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CASE REPORT

Myelodysplastic syndrome with fibrosis and complex karyotype arising in a patient with essential thrombocythaemia

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Abstract

Introduction: Essential thrombocythaemia (ET) is a chronic myeloproliferative neoplasm (MPN) characterised by persistent thombocytosis. It is an indolent disorder but transformation to myelofibrosis (MF), acute myeloid leukaemia (AML) or myelodyplastic syndrome (MDS) has been reported. *Case Report:* We described a patient with ET whose disease evolved into MDS with fibrosis and complex karyotype after 15 years of stable disease. She was asymptomatic and was on hydroxyurea (HU) treatment until recently when she presented with worsening anaemia. Physical examination showed mild splenomegaly. Full blood picture showed leukoerythroblastic picture with presence of 3% circulating blasts and background of dysplastic features such as hypogranular cytoplasm and nuclear hyposegmentation of neutrophils. The bone marrow aspiration was haemodiluted but revealed presence of 6% blast cells, trilineage dysplasia and predominant erythroid precursors (60%). Trephine biopsy showed no excess of blast cells and normal quantity of erythroid precursors, but there was increased in fibrosis (WHO grade 2) and presence of dysmegakaryopoeisis such as nuclear hypolobation, multinucleation and micromegakaryocytes. Cytogenetic study showed complex karyotype; monosomy of chromosome 2, chromosome 5, chromosome 18 and presence of a marker chromosome (42~44, XX,-2,-5,-18,+mar). Fluorescence *in situ* hybridisation (FISH) showed 5q deletion (CSF1R and EGR1). *Conclusion:* The findings were consistent with transformation of ET to MDS with fibrosis and complex karyotype. ET progression to MDS is considered rare. The presence of complex karyotype and fibrosis in MDS are associated with unfavourable outcome.

Keywords: Myeloproliferative neoplasm, essential thrombocythaemia, myelodysplastic syndrome, complex karyotype

INTRODUCTION

Essential thrombocythaemia (ET) is a chronic myeloproliferative neoplasm (MPN) in which megakaryocyte proliferation and overproduction of platelets lead to a sustained increase in number of circulating platelets. In the United States of America, it is the second most common MPN with a yearly incidence rate of approximately 0.2-2.3 cases per 100,000 persons per year.[1] The clinical course of the disease is relatively indolent with thrombosis and, less frequently, bleeding are complications of the disease because of inherent platelet dysfunction. Delayed occurrance of disease transformation into myelofibrosis, acute myeloid leukaemia and myelodysplastic syndrome have been reported, but such progression is uncommon.[2] In a large cohort study, median time from ET diagnosis to transformation was around 76 month.[3] The reported risk of transformation in patients with ET is diverse in the literature but the consequences remain poor.[4] We report a patient who was diagnosed with ET but after 15 years of stable disease evolved to myelodyplastic syndrome (MDS) with fibrosis and complex karyotype.

CASE REPORT

A 65-year-old Chinese lady was diagnosed with ET for 15 years. She initially presented in

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year 2002 with incidental findings of persistent thrombocytosis during her routine follow up for dyslipidaemia at a private hospital. Otherwise she was asymptomatic with no symptoms of bleeding or thrombosis. On examination there was no hepatosplenomegaly or lymphadenopathy noted.

Full blood count (FBC) at that time revealed thrombocytosis with platelet count of 1800x10^9/L. There were mild normochromic normocytic anaemia for age (haemoglobin of 11.2 g/dl) and normal total white blood cell (WBC) count of 10.5x10^9/L with absolute neutrophils count (ANC) of 7 x10^9/L. Full blood picture (FBP) showed thrombocytosis with absence of rouleux formation.

Bone marrow aspirate (BMA) at diagnosis showed increase in megakaryocytes and platelet production resulting in islands of platelet aggregates and megakaryocytes fragments. Trephine biopsy specimen consisted of a piece of marrow tissue about 1.2 cm long, which was hypercellular for age, and showed prominent proliferation of megakaryocytes. These megakaryocytes were described to be normal morphologically. There was minimal increase in reticulin deposition (WHO grade 1) and there were no dysplastic changes seen. BCR-ABL1 fusion gene was undetectable and JAK-2 V617F mutation by polymerase chain reaction (PCR) method was negative. Other cytogenetic studies such as karyotyping and fluorescence *in situ* hybridization (FISH) were not done at diagnosis. The investigation done at that time was consistent with ET and in retrospect does meet with the latest WHO 2017 diagnostic criteria for ET.

She was started on hydroxyurea (HU) 500 mg bd and was transferred to Universiti Kebangsaan Malaysia Medical Centre (UKMMC) in 2003 for further care and follow-up. The disease and haematological baseline has been stable with Hb around 10-11 g/dL, with normal white cell and platelet count. She had been managed at the haematology clinic UKMMC and the disease has remained stable while she was on HU. However, 15 years later in October 2017, during routine clinic follow up, she was found to have worsening of anaemia requiring regular blood transfusion. The Hb was reduced to 6-7 g/dl. The platelet count was 226 x10^9/L with absolute neutrophil count of 5.9 x10^9/L. She denied any bleeding tendencies. Physical examination revealed mild splenomegaly (1 cm below the costal margin).

FBP showed leukoerythroblastic picture with 3% circulating blast and features of dysplasia. Moderate anisopoikilocytosis were noted with presence of macrocytes, tear drop cells and pencil cells (Fig. 1). Some neutrophils showed cytoplasmic hypogranulation and nuclear hypolobation. Platelet anisocytosis and megakaryocytic fragments were also seen.

BMA revealed only 2 small fragments and

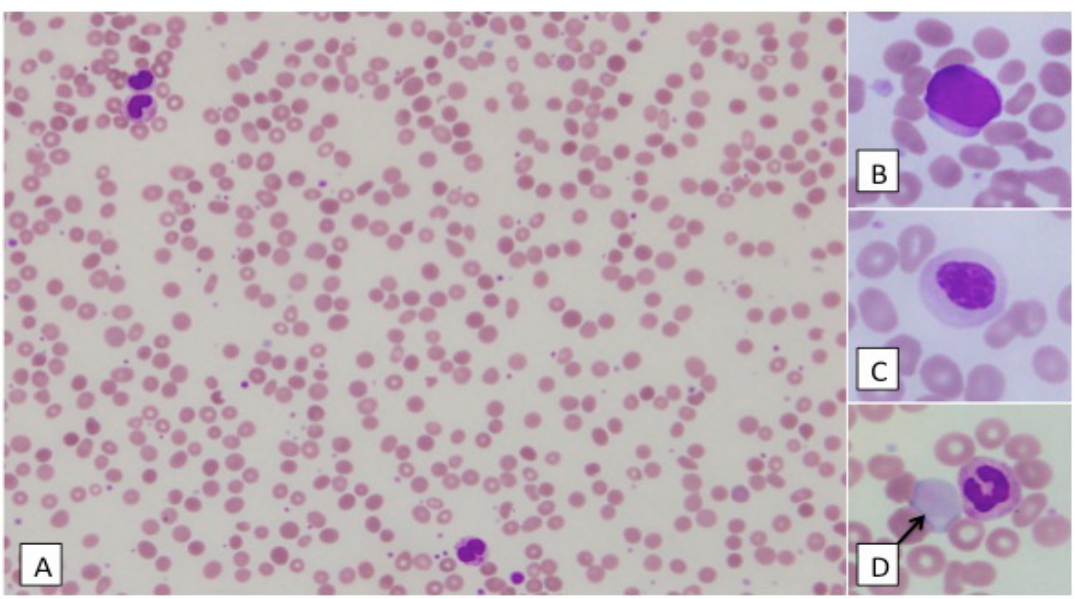

FIG. 1: FBP showed (A) red cells anisopoikilocytosis with macrocytic cells, tear drop cells, and pencil cells (Wrights' stain, x400). (B) Circulating blast cells (3%) (Wrights' stain, x400). (C) Dysplastic neutrophils with cytoplasmic hypogranulation and nuclear hypolobation (Wrights' stain, x400). (D) Megakaryocytic fragments (arrow) were also observed (Wrights' stain, x400).

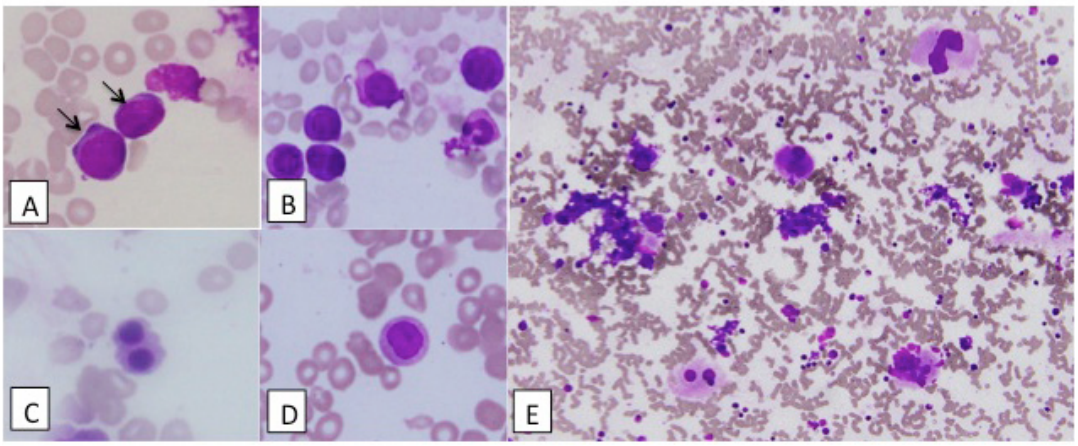

FIG. 2: BMA showed (A) presence of 6% blast cells (arrow) (May-Grünwald-Giemsa (MGG), x400). (B) Predominant erythroid precursors (MGG, x400) with (C) dyserythropoeisis such as binucleated erythroblast (MGG, x400). (D) Presence of dysgranulopoeisis such as cytoplasmic hypogranulation and nuclear hypolobation (MGG, x400). (E) Dysplastic megakaryocytes such as separated nuclei, and abnormal nuclear segmentation (MGG, x200).

haemodiluted sample. There were presence of 6% blast cells, trilineage dysplasia and predominant erythroid precursors (60%) (Fig. 2). No ring sideroblast seen on Perls' stain. Trephine biopsy sample was suboptimal with crushed marrow space with only few intact marrow spaces (Fig. 3). The marrow spaces were hypocellular with no increase in blast cells and normal quantity of erythroid precursors based on immunohistochemistry staining of CD34 and glycophorin C respectively. There were increase in fibrosis (WHO grade 2) and megakaryocytes, which showed dysplastic features such as hypolobation, multinucleation and micromegakaryocytes. Immunophenotyping showed inconclusive results. The combined morphology findings of full blood picture, bone marrow aspirate and trephine biopsy pointed towards the diagnosis of MDS with fibrosis.

Cytogenetic study of bone marrow cells showed complex karyotype (≥ 3 abnormalities); monosomy of chromosome 2, chromosome 5, chromosome 18 and presence of a marker chromosome (42~44,XX,-2,-5,-18,+mar) (Fig. 4). FISH showed 5q deletion (CSF1R and EGR1) (Fig. 5). These results together with the morphology findings were consistent with MDS, with fibrosis and complex karyotype.

Currently, she is still under haematology clinic follow up and was planned for chemotherapy, with Azacytidine (Vidaza®), a DNA methyl transferase inhibitor.

DISCUSSION

ET is generally considered a benign disease as it is associated with prolonged survival. Risk of thrombohaemorrhagic complications is more than risk of its transformation. Based on many studies, only about 10% transforms to myelofibrosis (MF) while less than 5% transforms to blast phase (i.e. AML) or MDS, and was reported to be likely related to cytotoxic therapy.[1,2,4] The median time to myelofibrotic transformation from time of ET diagnosis is roughly 7–16 years.[5]

In this patient, she had consumed HU for the treatment of ET for about 15 years whereby the disease had been stable during that period of time. Cytotoxic therapy such as exposure to radioactive phosphorus (P^{23}) and alkylating agents such as busulfan or melphalan had been associated with development of such transformation, but association with HU treatment has not been well established.[4,6] However, out of all the patients with MPNs who has transformed, 25% of patients were not exposed to cytotoxic therapy.[6] It was mentioned that complex cytogenetic abnormalities with loss of chromosomal materials in 5, 7, 17 and 20, could play an important role in disease transformation and progression regardless of specific drugs use such as HU or anagrelide in ET.[7] In this patient, other cytogenetic abnormality at initial diagnosis was not tested except for the BCR-ABL and JAK2 which were negative.

There was also a challenge in making a definitive diagnosis for the current presentation

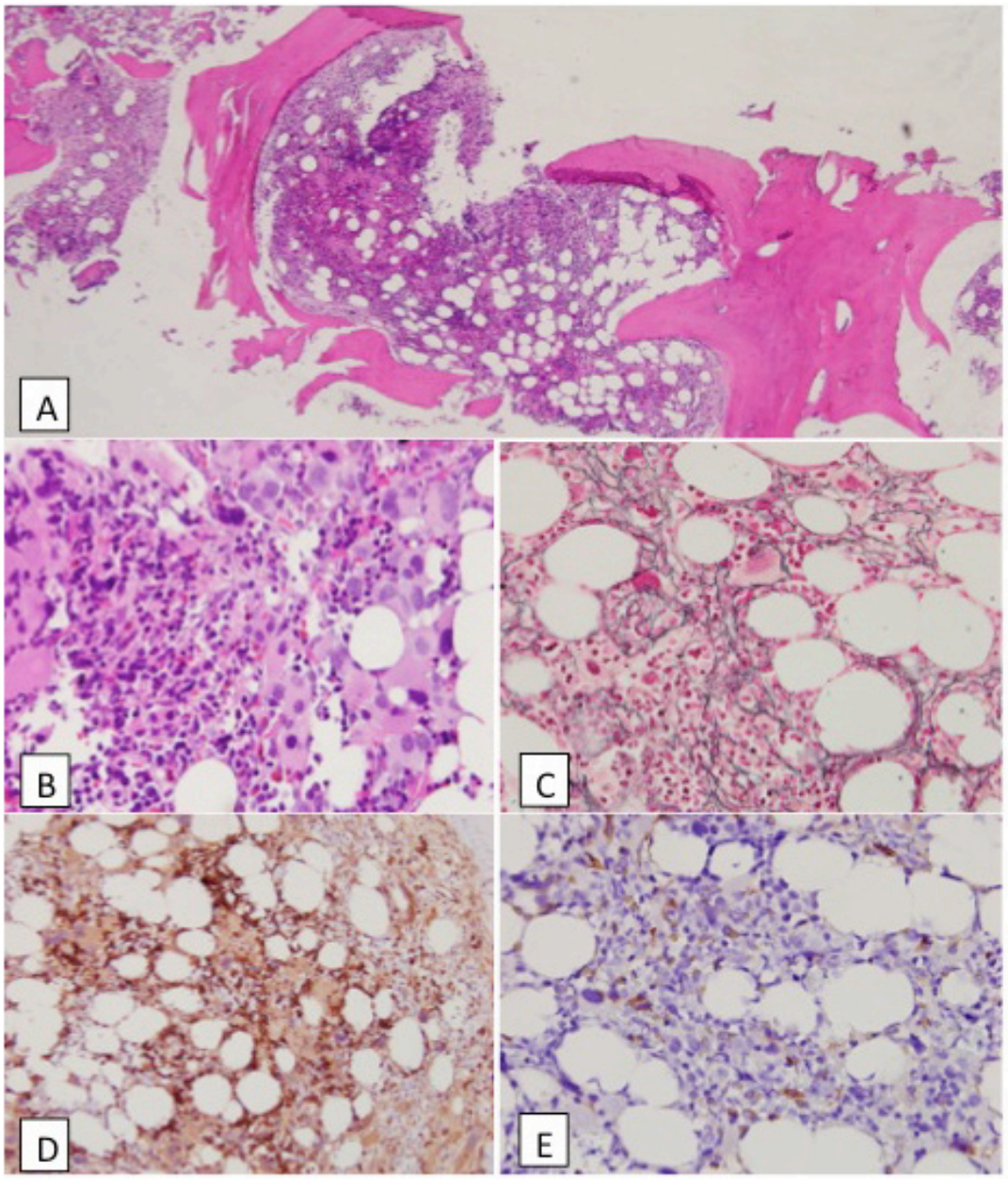

FIG. 3: Trephine biopsy: (A) Intact marrow spaces with crushed areas (Haematoxylin & eosin (H&E) stain, x40). (B) Increased in megakaryocytes which were small in size and showed dysplastic changes such as nuclear hypolobation and multinucleation. (H&E stain, x400). (C) Increased in reticulin fibres, WHO grade 2 (Reticulin stain, x400). (D) No increase in erythroid precursors (glycophorin C stain, x400). (E) No excess of blast cells (CD34 stain, x400).

due to some overlapping criterias. It was based initially on the latest edition of the 2017 WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues.[1] The differential diagnoses of which included post-ET myelofibrosis and MDS were considered. For post-ET myelofibrosis, the existing features in this patient include the presence of bone marrow fibrosis grade 2 with anaemia and leukoerythroblastosis. Furthermore, she had many risk factors for example advanced age, anaemia and absence of JAK2.[5] However, presence of trilineage dyplasia that is striking and consistently present in the morphology is not included in this classification.

MDS under the subclassification of MDS with multilineage dyplasia, MDS with excess blast

FIG. 4: Giemsa-banding of chromosomes showed a complex karyotypes monosomy of chromosome 2, chromosome 5, chromosome 18 (arrows) and presence of a marker chromosome (M) (44, XX, -2, -5, -18, +mar). A total of 22 metaphases were analysed and revealed an abnormal female chromosome complemented with the presence of two cell lines that showed variation from cell to cell in abnormalities thus forming a composite of all abnormalities as 42~44,XX,-2,-5,-18,+mar[cp20]/46,XX[2]. First cell line, constructed from 20 cells showed at least one of the abnormalities listed. The second cell line showed a normal female complement.

with erythroid predominance or MDS with excess of blast with fibrosis are the other diagnoses under consideration as this patient presented with many but somewhat overlapping features. In the marrow aspirate there was 6% blast with predominant 60% erythroid precursor, however,

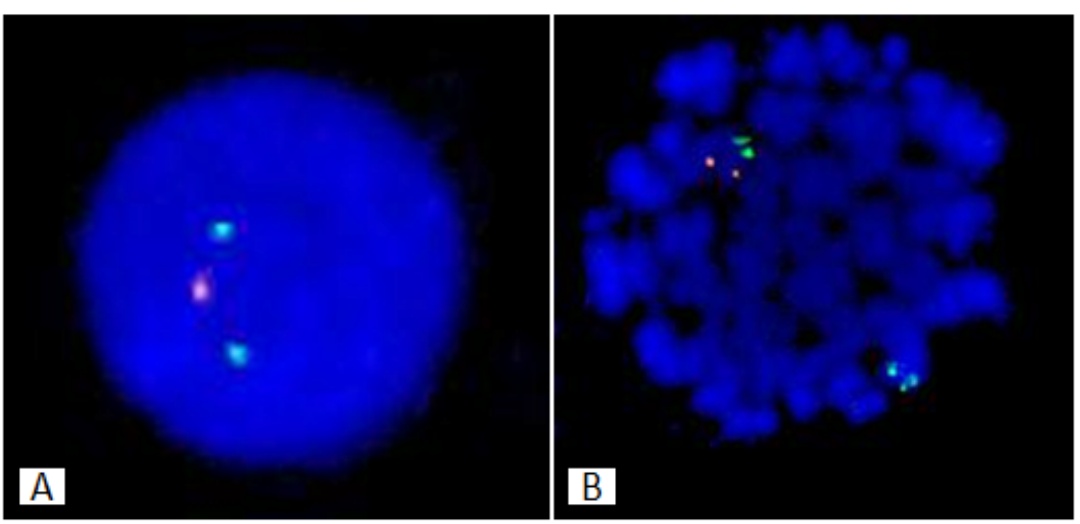

FIG. 5: FISH showed presence of 5q deletion. A total of 200 nuclei were analysed. (A) Positive CSF1R (5q33-34) deletion; 1 orange and 2 green signal patterns were observed in 86 nuclei analysed (43%). (B) Positive EGR1 (5q31) deletion. One orange and two green signal pattern were observed in 118 nuclei analysed (59%).

trephine biopsy showed no increase in blast and erythroid precursors by CD34 and glycophorin C immunohistochemistry respectively. These conflicting findings of the presence of excess of blasts in the bone marrow aspirate and trephine biopsy were difficult to rectify and was not supported by the flow cytometry findings which showed inconclusive results. Repeat bone marrow sampling performed 2 weeks later revealed similar findings.

Having considered all the above findings, we have concluded that the diagnosis of MDS with fibrosis (MDS-F) is currently the best fit for this patient. MDS-F is not recognised as a specific subtype in WHO classification[1], but this can occur in 10-15% of cases.[1,8] It is closely related with multilineage dysplasia, profound cytopenia, leading to high red cell and platelet transfusion needs, and poor cytogenetics.[1] Typically exhibits MDS type megakaryocytes morphology (ie micromegakaryocytes), other dysplastic changes and often increased in blast by CD34 immunohistochemistry.

Data pertaining to the treatment and prognosis in the MDS-F are very limited.[9] The survival of patients with moderate-to-severe fibrosis is significantly poor than that of patients with no or mild fibrosis, both because of an increase of nonleukaemic death (mainly a consequence of profound marrow failure) and because of the high rate of leukaemic evolution.[10] However, there are studies that showed that only the presence of severe bone marrow fibrosis (grade 3) was associated with worse outcome and reduced overall survival.[9]

Although MDS-F can share some features with myelofibrosis, cytogenetic features and molecular markers vary between the two diseases. Presence of complex karyotype in this patient further supported diagnosis of MDS. Fifty percent of MDS had recurrent cytogenetic abnormalities. Complex karyotype is defined as $\geq$ 3 abnormalities including numerical and structural aberrations, marker chromosomes and ring chromosomes and typically includes abnormalities of chromosome 5 and/or 7. In this patient, the cytogenetic results revealed monosomy of chromosome 2, chromosome 5, chromosome 18 and presence of a marker chromosome. Cytogenetic studies play a major role in the evaluation of patient with MDS especially with regards to prognosis. Patient with complex cytogenetic abnormalities involving chromosome 5 generally has a poor prognosis. Their well-known association with bad prognosis might be tied to the fact that these abnormalities often involve gross chromosomal loss.[11]

The median overall survival (OS) for MDS-F from time of transformation was 14 months (5-22.9 months).[4] ET patients with transformation to MDS has longer OS (median: 35 months from time of transformation) than those with AML (median 10 months). Increased age, myelofibrosis (grade 2-3), and leukocytosis at the time of transformation were associated with shorter OS from transformation. Surprisingly, patients with complex cytogenetic abnormalities versus patients with non-complex cytogenetic abnormalities at the time of transformation showed no difference in OS.[4]

CONCLUSION

The progression of ET is uncommon and only about 10% transforms to myelofibrosis while less than 5% transforms to blast phase (i.e. AML) or MDS. ET transformation to MDS-F with complex karyotype is a rarer case and hardly been reported as compared to the other type of ET transformation. The under reporting of MDS-F could be the reason why it is not part of the WHO classification distinctively. It is associated with an aggressive clinical course and patient with complex cytogenetic abnormalities involving chromosome 5 generally has a poorer prognosis.

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