Evolution of Janus Kinase 2 V617F-negative idiopathic myelofibrosis into Philadelphia+ chronic myeloid leukemia

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Abstract

Limited data exist on the mechanisms promoting clonal expression of BCR-ABL1 cells in various myeloproliferative disorders. We present a patient whose Janus Kinase (JAK) 2 V617F-negative idiopathic myelofibrosis (IMF) transformed to Philadelphia-positive chronic myeloid leukemia (CML). A 55-year-old man had anemia and splenomegaly. Trephine biopsy showed excess fibrosis without a JAK2 V617F mutation. Diagnosis of high-risk IMF with t(3;12) and del(16q) was made. Five years later a repeated trephine biopsy showed extensive fibrosis and t(9;22) with der(22)t(9;22). BCR-ABL1 fusion gene with typical p210 fusion transcript was found resulting in the diagnosis of CML. A modest treatment response was achieved with tyrosine kinase inhibitor (TKI) therapies, but the disease eventually progressed to a myeloid blast phase. With AML-based chemotherapy plus azacytidine and a second generation TKI the patient survived for years but succumbed 11 years after the initial diagnosis.

Clonal evolution may cause atypical disease characteristics or a poor response to targeted therapy in myeloproliferative disorders.

Introduction

Idiopathic myelofibrosis (IMF) is one of the BCR-ABL1-negative clonal disease entities of cells of myeloid origin in addition to polycythemia vera and essential thrombocythemia. The incidence of this rare and heterogenous disease varies from 0.3 to 2 cases per 100 000 individuals annually. Even though the exact pathophysiological mechanisms for fibrosis development are unknown, megakaryocytes are potential advancers for its development. Megakaryocytes have been shown to produce both growth factors and cytokines, which have been proven to result via fibroblast proliferation in the formation of myelofibrosis. Bone marrow fibrosis in conjunction with osteosclerosis have been suggested to express stromal responses for disturbances in clonal hematopoiesis in patients with IMF.

About 50% to 60% of the patients with IMF have a mutation in the exon 14 of Janus Kinase 2 gene in chromosome 9 resulting in valine-to-phenylalanine substitution (JAK2 V617F) identified in 2005. This mutation has been linked to...
activation of a tyrosine kinase function which dysregulates JAK-STAT signaling. About 5 to 10% of IMF patients carry activating mutations of the thrombopoietin receptor gene (MPL). Of note, a previous retrospective study concluded that 88% of both JAK2 V617F and MPL-negative patients with IMF had a somatic calreticulin gene mutation (CALR), which has been associated with a favorable clinical course\(^3\). Thus, the great majority of patients have one of these driver mutations that have an impact on outcome. Of note, IMF patients without any somatic mutations (10%) have poorer outcome\(^3\). In addition to the mutations, up to half of the patients have cytogenetic findings of which del(20q) and del(13q) have been considered to associate with better prognosis\(^3\).

Moreover, with the progressive bone marrow fibrosis, typical characteristics of IMF include palpable splenomegaly, ineffective and extramedullary hematopoiesis and general symptoms caused by excessive production of various inflammatory cytokines. To fulfill WHO 2016 diagnostic criteria for IMF, a patient should have megakaryocyte proliferation and atypia with reticulin formation in trephine biopsy, presence of a driver mutation or another clonal marker and no findings of other myeloproliferative diseases plus anemia or leukocytosis or elevated levels of lactate dehydrogenase (LDH)\(^3\). A widely used Dynamic International Prognostic Scoring System plus (DIPSS plus) calculator for prognosticication of IMF takes into account karyotype findings in addition to older age, hemoglobin level lower than 100g/L, white blood cell (WBC) count over 25 x 10\(^9\)/L, constitutional symptoms and circulating blasts of at least 1%\(^6\). However, this IMF scoring system does not include the aforementioned mutated driver genes that potentially influence on outcome.

The incidence of chronic myeloid leukemia (CML) is only 1-2 cases per 100 000 adults per year. There are some reports concerning transformation of JAK2 V617F-positive and especially JAK2 V617F-negative myeloproliferative disorders into CML\(^7\)-\(^9\). In addition, a case report has been published of a patient with Philadelphia chromosome positive (Ph+) CML who proceeded to JAK2 V617F-positive myelofibrosis\(^10\). To the best of our knowledge, this is the first case report of a patient whose JAK2 V617F negative IMF transformed to Ph+ CML with a blast phase.

**Case report**

**Investigations**

A 55-year-old man was evaluated for night sweating, splenomegaly (long axis 21.5 cm) and anemia in 2010. The initial hemoglobin concentration (Hb) was 113g/L, WBC count was 4 x 10\(^9\)/L with 6% circulating blasts in the differential and platelets were 285 x 10\(^9\)/L. The serum LDH level was elevated up to 537 U/L. The patient did not have JAK2 V617F mutation as analyzed by TaqMan® method from a peripheral blood sample.

**Diagnosis**

Bone marrow aspirate yielded a dry tap. Trephine biopsy showed 50% cellularity and excess fibrosis. Cytogenetic analysis by G-banding revealed t(3;12) (100%) and del(16q) (100%) when 20 cells were analyzed. Calreticulin or MPL mutation analyses were not available at that time. The diagnosis of high-risk IMF was done according to the DIPSS score.

**Treatment**

Hydroxyurea was started a year after the diagnosis of IMF due to symptomatic splenomegaly and leukocytosis (WBC 31 x 10\(^9\)/L) Erythropoietin injections were started for symptomatic anemia with a hemoglobin level of 90 g/L. In the next year ruxolinitib was initiated at a dose of 20mg daily to reduce the spleen size with a minimal response. Five years after the initial diagnosis of IMF, WBC count rose
up to 210 x 10⁹/L, differential showed 4% of blasts, platelets were 135 x 10⁹/L and serum LDH was as high as 985 U/L. A new trephine biopsy showed extensive fibrosis without signs of blast transformation and blood flow cytometry analysis showed only 1% myeloid blasts. In chromosomal analysis by G-banding both the t(3;12) and t(9;22) were found in all 20 cells analyzed. In addition, der (22)t(9;22) was detected in 15 out of 20 cells analyzed. Molecular analysis showed a BCR-ABL1 fusion gene with typical p210 fusion transcript in quantitative real-time RT-PCR test of isolated RNA (5.8%), resulting in the diagnosis of CML.

Hydroxyurea was continued and one high-dose of cytarabine was given to reduce the high leukocyte counts. Packed red cells were given due to symptomatic anemia (Hb 72 g/L) before imatinib was started. Three months later imatinib was replaced by nilotinib due to a rising amount of BCR-ABL1 fusion transcripts up to 24% according to the International Scale. Five months later 1.3% residual disease in molecular analysis was found. However, disease progression was inevitable and ponatinib was started instead. Due to marked side effects manifested by pericardial effusion and chest pain, ponatinib was stopped early. Hydroxyurea combined with interferon α injections was started.

Follow-up and outcome

A month later a repeated bone marrow analysis showed a disease progression to the myeloid blast phase. Induction chemotherapy with idarubicin and cytarabine followed by three consolidation courses combined with dasatinib as a TKI at a dose of 100 mg daily was started. This therapy resulted in a cytogenetic remission by FISHMRD study with a residual disease of 0.5% in the RT-PCR analysis. The patient refused allogeneic stem cell transplantation, and dasatinib alone was continued. Almost 1.5 years after the last chemotherapy course an acceleration was detected and azacytidine was administered for six cycles, which resulted in a good response with residual disease of 0.04% in the molecular analysis. The patient remained transfusion-dependent during dasatinib treatment. Later, due to severe heart failure, dasatinib was stopped a few months before the patient died 11 years after the initial diagnosis of IMF.

Discussion

This case report addresses for the first time the evolution of JAK2 V617F- and BCR-ABL-negative IMF to Ph⁺ CML with a blast phase. The exact pathologic mechanism for IMF, a disorder of clonal hematopoietic stem cells, is unknown. Either a combination of many molecular level defects of multipotent stem cells or separate single pathologic stem cell clones may result in the unusual heterogeneity of IMF manifestations. However, the exact events that promote clonal expression of BCR-ABL1-positive cells in various myeloproliferative disorders are still unknown. Stem cell defects, simultaneous abnormal stem cell clones, or driver mutations are probably causes for the clonal expansion. In some previous publications distinct cytogenetic abnormalities situated in different clones have been suggested as potential markers of two rare coexisting independent myeloproliferative disease entities instead of clonal evolution.

Mutated genes have a well-known prognostic significance in IMF. At least half of the IMF patients have the JAK2 V617F mutation, which results in tyrosine kinase activation promoting sensitivity of hematopoietic cells for growth and cytokines. An Italian registry data showed that patients with the JAK2 V617F mutation at diagnosis had an over five-fold higher risk for leukemic transformation compared to the patients without mutation. However, the potency of activation of JAK2 to enhance the clonal manifestation of BCR-ABL1 cells remains unclear. The mechanisms for leukemic transformation or for the dismal prognosis (medial OS of 37 months) in those patients with JAK2
V617F, MPL and CALR triple negativity is unknown. Overexpression of SRSF2, SETBP1, IDH2 and GNAS mutations established by extended genomic profiling in these patients may be in part causative for clinical course of the disease\textsuperscript{15}. Our patient did not have the JAK2 V617F mutation in repeated samples and data on other driver mutations are not available.

The bone marrow microenvironment may play a role in a differentiation of various myeloproliferative diseases and possibly impacts also on the patient outcome\textsuperscript{2}. Not only increased fibrosis, but also proliferation of angiogenesis and variations of bone trabecular thickness may assist to determine the phenotype of disease\textsuperscript{2}. Vascular endothelial growth factor (VEGF) is one of the cytokines that are involved in the neovascularization and also in the disease progression, albeit so far no cytokine storm has been proven to associate with the evolution of one myeloproliferative disease into another\textsuperscript{2}. In addition, environmental factors, genetic interactions, immunological profile and increased age have been linked to production of mutant clones and disease evolution\textsuperscript{16}.

Imatinib mesylate alone as a first-line treatment or second generation TKIs have produced deep molecular responses for most patients with CML\textsuperscript{17}. However, the great majority of patients with CML and simultaneous excess marrow fibrosis have been shown to be imatinib mesylate-resistant\textsuperscript{18-19}. Our patient did not have a point mutation in the ABL 1 kinase domain of BCR-ABL1. Possibly the primary diagnosis of IMF was the reason for the modest response both to first- and second-generation TKIs. It should be kept in mind that persisting splenomegaly during tyrosine kinase inhibitor treatment for CML may be a marker of another simultaneous myeloproliferative clone.

To conclude, stem cell defects, simultaneous abnormal stem cell clones or driver mutations may present mechanism that promote clonal expansion of BCR-ABL1-positive cells in different myeloproliferative diseases. Due to potential clonal evolution repeated karyotypic and molecular testing is suggested to detect co-existing myeloproliferative disorders in cases with atypical disease characteristics or a poor response to targeted therapy.

Author contributions
A.P. designed and wrote the manuscript, E.J. reviewed the manuscript.

Conflict of Interest Statement
Dr. Partanen reports honoraria from Behring and Abbvie and has participated in Scientific Advisory Board meetings organized by Abbvie, Janssen-Cilag, Novartis and Takeda.

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Statement of Ethics
An informed consent was obtained from the patient’s relative for publication of this case report. The case report was produced according to the declaration of Helsinki.

Data availability statement
The data that supports the findings of this case report are not publicly available as they contain information that could compromise the privacy of patient.

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