF3	Blasts 4%; MF3	na
Treatment						
First line	none	none	none	none	lenalidomide	none
Lenalidomide ever used	yes	yes	yes	yes	yes	no
N. of lines required	4	2	5	4	5	0
Other drugs	hydroxyurea, peg-ifn, JAK2-inhibitor	rituximab	epo, thal, aza, cyclo	epo, aza	busulfan, peg-ifn, JAK2-inhibitor, dec	none
Follow up						
Status; FU (months)	alive on JAK2-inhibitor; 106	dead (unknown cause); 96	lost to FU; 96	lost to FU; 133	dead of AML; 40	alive, no therapy; 2

MCV: mean corpuscular volume; WBC: white blood cells; LDH: lactate dehydrogenase (reference range: 118-230 IU/L); ↑: increased; na: not available; BM: bone marrow; M:E ratio: myeloid to erythroid ratio; Mega: megakaryocytes; BMA: bone marrow aspirate; MF: marrow fibrosis (World Health Organization grading system); peg-ifn: pegylated interferon; epo; erythropoietin; thal: thalidomide; aza: azacitidine; cyclo: cyclosporine; dec: decitabine; FU: follow up; AML: acute myeloid leukemia.

follow-up of these six cases is reported in Table 1. The cohort with the *JAK2* V617F mutation comprised five women and one man, with a median age at diagnosis of 76.5 years (range, 50-81). The mean hemoglobin level was 11.9 g/dL, with a mean corpuscular volume of 90.5 fL. Mean white blood cell and platelet counts were 9.9x10^9/L and 524.5x10^9/L, respectively. Three patients had information available on the status of the spleen at diagnosis; all of them showed splenomegaly (mean splenic diameter: 14.6 cm; range, 13.8-16 cm). The mean lactate dehydrogenase concentration was 490.3 IU/L.

BM samples at diagnosis were available for review in five of the six cases (Figure 1). Cellularity was increased in four of these five patients. In all the cases megakaryocytes were increased and pleomorphic, displaying a combination of small to medium forms with hypolobated/monolobated nuclei as well as large forms with hyperlobated nuclei. Dyserythropoiesis and dysgranulopoiesis were not observed. The myeloid to erythroid ratio in BM aspirate smear counts was within normal limits (i.e. 3:1), as observed in the *JAK2* wildtype (wt) cases. BM fibrosis ranged from MF1 to MF2, according to the WHO grading system.[1] The blast count in BM aspirates was 1% in four cases, 3% in one case and 4% in one case; circulating blasts were not seen in the peripheral blood in any of the cases. On iron staining, ring sideroblasts were not noted in any of the cases. We compared the original BM specimens with subsequent samples in four cases. All showed a later increase in the blast count and/or worsening of fibrosis.

Besides del(5q), no additional cytogenetic abnormalities were detected (*Online Supplementary Table S1*).

Targeted next-generation sequencing was performed on DNA extracted from unstained and unfixed BM aspirate smears (see *Online Supplementary Material*).

JAK2 variant allele frequency (VAF) was available for five of the six cases. Excluding one case with a VAF of 2%, the VAF ranged from 28% to 48.3% (mean 38.8%). Additional mutations other than *JAK2* were not detected in any of the cases. Among the *JAK2* wt cases (n=41), 13 had at least one additional mutation (see *Online Supplementary Material*).

Among the five of six patients who required disease-related treatment, all received lenalidomide, but were eventually allocated to other agents; two received a *JAK2* inhibitor.

A comparison of the main clinical and pathological data from *JAK2* wt *versus JAK2* mutated cases is reported

Table 2. Summary of clinical and pathological data at diagnosis and follow-up data from the *JAK2* wildtype *versus JAK2* mutated cases.

	JAK2 wt	*JAK2* mut	*P*
Clinical data			
Age, median (range), years	74.3 (44-87)	76.5 (50-81)	0.6
Sex (male/female)	10/31	1/5	0.7
Hemoglobin, mean (g/dL)	9.5	11.9	0.006
MCV, mean (fL)	100.9	90.5	0.01
WBC, mean (x10^9/L)	5.3	9.9	0.001
Platelet count, mean (x10^9/L)	258	524	0.005
Bone marrow findings			
BM cellularity (normal/increased)	9/20	1/4	0.6
Blasts count BMA (%)	2.5%	1.5%	0.08
BM fibrosis, mean	0.7	1.9	0.002
Dysmyelopoiesis, yes (%)	13.3%	0%	0.3
Dyserythropoiesis, yes (%)	3.3%	0%	0.7
Dysmegakaryopoiesis, yes (%)	100%	100%	1
Mega hypolobated, yes (%)	100%	100%	1
Mega hyperlobulated	3.3%	80%	<0.0001
Follow up			
Median time to FU (months)	50 (2-144)	96 (2-133)	0.09
Progression to AML, n (%)	7 (25.9%)	2 (20%)	0.8
Number of deaths	4 (12.1%)	2 (33%)	0.2

wt: wildtype; mut: mutated; MCV: mean corpuscular volume; WBC: white blood cell count; BM: bone marrow; BMA: bone marrow aspirate; ↑ : increased; Mega: megakaryocytes; FU: follow up; AML: acute myeloid leukemia.

in Table 2. No differences were observed in relation to age at diagnosis or sex. *JAK2* mutated cases had significantly higher hemoglobin levels than those of wildtype cases (11.9 *vs.* 9.5 g/dL; *P*=0.006) with normal mean corpuscular volume (90.5 *vs.* 100.9 fL; *P*=0.01), higher white blood cell counts (9.9 *vs.* 5.3 x10^9/L; *P*=0.001) and higher platelet counts (524 *vs.* 258 x10^9/L; *P*=0.005). On BM examination, *JAK2* mutated cases showed a pleomorphic population of megakaryocytes with a combination of small, hypolobated/monolobated forms and large megakaryocytes with hyperlobated nuclei; the latter were absent in the *JAK2* wt cases. *JAK2* mutated cases displayed greater reticulin fibrosis compared to *JAK2* wt cases (1.9 *vs.* 0.7; *P*=0.002). A tendency toward a higher blast count on BM aspirates was seen in the *JAK2* wt cases (2.5% *vs.* 1.5%; *P*=0.08). No significant differences were noted in other microscopic parameters.

On follow-up, there were not statistically significant differences in progression to acute myeloid leukemia or overall survival between the *JAK2* wt and *JAK2* mutated subgroups (using Kaplan-Meier analysis and the log-rank test).

Few studies have investigated the significance of *JAK2* mutations in MDS del(5q).[5-7] Analyzing a cohort of 78 patients with MDS del(5q), Patnaik *et al.* found *JAK2* V617F in 6.4% of cases. No differences in blood counts or clinical outcome were observed in *JAK2* wt *versus* mutated cases.[6] Ingram *et al.* detected *JAK2* V617F in 6.2% of the cases they studied. However, their study included cases of MDS other than MDS del(5q) and none of the *JAK2* mutated cases fell in the MDS del(5q) category.[5] In a cohort of 123 MDS del(5q), Meggendorfer *et al.* found that the incidence of *JAK2* mutations was 6% and reported a correlation between mutated *JAK2* and thrombocytosis.[7] However, none of these studies provided information on BM morphology and clinical management.

Our study brings new insights into the significance of *JAK2* mutations in the context of myeloid neoplasm with del(5q). We found an incidence of *JAK2* V617F mutation of 12.7%, which is higher than previously reported. In our cohort, *JAK2* mutated cases presented with more heterogeneous cell blood counts than expected for conventional MDS del(5q). Indeed, three patients (cases 1, 4 and 6) did not have any cytopenia and therefore strict WHO criteria for MDS del(5q) were not met. Similarly, mean corpuscular volume was mostly within limits, while white blood cell and platelet counts were higher than those normally observed in *JAK2* wt MDS del(5q). On BM examination, *JAK2* mutated cases showed a combination of megakaryocytes with "del(5q)-like" features and large forms with hyperlobated nuclei, as typically seen in some myeloproliferative neoplasms, such as essential thrombocythemia. Reticulin fibrosis was more prominent in *JAK2* mutated cases; however, no megakaryocytes with bulbous, hyperchromatic and bizarre nuclei or tight clusters of megakaryocytes were seen. This is important in the context of the differential diagnosis of the so-called "*JAK2* positive myeloid neoplasms" as del(5q) is not specific to MDS, but can be detected in other myeloid neoplasms, particularly in primary myelofibrosis.[8] del(5q) primary myelofibrosis affects younger patients with a similar male and female distribution, whereas MDS del(5q) presents mostly in elderly women. An important diagnostic feature is the presence of large, tight clusters of megakaryocytes with bulbous and/or atypical nuclear morphology in primary myelofibrosis, which are absent in MDS del(5q) even in the cases with a *JAK2* mutation. Another entity to consider is essential thrombocythemia, which carries *JAK2* V617F mutations in around 50% of cases. An abnormal karyotype is found in 5-10% of cases of essential throm-

bocythemia, but del(5q) is only rarely observed.[1,9] Essential thrombocythemia presents most commonly with isolated thrombocytosis; BM is generally normocellular with giant megakaryocytes with hypersegmented nuclei; "del(5q)- like" megakaryocytes are not observed.

Our study highlights how the diagnosis of *JAK2* mutated myeloid neoplasm with isolated del(5q) is controversial, as these cases show clinical and pathological overlapping myeloproliferative and myelodysplastic features. In our cohort, three patients did not have any cytopenia, a sine qua non criterion for the diagnosis of MDS. BM showed both MDS del(5q)-like megakaryocytes as well as large megakaryocytes with hyperlobated nuclei. Perhaps the *JAK2* mutation imparted a partial "proliferative" phenotype, both in terms of blood counts (i.e. lack of cytopenia) and BM features. At the same time, none of the cases showed the classical features of primary myelofibrosis, with particular reference to the absence of large megakaryocytes with abnormal chromatin clumping, bulbous or "cloud-like" nuclei, or "bare" megakaryocytic nuclei. As such, a diagnosis of myeloproliferative neoplasm, unclassifiable or myelodysplastic/myeloproliferative neoplasm, unclassifiable may be more appropriate for the subgroup of myeloid neoplasms with concurrent *JAK2* mutation and isolated del(5q), particularly for those cases which do not meet the WHO criteria for other myeloid neoplasms.

Excluding one case, the mean *JAK2* VAF was 38.8%. In our cohort *JAK2* was detected upfront in all the cases and, given the high number of metaphases showing del(5q), we can speculate that both events occurred in the same clone.

The correct identification of MDS del(5q) is important for clinical management, since this disease shows a favorable response to lenalidomide. However, the usefulness of lenalidomide in MDS del(5q) with concomitant *JAK2* mutation has never been extensively studied. Musto *et al*. reported the case of an 84-year old man with MDS del(5q) and *JAK2* mutation who showed a good response to lenalidomide in terms of improved anemia and thrombocytosis.[10] In our cohort, all the patients who required treatment received lenalidomide but were eventually switched to other drugs. The administration of *JAK2* inhibitors may be clinically useful in this subset of patients.

Finally, we did not see any difference in leukemic transformation or overall survival between the *JAK2* wt and *JAK2* mutated cases.

Our observations are based on a small cohort from a single institution, thus limiting their generalizability and prompting the need for further studies. However, myeloid malignancies with "MDS-like" features and del(5q) require careful investigation when they co-occur with "myeloproliferative neoplasm-like" features and a *JAK2* V617F mutation, as the latter may confer more clinical and pathological "proliferative" features.

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