

Isolated deletion of the long arm of chromosome 20 [del(20q12)] in myelodysplastic syndrome: a case report and literature review

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ABSTRACT Isolated deletion of the long arm of chromosome 20 [del(20q12)] is a rare abnormality in patients with *de novo* myelodysplastic syndrome. It is characterised by refractory thrombocytopenia, minimal haematological dysplasia and a lower risk for progression to acute myeloid leukaemia. Its distinction from chronic autoimmune thrombocytopenia, although clinically and morphologically difficult, is critical. We report a case of refractory cytopenia and unilineage dysplasia in an elderly woman with isolated del(20q12), identified via fluorescence *in situ* hybridisation analysis of her bone marrow. In order to avoid a misdiagnosis, we suggest that cytogenetic analysis be performed on all patients suspected to have myelodysplastic syndrome with predominant thrombocytopenic presentation.

Keywords: del20q12, FISH, myelodysplastic syndrome, thrombocytopenia

INTRODUCTION

Myelodysplastic syndromes (MDS) are a group of clonal haematopoietic stem cell disorders characterised by peripheral blood cytopenia, profound morphologic heterogeneity involving one or more major myeloid cell lines, ineffective haematopoiesis due to increased apoptosis, and increased risk of progression to acute myeloid leukaemia (AML).⁽¹⁾ Clonal chromosomal abnormalities, identified using metaphase cytogenetics, are seen in 30%–50% of patients with *de novo* MDS, and in up to 80% of cases with therapy-related MDS. These abnormalities, demonstrated using conventional cytogenetics (CC) and/or fluorescence *in situ* hybridisation (FISH), are critical for diagnosis and prognosis.^(1,2) Deletion of the long arm of chromosome 20 [del(20q)] may be an early and primary genetic event in several haematological disorders such as MDS (most common); myeloproliferative neoplasms (MPN) such as polycythaemia rubra vera and chronic neutrophilic leukaemia; AML; angioimmunoblastic lymphadenopathy associated T-cell lymphoma with dysproteinaemia; and pure red cell aplasia.⁽³⁾ The occurrence of del(20q) as a sole event [i.e. isolated del(20q)] is a favourable prognostic marker in patients with MDS. It is characterised by an indolent clinical course and a significantly lower risk of progression to AML. Patients with isolated del(5q) have a longer survival period than patients with del(20q).^(1,4-12) FISH and CC analyses may complement each other in delineating this subtle abnormality and confirming the true nature of the disease process.⁽¹³⁻¹⁵⁾ In this report, we describe a case of refractory cytopenia with unilineage dysplasia (RCUD) that harboured an isolated del(20q12), and present a review of the literature.

CASE REPORT

A 65-year-old postmenopausal woman presented with a six-month history of fatigue, dyspnoea on exertion and palpitation, which were followed by the onset of a two-month-long generalised purpuric rash and occasional passage of blood in urine. The patient was a normotensive diabetic on oral hypoglycaemic drugs for the past eight years. Her fasting plasma glucose at presentation was 112 mg/dL and her haemoglobin A1c was 5.7%. She had no history of prior blood transfusion, paroxysmal nocturnal dyspnoea, coronary artery disease, cerebrovascular accident, bronchial asthma, collagen vascular diseases or exposure to any known environmental toxins/allergens. Ten years prior to this presentation, the patient had undergone total abdominal hysterectomy with bilateral salpingo-oophorectomy for uterine fibroid. Physical examination revealed that the patient had marked conjunctival pallor, mild scleral icterus, a blood pressure reading of 124/88 mmHg, generalised purpuric rash and mild splenomegaly (2 cm below the left costal margin). The patient was found to be afebrile. Systemic evaluation and radiological examinations of the patient's chest and abdomen were not remarkable.

Serial evaluations of the patient's laboratory parameters on admission and during follow-up sessions are presented in Table I. Haemogram findings revealed bicytopenia with an increased reticulocyte count, but no dyspoiesis, blasts or fragmented cells. Bone marrow aspirate smears and trephine biopsy sections on Day 2 of admission, and at one and three months post admission showed a similar picture: increased cellularity; erythroid hyperplasia (myeloid to erythroid ratio of 1:1.5); focal megaloblastic nuclei; cytoplasmic periodic

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Table I. Laboratory parameters of the patient on Day 2, 5 and 7 post admission, and at follow-up sessions 1, 2 and 3 months post admission.

Laboratory parameter	Day 2	Day 5	Day 7	1 mth	2 mths	3 mths
Haemoglobin (g/L; RR 110–140)	70	85*	90*	90*	92*	95
Haematocrit (%)	23	25	27	27	29	32
Corrected reticulocyte count (%)	2	1.8	1.8	1.6	1.7	1.2
TLC ($\times 10^9/L$; RR 4–11)	7	88	9	9.2	10	8
TPC ($\times 10^9/L$; RR 150–450)	15	22*	25*	30*	60*	50*
Absolute neutrophil count (/L)	4,500	4,500	5,000	5,200	4,700	5,700
Red cell indices#	WNL	WNL	WNL	WNL	WNL	WNL
Peripheral smear	A+T, no dyspoiesis, blast or parasites	Same	Same	Same	Same	Same
Urine (routine, microscopic, culture)	No cast, dysmorphic RBCs or growth	–	–	–	–	–
Bone marrow study	S/o RCUD, 2% RS, 4% blast, grade 2 reticulatin	–	–	S/o RCUD, 5% blast, 3% RS	–	S/o RCUD, 6% blast, 3% RS
Vitamin B ₁₂ (pg/mL)/folate(ng/mL)/LDH (IU/L)/ferritin (ng/mL)	1,259/> 20/900/433	–	–	–	–	–
Total bilirubin/direct bilirubin/urea/creatinine (mg/dL)	5/1.5/25/0.5	–	–	–	–	–
Urine haemosiderin	–ve	–ve	–ve	–	–	–
Flow cytometry for CD55, CD59	–	–	–	–ve	–	–
G-banding (peripheral blood)	–	–	–	–	–	46, XX
Fluorescence <i>in situ</i> hybridisation (bone marrow aspirate)	–	–	–	–	–	46, XX Isolated del (20q12)

*With packed cell transfusion. †With transfusion and steroids. #Includes mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and red cell distribution width.

A+T: anaemia and thrombocytopenia; LDH: lactate dehydrogenase; RCUD: refractory cytopenias (anaemia and thrombocytopenia) with unilineage dysplasia; RR: reference range; RS: ring sideroblast; S/o: suggestive of; TLC: total leukocyte count; TPC: total platelet count; WNL: within normal range

acid-Schiff positivity; nuclear budding (< 10% of cells); occasional ring sideroblasts (2% of cells); normal myeloid morphology; increased megakaryocytes with few hypolobated forms and focal clustering (15% of cells); and an insignificant number of blasts (4%) (Fig.1). Flow cytometric analysis was negative for CD55 and CD59, and urinary haemosiderin test was also negative. Bone marrow morphology, in correlation with the patient's routine haematological and biochemical parameters (raised unconjugated bilirubin, vitamin B₁₂, folate, lactate dehydrogenase and ferritin), was suggestive of RCUD. However, chronic immune thrombocytopenia (ITP) could not be ruled out with certainty.

Peripheral blood G-banding cytogenetics performed at three months post admission showed normal female karyotype (Fig. 2). FISH analysis was performed on the heparinised bone marrow aspirate sample using the following probes: (a) D20S108 (del 20q12) (Vysis, Abbott Laboratories, Abbott Park, IL, USA); (b) DS523 and D5S723 (5q33-34) (Vysis, Abbott Laboratories); (c) D7S486 (7q31) (Vysis, Abbott Laboratories); and (d) AML/ETO (Religare SRL Diagnostics, Mumbai, India). Analysis of 200 metaphase nuclei demonstrated del(20q12) (12% positive; cutoff > 5%) as the sole aberration. As cytogenetic analysis in correlation with bone marrow morphology was suggestive of RCUD, the patient was managed with transfusion support and antibiotics.

DISCUSSION

A dominant thrombocytopenic presentation in MDS is rare (3%–7%) and difficult to differentiate from ITP using solely clinical and morphological features; hence, cytogenetic analyses may be necessary.^(1,9) When del(20q12) occurs as a primary or isolated event, it is associated with minimal dysplasia involving erythroid and/or megakaryocytic lineages (morphologically, as RCUD or refractory anaemia with ring sideroblast [RARS]), and a favourable outcome. However, when del(20q12) occurs with one or more cytogenetic abnormalities, it suggests genetic instability, which is featured by the presence of refractory cytopenia multilineage dysplasia, increased blasts with or without transformation, and an increased risk for leukaemic transformation (Table II).^(5-9,11)

A recent French study⁽⁶⁾ retrospectively analysed 98 patients with del(20q). In 62 patients, del(20q) was found to be the sole abnormality, whereas it was associated with other chromosomal abnormalities in the remaining patients. The researchers found that compared to patients with multiple chromosomal abnormalities and patients without del(20q), those with isolated del(20q) had significantly lower platelet counts, fewer (< 5%) blasts in the marrow and higher immature red blood cell counts (higher reticulocyte count). At a median follow-up time of 3.8 years, approximately 14% of patients with isolated del(20q) progressed to AML, compared to 11%

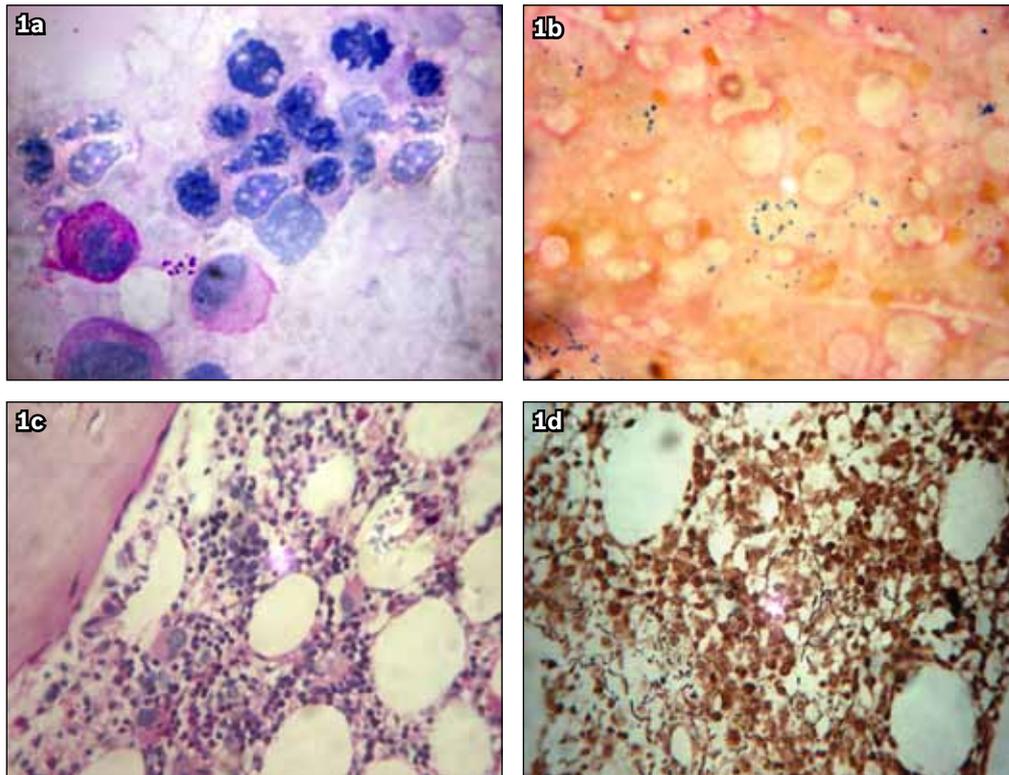


Fig. 1 Photomicrograph of (a) bone marrow aspirate smear shows diffuse cytoplasmic positivity in erythroid precursors, suggestive of dyspoiesis (PAS, $\times 400$); (b) bone marrow aspirate smear shows the presence of ring sideroblasts (Prussian blue, $\times 400$); (c) trephine section of the bone marrow shows scattered megakaryocytes with hypolobated nuclei in a paratrabeular location, and focal clustering evident in other foci, suggestive of dyspoiesis (Haematoxylin & eosin, $\times 200$); (d) trephine section of the bone marrow shows grade 2 reticulin condensation (Reticulin, $\times 200$).

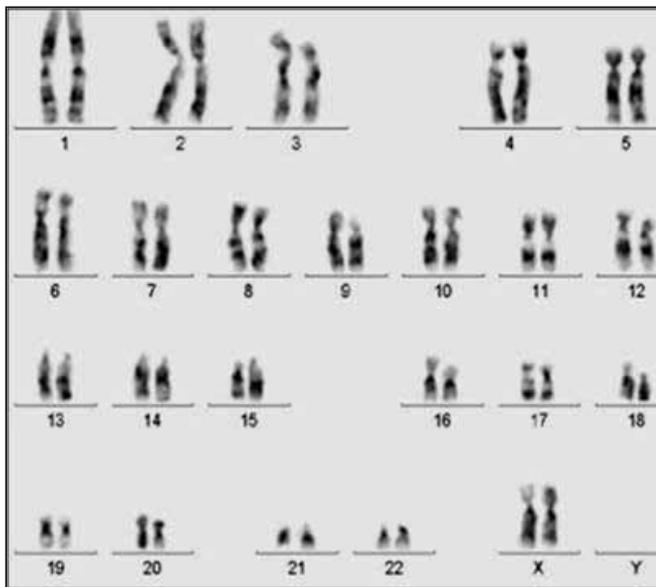


Fig. 2 Peripheral blood G-banding cytogenetics conducted three months post admission shows a normal female karyotype (46, XX), with no obvious structural abnormality.

of patients with one additional abnormality and 24% of patients with several additional abnormalities.⁽⁶⁾

Isochromosome of the long arm of chromosome 20 with interstitial loss of material [ider(20q)] is a rare [a tenth that of del(20q)] and recently described cytogenetic abnormality in MDS. Due to the small size and ambiguous G-band pattern, ider(20q) is often missed in routine cytogenetic studies,

although FISH may detect this abnormality.^(5,8) A retrospective multicentric study,⁽⁵⁾ which analysed 13 patients with ider(20q) using FISH and compared their morphology and prognosis with 21 patients with del(20q), found that prominent granulocytic dysplasia with hypogranular vacuolised neutrophils and neutrophil erythrophagocytosis was a sensitive and specific morphological marker of ider(20q) and was significantly associated with survival.

Gupta et al,⁽⁹⁾ who analysed nine MDS patients with isolated del(20q) and compared them with 17 adult patients with ITP, reported that seven of the nine patients had thrombocytopenia and six had mild anaemia (Hb > 11 g/dL). All nine patients had minimal haematopoietic dysplasia and fewer blasts (< 5%) in the marrow. According to the authors, the identification of del(20q) was crucial to avoid misdiagnosing this entity as ITP.⁽⁹⁾ Another study from China⁽⁷⁾ described 29 MDS patients with del(20q) – 11 patients had isolated del(20q), while 18 had del(20q) that was associated with other abnormalities. Of the 11 patients with isolated del(20q), 9 were categorised as refractory anaemia (RA)/RARS with breakpoint in 20q11, and two were categorised as refractory anaemia with excess blasts/refractory anaemia with excess blasts in transformation (RAEB/RAEB-t) with breakpoint in 20q12. Additional chromosomal abnormalities and complex karyotype were more frequent in the RAEB/RAEB-t group than the RA/RARS group. Aberrant expression of lymphoid antigens (18%) and trilineage dyspoiesis were important findings in this study.⁽⁷⁾

Table II. Review of the literature on the presence of del(20q), and its associated morphological features and effect on the prognosis of patients with *de novo* myelodysplastic syndrome (MDS).^(5-9, 11)

Author, year	No. of MDS patients	No. (%)		Bone marrow morphology	Peripheral blood findings	Remarks/prognosis
		Isolated del(20q)	del(20q)*			
Mullier et al, ⁽⁵⁾ 2012	34	del(20q): 21 (61.8) ider(20q) [†] : 13 (38.2)	–	ider(20q): granulocytic dysplasia, neutrophil hypogranularity, vacuolation, erythrophagocytosis	Not described	Neutrophil abnormalities: morphologic marker for ider(20q), detection requires FISH analysis, no difference in survival between del(20q) and ider(20q) groups
Braun et al, ⁽⁶⁾ 2011	1,433	62 (4.3)	36 (2.5)	Minimal blasts (< 5%)	Isolated del(20q): low platelet; Hb > 11 g/dL; high reticulocyte count	Isolated del(20q) favourable; del(20q) with other abnormalities unfavourable; progressed to AML (n = 8/62 [14%])
Qin et al, ⁽⁷⁾ 2004	29	11 (37.9)	18 (62.1)	Prominent dysplasia (trilineage)	Pancytopenia	RA/RARS (-20q11) (n = 9/11); RAEB/RAEB-t (-20q12) and expression of lymphoid antigens (n = 2/11, 18%); progressed to AML (n = 2)
Smoley et al, ⁽⁸⁾ 2007	8	ider (20q)+: 8 (100)	–	Multilineage dysplasia (prominent granulocytic)	Not described	Ider (20q) rare [1/10th the incidence of del (20q12)]; strong association with MDS and AML
Gupta et al, ⁽⁹⁾ 2007	9	9 (100)	–	Minimal dysplasia (erythroid and megakaryocytic), few blasts	Low platelet (n = 7); mild anaemia, increased reticulocyte count, and no dysplasia (n = 6)	Favourable prognosis; clinical and morphologic resemblance with ITP
Wattel et al, ⁽¹¹⁾ 1993	392	5 (1.2)	3 (0.7)	RA (n = 4); RARS (n = 2); RAEB (n = 2)	Hb > 110 g/L (n = 5); neutropenic (< 500/L) (n = 1); low platelet (< 50 × 10 ⁹ /L) (n = 0)	Progressed to AML (n = 1); increased blasts (n = 2); stable disease (n = 5); overall favourable prognosis
Present case	1	del(20q12)	–	Mild dysplasia (erythroid and megakaryocytic); < 5% blast; 2% ring sideroblast	Anaemia (Hb < 10 g/dL); platelet < 50,000/cmm; reticulocyte (2%); no dyspoiesis	Dominant thrombocytopenic presentation mimicking ITP on follow up

*del(20q) in association with other chromosomal abnormalities. [†]Isochromosome of the long arm of chromosome 20 with interstitial loss of material.

FISH: fluorescence *in situ* hybridisation; AML: acute myeloid leukaemia; Hb: haemoglobin; ITP: immune thrombocytopenic purpura; RA: refractory anaemia; RAEB: refractory anaemia with excess blasts; RAEB-t: refractory anaemia with excess blasts in transformation; RARS: refractory anaemia with ring sideroblast

The clinical significance of del(20q) was studied in 26 patients with myeloid malignancies (4 MPN, 15 MDS, 7 acute leukaemia).⁽¹²⁾ The median survival was highest in patients with MPN (all alive at 18–184 months post diagnosis) and lowest in patients from the leukaemia group (five months following chemotherapy). The median survival of the 15 MDS patients was only 12 months. Out of the 15 patients, 7 developed acute leukaemia, including 3 of the 4 patients with RARS. These results indicated that del(20q) in MDS was associated with a poor prognosis and a higher rate of transformation to acute leukaemia. In *de novo* acute leukaemia, del(20q) was associated with a poor response to treatment and reduced survival.⁽¹²⁾

Brezinová et al⁽⁴⁾ analysed the del(20q) in 36 patients with haematological malignancy (MPN/MDS/AML) by means of routine G-banding cytogenetics and FISH probes directed against 20q12. The del(20q) was found to be the sole cytogenetic aberration in 23 patients and associated with other chromosomal aberrations in the remaining 13. The duration of survival was found to be significantly correlated with the pattern of cytogenetic abnormality.⁽⁴⁾

Detailed molecular analysis of 20q in several haematological disorders has revealed heterogeneity of centromeric and telomeric breakpoints, supporting the existence of a tumour suppressor gene that contributes to the regulation of normal multipotent haematopoietic progenitors. Most of the small deletions on 20q involve the proximal G(+) band.⁽³⁾ FISH complements CC since it is able to evaluate large numbers of interphase and metaphase nuclei, as well as overcome the inaccuracy of CC in defining subtle chromosomal aberrations such as del(20q) and ider(20q) in MDS.^(5,6,9) However, there are limitations to FISH, as highlighted by other studies.⁽¹³⁻¹⁵⁾ Clinically, practical FISH strategies are limited to detection of known lesions, and the commercial FISH probe D20S108 may not always distinguish among del(20q), ider(20q) and monosomy 20.⁽¹³⁾ FISH adds little, if any, relevant information to adequate routine G-banding cytogenetics.⁽¹⁴⁾ If available, single nucleotide polymorphism array-based karyotyping can reveal unbalanced defects with resolution that is superior to FISH.⁽¹⁵⁾ Apart from the limitations of these techniques, availability and financial constraints are the other major

limiting factors in resource-poor laboratories. Therefore, FISH is recommended only in patients with inadequate or inconclusive CC results, as was the case with our patient.

Due to the rarity of predominant thrombocytopenic presentation in MDS, it may be confused with ITP, microangiopathic haemolytic anaemia (when kidney involvement is present), paroxysmal nocturnal haemoglobinuria (when intravascular haemolysis is dominant) or even acute leukaemia. All the aforementioned conditions were ruled out in the present case by means of appropriate laboratory tests (Table I). FISH played an instrumental role in confirming the diagnosis, especially when peripheral cytogenetics was inconclusive.

In conclusion, cytogenetic analysis should be an integral component of evaluation in adults with refractory thrombocytopenia, in order to confirm a diagnosis of MDS. Morbidity and mortality in MDS patients with isolated del(20q) is due to low platelet counts; hence, platelet transfusion should be offered if the patient is symptomatic and transfusion-dependent.

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