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Tyrosine kinase inhibitors induced immune thrombocytopenia in chronic myeloid leukemia?

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Abstract

The outcome and quality of life of chronic myeloid leukemia (CML) patients has remarkably changed with the treatment of tyrosine kinase inhibitors (TKIs). Currently, hematopoietic stem cell transplantation (HSCT) is considered mainly as a third line salvage therapy in cases of TKIs resistance or intolerance. Here we describe a patient with chronic phase CML who developed both resistance and late occurrence of s severe thrombocytopenia on first and second generation TKIs and eventually underwent HSCT. Although the mechanism of the myelosuppression is not fully understood, we showed for the first time the development of dose dependent platelet antibodies in the presence of TKIs, suggesting the possibility of TKIs induced thrombocytopenia. Our case emphasizes that late development of severe myelosuppression during imatinib treatment is probably an important indication for consideration of early HSCT.

Introduction

Chronic myeloid leukemia (CML) accounts for approximately 20% of all adult leukemia. It is a clonal myeloproliferative disorder, which is caused by the Philadelphia (Ph) chromosome that produces the constitutively active BCR-ABL tyrosine kinase. Tyrosine kinase inhibitors (TKIs) act by competitive inhibition of adenosine triphosphate (ATP) that binds to BCR-ABL. This process prevents the downstream signal transduction pathways.[1,2] Imatinib is the first tyrosine kinase inhibitor to have achieved long term disease control in the majority of patients with CML.[2] In an internationally randomized study of interferon versus ST1571 (IRIS), 63% of all imatinib patients' showed a complete cytogenetic response (CCyR) and the estimated event free survival (EFS) was 83%.[3] The degree of treatment response at earlier time points in the CML correlates with EFS and time to progression to accelerated phase/ blast crisis (AP/BC).

However, imatinib use is complicated by the development of resistance or intolerance,[3-7] which leads to suboptimal responses or treatment failure.[2] Imatinib resistance appears to be multifactorial.[8,9] The most common mechanisms for the development of this resistance are the occurrence of a mutation in the BCR-ABL tyrosine kinase domain that inhibits imatinibs ability to bind to ABL and poor patient compliance.[9,10]

Most side effects of imatinib are mild to moderate and are often self limiting. Grade ≥3 neutropenia and thrombocytopenia were reported in 17% and 9% of the patients respectively, and most of them occurred within the first two years of imatinib treatment.[7] Furthermore, most myelosuppression is dose-dependent, reversible, and can be managed by dose interruption or reduction. In the IRIS study, 4% of the imatinib group discontinued therapy owing to an adverse event.

The second generation TKIs - nilotinib or dasatinib - are more potent than imatinib and can overcome most imatinib resistant BCR-ABL mutations.[2] In a two year follow up of a phase 2 study, nilotinib was given to patients with chronic phase (CP) or advanced phase CML who had failed, or were unable to tolerate, initial imatinib therapy. Cross-intolerance with imatinib (defined as occurrence of a grade ≥3 adverse event (AE) or persistence or recurrent grade 2 of the same AE that previously led to discontinuation of imatinib) was observed in a sub-analysis of 121 out of 459 patients with CP and AP. All of the seven patients who discontinued nilotinib did so due to thrombocytopenia.[11]

Additional data suggests that patients who develop severe hematological toxicity with imatinib treatment, are at high risk of having a suboptimal response or hematological cross-intolerance with a second generation TKI.[12]

Currently, the general assumption is that an initial trial of second-generation TKIs, before proceeding to hematopoietic stem cell transplantation (HSCT), is reasonable in nearly all patients in whom imatinib treatment fails, particularly, because HSCT is available only to a minority of patients and is associated with significant morbidity and mortality.[13]

The *Hammersmith score* helps predict the response to second generation TKI's and is based on calculating the cytogenetic response to imatinib, the Sokal high risk group and the recurrence of neutropenia.[14] It is still unclear whether a small, select group of young CML patients in CP who fail imatinib treatment should undergo allogeneic HSCT without being first treated with second-line TKIs.

Here we present a CP CML patient who

developed severe thrombocytopenia during treatment with all three TKI's and we raise the question as to whether an early HSCT should have been performed.

Case Report

A 34-year-old man was diagnosed with Ph-positive CML in May 2009, after presenting the preceding year with weight loss and a white blood cell count (WBC) of 188×10^9/L. The patient received hydroxyurea for three weeks followed by imatinib 400 mg/day. Within a month he achieved a complete hematological response (CHR).

The patient entered a clinical study that assessed therapeutic levels of imatinib and he was found to have a sub-therapeutic level of 640 ng (the study estimated an optimal therapeutic level of approximately 1000 ng).[15] After three months of imatinib treatment the BCR-ABL transcript level decreased by only 0.2 logs as compared to the test performed at diagnosis. In November 2009, the patient complained of *flu like* symptoms and developed severe thrombocytopenia with a platelet (PLT) count of 22×10^9/L, WBC of 4.4×10^9/L with absolute neutrophil count of 0.8×10^9/L, and hemoglobin of 12.8g/dL. A peripheral blood smear showed atypical lymphocytes with toxic granulation, suggestive of acute viral infection. The following tests were normal or negative; herpes simplex virus, herpes zoster, cytomegalovirus, epstein barr virus, IgM antibodies, hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (HBsAb), hepatitis c virus antibody , antinuclear antibody and human immunodeficiency virus antibody. Bone marrow (BM) aspiration and biopsy revealed a hypoplastic mar-

row with a decreased number of megakary-ocytes, no blast cells and no excess of reticulin. Cytogenetic studies demonstrated 93% mitotic cells with Ph chromosome and fluorescent in situ hybridization (FISH) showed 83% of the cells with BCR-ABL. Mutation analysis did not reveal a mutation in the BCR-ABL. The treatment with imatinib was stopped for two weeks and Nilotinib 400 mg twice daily was started after the platelets rose above 50,000 µL. Within another two weeks, there was a decline in his blood count with PLT 15×10^9/L, normal levels of WBC (5.6×10^9/L), ANC of 1×10^9/L and hemoglobin of 12.1g/dL. Of note, severe thrombocytopenia was also observed with a lower dose of Nilotinib (200 mg twice daily). The treatment with nilotinib was discontinued and the patient was treated empirically with prednisolone 80 mg daily. Within 2 weeks, the PLT level increased to 110,000 µL. After the treatment failure with nilotinib, dasatinib100 mg once daily was started, with a similar decrease in PLT level to 21×10^9/L. At this point, studies to evaluate the possibility of drug (TKI's) induced thrombocytopenia were performed.

Tests for anti-human antibodies IgM, IgG and IgA against PLTs were negative. However, after adding the TKI drugs, drug-dependent antibodies against PLT were found using an immunofluorescence test applying flow cytometry (Figure 1). Moreover, the presence of these antibodies was dose dependent and was thus raised the possibility of drug induced thrombocytopenia.

In August 2010, the status of his disease was again re-evaluated. Cytogenetic studies
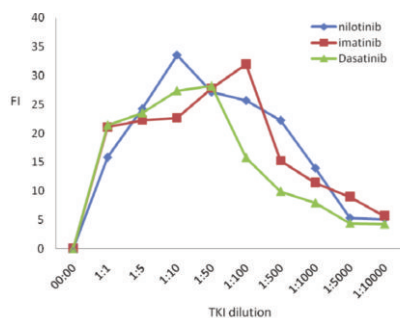

Figure 1. Dose dependent anti platelets antibodies in the presence of tyrosine kinase inhibitors. The presence of antibodies against platelets was detected using platelet immunofluorescence test (PIFT) and the measuring unit was mean fluorescence intensity (FI). Normal group O platelets from 6 different donors were washed and incubated with patient serum or control serum and with either PBS or TKIs diluted in PBS from 1:1 up to 1:10000. After washing platelets were incubated with FITC labeled goat anti-human IgG and analyzed using flow cytometer. Point zero demonstrates that in the absence of TKIs there are not any antibodies.

showed 100% mitosis with Philadelphia chromosome along with only one log reduction in BCR-ABL load. Bone marrow aspiration and the peripheral counts revealed no blasts thereby implying that the patient was still in the CP of disease. Since the patient was intolerant to all TKI's, therapy with interferon alpha 3MU three times weekly was started before proceeding to HSCT. During the interferon therapy, there was a minor decrease in PLT to 126×10^9/L.

In September 2010 the patient underwent an *uneventful*, allogeneic, peripheral blood stem cell transplantation from a fully matched unrelated male donor after conditioning with BU-CY (busulfan- cyclophosphamide) and standard GVHD prophylaxis. Engraftment was documented on day 13. RT-qPCR for BCR/ABL transcripts performed on day 28 showed 6 log decrease. A repeated test on day 72 showed an increase of BCR-ABL/ABL ratio to 10^{-4} and cyclosporine was discontinued. During the following month the patient presented with skin rash, diarrhea and elevated liver enzymes, compatible with acute GVHD. There was a concomitant decrease of BCR-ABL/ABL to 10^{-6}, 46XY karyotype and 100% donor chimerism.

Today the patient receives corticosteroids and tacrolimus for chronic limited GVHD. His blood count is normal, with complete donor chimerism and undetectable BCR/ABL.

Discussion

Our CML patient presented with an interesting combination of resistance and intolerance to TKIs that was manifested mainly as grade 3 thrombocytopenia. Interruption of therapy, dose reduction and therapy with second generation TKIs, did not prevent the recurrence of the adverse hematological events. Mutations in the BCR-ABL tyrosine kinase domain were not found in our patient and he was compliant with medication.

In CML, the hematopoiesis is mainly derived from the Ph+ stem cells and myelosuppression is usually observed within the first weeks of TKI treatment. The latter is probably due to delayed recovery of the normal Ph− stem cells. The reduced reserve of residual, healthy Ph - bone marrow (BM) is incapable of reconstituting the peripheral blood count.[16,17] In the case of our patient, the cytopenia occurred six months after the initiation of imatinib therapy and the Ph+ clone was not suppressed, as shown by the 100% Ph+ mitotic cells in the karyotype analysis.

The demonstration of anti-platelets antibodies in the patients' serum only in the presence of TKIs implies that the possible mechanism is indeed drug (TKIs) induced thrombocytopenia.

However, the full significance of this finding is unclear since, unlike in ITP (Immune Thrombocytopenic Purpura), there were decreased numbers of megakaryocytes in the BM, and since no TKI induced immune thrombocytopenia has been previously reported.

HSCT is effective therapy for patients with CP-CML but in the current era of second generation TKI's it is mainly indicated for patients who develop resistance. Less than 5% of patients develop repeated severe myelosuppression after only a few months of TKI therapy. Late development of severe myelosuppression during imatinib treatment is probably an important indication for consideration of early HSCT. Future studies are needed in order to understand the pathogenesis of recurrent severe myelosuppression during TKI therapy.

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