Peer Review File

Article Information: http://dx.doi.org/10.21037/acr-20-107.

REVIEWER

1. Response to Reviewer

Comments 1

The reviewer commented "The most significant concern is the deduction of "subsequent" Gilbert's Syndrome. Though the PCR technique confirmed the disease, it is not convincing enough to make such a conclusion. Some suggestions: (1) Add tables to show the hyperbilirubinemia and PCR result. (2) In the discussion, discuss this subsequent possibility in-depth."

Authors response

We thank the reviewer for this important suggestion related to Gilbert's Syndrome in the patient.

We summarized some clinical and laboratorial data, including CML diagnosis parameters (bone marrow cytogenetics analysis and real-time PCR analysis), hematological data (at diagnosis, three, six, one year, one year and a half, two years and two years and a half), biochemistry analysis, Gilbert Syndrome analysis, on the Table 1 (page 5).

Gilbert's Syndrome is a common liver disease caused by a mutation to the UGT1A1 gene that is able to synthesize a liver enzyme required for the conjugation and subsequent excretion of bilirubin. In this way, the process of bilirubin occur inappropriately and elevated levels of bilirubin (hyperbilirubinemia) is observed. Gilbert's syndrome patients could present serum bilirubin levels ranging from 0.6 to 3.0mg/dL. Usually, individuals with Gilbert Syndrome are asymptomatic or exhibit mild yellowing of the skin, mucous membranes, and whites of the eyes. Interestingly, some tyrosine kinase inhibitors, as nilotinib, are a potent noncompetitive inhibitor of human UGT1A1 activity (Fujita et al., 2011). Thus, undiagnosed Gilbert's Syndrome patients with bilirubin levels less than 3.0mg/dL that initialized treatment with tyrosine kinase inhibitor, could have additional reduction of UGT1A1 activity, resulting in an increase in bilirubin levels.

In this study, we reported a case of a patients that was diagnosed with CML by bone marrow cytogenetics analysis with the Philadelphia chromosome (t(9;22)(q34;q11.2)) and confirmation by real-time PCR analysis (b2a2 and b3a2 mRNA and $p210^{BCR/ABL}$ – 221.7%). At the time of diagnosis, bilirubin level was 1.0mg/dL and Gilbert's Syndrome polymorphism was not investigated. After diagnosis, the patient was initially treated with Imatinib and presented a response. However, the patient lost response to Imatinib over the evolution of treatment, requiring an alternative therapy and, therefore, Nilotinib was prescribed. A reduction in the BCR-ABL translocation was detected and a slight hyperbilirubinemia was observed. Thus, a PCR analysis was done, revealing the presence of Gilbert's Syndrome (TA7 /TA7 allele presence of UGT1A1 gene). TKIs are also inhibitors of the enzyme UGT1A1, which acts on hepatic phase II metabolism, converting unconjugated bilirubin into conjugated bilirubin (Lvet al., 2019). A reduced expression of the UGT1A1 enzyme leads to deficient glucuronidation, characterizing Gilbert's Syndrome, in which there is a mild hyperbilirubinemia (Sun et al., 2017). Nilotinib is a potent non-competitive inhibitor of UDPGT1, which can lead to the worsening of this condition (Cheng et al., 2017). Interestingly, although a gene alteration was found and two coexisting conditions that predisposes to hyperbilirubinemia occurred, the patient did not present symptoms of jaundice.

Fujita K. , Sugiyama M., Akiyama Y., Ando Y., Sasaki Y. The small-molecule tyrosine kinase inhibitor nilotinib is a potent noncompetitive inhibitor of the SN-38 glucuronidation by human UGT1A1 Cancer Chemother Pharmacol. 2011;67(1):237-41. doi: 10.1007/s00280-010-1445-3.

Location of changes

We have added some clinical and laboratorial data in a table, included on page 5 and line 1.

Table 1. Clinical data of one patient with a diagnosis of CML and Gilbert's Syndrome.

Case	
Gender	Female
Age at diagnosis	34

CML diagnosis parameters	
BM cytogenetic analysis	t(9;22)(q34;q11.2)
PCR analysis	b2a2 and b3a2 mRNA / $p210^{BCR/ABL}$ – 221.7%
Hematological analysis	
at diagnosis	leukocytocytes - 165,190 / mm^3 (monocytes 11,557 / mm^3, segmented neutrophils 119,223 / mm^3, band neutrophils 1,651 / mm^3, eosinophils 8,255 / mm^3; basophils 8.0%; lymphocytes 24,765 / mm^3); blasts - 6.0%; platelets - 626,000 / mm^3); erythrocytes (3.70 10^6 / mm^3); hemoglobin (11.7g/dL)
three months after diagnosis	leukocytocytes - 5,100 / mm^3; blasts - 0.0%; platelets - 129,000 / mm^3; erythrocytes (4.52 10^6 / mm^3); hemoglobin (13.54g/dL)
six months after diagnosis	leukocytocytes - 5,100 / mm^3; blasts - 0.0%; platelets - 184,000 / mm^3; erythrocytes (4.66 10^6 / mm^3); hemoglobin (14.00g/dL)
one year after diagnosis	leukocytocytes - 9,400 / mm^3 (monocytes 658 / mm^3, segmented neutrophils 6,768 / mm^3, band neutrophils 94 / mm^3, eosinophils 470 / mm^3; basophils 0.0%; lymphocytes 1,410 / mm^3); blasts - 6.0%; platelets - 626,000 / mm^3); blasts - 0.0%; platelets - 219,000 / mm^3; erythrocytes (4.84 10^6 / mm^3); hemoglobin (14.30g/dL)
one year and a half after diagnosis	

two year after diagnosis	leukocytocytes - 6,600 / mm^3; blasts - 0.0%; platelets - 180,000 / mm^3; erythrocytes (4.51 10^6 / mm^3); hemoglobin (14.00g/dL)
two year and a half after diagnosis	leukocytocytes - 5,800 / mm^3; blasts - 0.0%; platelets - 187,000 / mm^3; erythrocytes (4.69 10^6 / mm^3); hemoglobin (14.30g/dL)
	leukocytocytes - 6,400 / mm^3; blasts - 0.0%; platelets - 194,000 / mm^3; erythrocytes (4.70 10^6 / mm^3); hemoglobin (14.40g/dL)
Biochemistry analysis	
at LMC diagnosis	total bilirubinemia 1.0 mg/dL
one year after diagnosis	total bilirubinemia 1.6 mg/dL
Gilbert Syndrome diagnosis parameters	
PCR result	TA7 /TA7 allele of UGT1A1 gene

We have rephrased the information in the 3- Discussion on page 7 and line 10-31.

"… We reported a case of a patient that was diagnosed with CML by bone marrow cytogenetics analysis with the Philadelphia chromosome (t(9;22)(q34;q11.2)) and confirmation by PCR analysis (b2a2 and b3a2 mRNA and p210BCR/ABL – 221.7%). At the diagnosis, bilirubin level was 1.0mg/dL and Gilbert's Syndrome polymorphism was not investigated due to the absence of hyperbilirubinemia. After diagnosis of CML, the patient was initially treated with Imatinib, and presented response to treatment indicated by a decrease in the leukocytes levels. However, response to Imatinib was lost over the evolution of treatment, requiring an alternative therapy and, therefore, Nilotinib, a second generation of tyrosine kinase drug, was prescribed. A reduction in the BCR-ABL translocation was detected with Nilotinib treatment while a slight hyperbilirubinemia (1.6 mg/dL) was observed. In this way, a PCR analysis for UGT1A1 gene polymorphism was done, revealing the presence of

Gilbert's Syndrome (TA7 /TA7 allele presence of UGT1A1 gene). Gilbert's Syndrome is a common liver disease caused by a mutation on the UGT1A1 gene. This gene normally enables the synthesis of a liver enzyme, which acts on hepatic phase II metabolism, converting unconjugated bilirubin into conjugated bilirubin (12). In the Gilbert's syndrome, the process of bilirubin occur inappropriately due to deficient glucuronidation, causing elevation in the levels of bilirubin and a consequent mild hyperbilirubinemia (13). Gilbert's syndrome patients could present serum bilirubin levels ranging from 0.6 to 3.0mg/dL. Usually, individuals with Gilbert's Syndrome are asymptomatic or exhibit mild yellowing of the skin, mucous membranes, and whites of the eyes. Furthermore, TKIs, as Nilotinib, are also a potent non-competitive inhibitors of the enzyme UGT1A1, which can lead to the worsening of Gilbert's Syndrome (14). Interestingly, although a gene alteration was found and two coexisting conditions that predisposes to hyperbilirubinemia occurred, the patient did not present symptoms of jaundice..."

Comments 2

The reviewer requested “Draw a timeline to outline the entire case report. Make sure all critical information is included, including the clinical manifestation, diagnosis, treatment details, and prognosis etc. A precise time is needed at every step. Accordingly, time is required in the manuscript too..”

Authors’ response

We thank the reviewer for the opportunity to clarify the timeline information about the case report. Thus, we have added a figure, including the clinical manifestation, diagnosis, treatment details, and prognosis.

Location of changes

We have added a figure on the 2 - Case, on Page 6, Line 6-17.

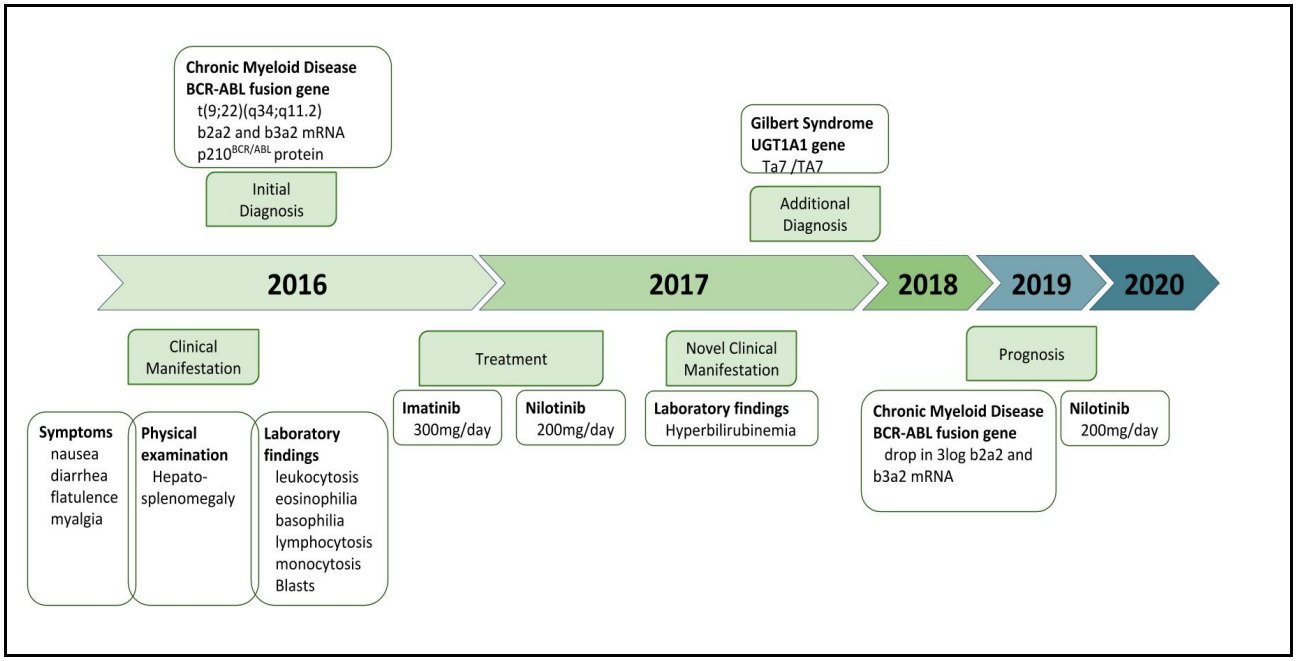

Figure 1: Timeline of the case report of a patient that was diagnosed with Chronic Myeloid Leukemia (CML) and subsequently, with Gilbert Syndrome. Initially, A 34-year-old female was diagnosed with Chronic Myeloid Leukemia (CML) due to the presence of the Philadelphia chromosome (t(9;22)(q34;q11.2 / b2a2 and b3a2 mRNA / $p210^{BCR/ABL}$ protein. Immediately, the patient started chemotherapy using Imatinib (300mg/day). One year after the beginning of treatment, due to partial response to Imatinib, the therapy was changed to Nilotinib (200mg/day). Despite the slight hyperbilirubinemia, it was found the presence of the Ta_7 /TA_7 allele of UGT1A1 gene, indicating positivity for Gilbert's Syndrome. Two years later, the translocation test of the BCR-ABL gene was positive for the isoforms b2a2 and b3a2 mRNA, suggesting that the patient went into a partial molecular remission of CML still demanding continuance of chemotherapy. Nowadays, the patient continues to be treated with Nilotinib drug and has related no symptoms.

Comment 3

The author suggested "Both strengths and limitations are required in the discussion."

Authors' response

We thank the reviewer for the important comment. In medicine, a case report is a detailed report of the symptoms, signs, diagnosis, treatment, and follow-up of an

individual patient. It is the investigation and exploration of an event thoroughly and deeply, providing detailed (rich qualitative) information about a specific and rare case of two coexisting conditions that could cause hyperbilirubinemia, did not have bilirubin alterations. Moreover, it highlights the importance of having genetic investigations in cancer patients, in order to identify secondary diseases that could worsen the course of treatment, providing insight for further research. Although, there are some limitations, as the difficult to replicate and the conclusions drawn from a particular case may not be transferable to other settings.

Location of changes

We have added the following information in the 3- Discussion on page 8, line 6-12.

"…This thorough and deep investigation and exploration provided detailed (rich qualitative) information about a specific and rare case of two coexisting conditions that could cause hyperbilirubinemia, in absence of bilirubin alterations. Moreover, it highlights the importance of having genetic investigation in cancer patients, in order to identify secondary diseases that could worsen the course of treatment, providing insights for further researches. However, there are some limitations, as the difficulty to replicate and the conclusions drawn from a particular case may not be transferable to other settings..."