Author's response to reviews

Title: Acute portal vein thrombosis precipitated by indomethacin in a HCV-positive elderly patient

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Author's response to reviews: see over
Report to Referee 1

Query 1
I have the pleasure to read this interesting report on a not previously described side effect due to indomethacin administration in a HCV patient. The Authors describe a possible association between an acute use of indomethacin and PTV. The case is well describe and informative. However, I have several doubts about this association and in my opinion this association is only casual.

Reply 1
We wish to thank the Reviewer 1 for his interest in our work. We agree with the Reviewer about the difficulty of clearly identifying and discussing the causal relationship linking indomethacin to the occurrence of PVT in the context of such a complex clinical picture. For this reason, we have made further efforts of reviewing with more detail the patient is clinical history and his medical documentation. Through this approach, we have been able to retrieve previous clinical records, since over the recent past the patient has undergone frequent medical visits and blood examinations to monitor his liver function. Accordingly, new information have been introduced throughout the Case presentation section (see page 3, lines 12-15), and the Discussion has been integrated (see page 5, lines 2-15), in order to better substantiate our contention that indomethacin acted as the precipitating factor of PVT (see also our reply to your Query no. 3).

Query 2
Also the possible discussed physiopathological mechanisms are plausible but they are not convincing.

Reply 2
We wish to kindly bring to the Reviewer’s attention the circumstance that, since our initial assessment of this clinical case, we regarded two main possible mechanisms as supportive of the causative role played by indomethacin in triggering PVT: a) the known interference by some NSAIDs on endothelial arachidonic acid metabolism, with possible loss of production of antithrombotic prostanoid mediators at vascular level; b) the ability of most NSAIDs of injuring the lower digestive tract, with a consequent risk of infectious systemic attack by enteric bacteria. With regard for the latter mechanism, please note also the following points: 1) NSAID-induced enteropathy is a well known condition, which can be associated with an increase in: systemic inflammation markers (including C-reactive protein), mucosal permeability, protein loss, neutrophil activation, bacterial invasion of intestinal wall, and intestinal bleeding, all these alterations being usually proportional to the severity of the resulting enteric inflammation (see references n. 18-23 in the revised manuscript). The mechanism of NSAID-induced enteropathy was addressed in the Discussion section (see page page 8 lines 1-15). However, in order to facilitate understanding of the proposed mechanisms linking indomethacin to PVT, a diagram (Figure 1) has been introduced, as
suggested by Reviewer 2 (see query no. 4 by Reviewer 2; this diagram has been quoted in the text at page 7 lines 11 and page 8 lines 1 and 12).
2) The present patient showed a clinical picture consistent with a NSAID-induced enteropathy (increased neutrophil count and C-reactive protein, bloody stool) (see references n. 18-23 in the revised manuscript). These signs were already reported in the Case presentation section (see page 3, lines 3-5 and page 4, lines 3-6), and have been further commented in the Discussion of the Revised manuscript (see page 8, lines 20-26)

3) The correlation of enteropathy and inflammatory bowel diseases with thrombotic conditions (such as PVT) is known as well. It was already mentioned in the Introduction (see page 2, line 4), and it has been better addressed in the Discussion of the revised manuscript (see page page 8 lines 1-15; see also references no. 2, 3, 4, 25 in the revised manuscript).

4) We have been able of retrieving 4 unpublished cases of PVT in patients taking indomethacin, by exploring the FDA and user community through the eHealthMe searching system (eHealthMe – Real World Drug Outcomes. http://www.ehealthme.com/. Accessed on 02/08/2012) (see page 6, lines 15-18 and reference 11)

Query 3
For example, the Authors describe that the patient had a "moderate reductions of platelet count, activated partial thromboplastin time and antithrombin (III?)..." In my mind this description reflects a prothrombotic state (may be due to chronic liver disease in the presence of an acute inflammatory status as suggested by WBC count and CRP values). In which way they excluded it?

Reply 3
In the attempt of excluding the presence of a prothrombotic state, we have carefully reviewed the laboratory examinations recorded before the PVT onset, since the patient was chronically managed at our Hospital for his liver disease. According to our records, the most recent laboratory assessment before hospitalization was performed on March 2010 (2 months earlier his admission; 45 days before starting indomethacin; a new column has been added to table 1 to display the last available laboratory parameters before the patient admission). These laboratory data do not support the existence of a prothrombotic state in the two months preceding the abdominal symptoms that led to hospitalization (platelet count, 355 10^3/μl; activated partial thromboplastin time 29.8 sec; antithrombin III, 30.9%); likewise, an inflammatory state appeared unlikely (white blood cell count, 9.15 10^3/μl; neutrophils, 4.21 10^3/μl; but, unfortunately, C-reactive protein was not assessed). Although these data do not allow to exclude that a prothrombotic state developed during the 2 months ranging from the last available laboratory testing to the onset of PVT symptoms, they do support however a mild, if any, contribution of the underlying liver disease. Moreover, the circumstance that the patient recovered after indomethacin dechallenge and the start of adequate anticoagulant therapy should not be neglected. Finally the patient has not experienced any thrombotic event from his discharge up to now (about 2-year follow-up) (see page 4, lines 23-24). All the above considerations have been introduced into the
Case presentation (see page 3, lines 11-14) and commented in the Discussion (see page 5, line 1-15) of the revised manuscript. We have made also clear that ‘antithrombin III’ was actually assayed in the present case (see page 3, line 9).

**Query 4**
A short aPTT (as reported at the study entry) represents a possible risk factor for hypercoagulability with an increased incidence of thromboembolic events. Did the Authors exclude this possible condition?

**Reply 4**
As mentioned above, the aPTT value, recorded about 2 months before the PVT presentation, was within the normal range. However, we can not exclude an alteration of this parameter in close proximity of PVT onset (see the new column added to Table 1; see also our reply to query no. 3 of Reviewer 1). We wish also to note that, according to current literature, the slight alterations of coagulation parameters recorded at admission could be explained by their consumption in the acute phase of thrombosis as well as by the reduced hepatic blood flow induced by PVT. This possibility lends further support to the hypothesis of indomethacin as the triggering agent of PVT (see page 6, lines 4-8, and reference no. 10)

**Query 5**
At the same time short aPTT is a marker of an increase in thrombin generation. Did they assayed F1+2 levels?

**Reply 5**
Unfortunately, the clinicians did not assay the prothrombin fragment F1+2 and therefore we are unable to provide clear information about the status of thrombin generation in our patient at the time of the event. This point of weakness has been acknowledge in the text of the revised manuscript (see page 3, lines 9-11).

**Query 6**
The authors suggest a possible activation of extrinsic pathway however a reduction in aPTT should suggest a prevalent intrinsic pathway activation. In which way they can explain it?

**Reply 6**
Since the patient had a mild liver disease, without signs of decreased synthesis of coagulant/anticoagulant factors before the onset of PVT (see the Case presentation at page 3, lines 5-9, and Table 1), we argue that the leakage of bacterial endotoxins and other toxic molecules from the intestinal tissues into the portal circulation, due to indomethacin-enteropathy, stimulated the expression of adhesion molecules and tissue factor in the vascular endothelium, with consequent activation of the extrinsic pathway. These mechanisms have been addressed in the Discussion of the revised manuscript (see pages 4-8)
**Final comment**
From these points of view, the causal effect of indomethacin appears less evident and only as a casual role. The Authors should discuss these points.

**Reply to final comment**
We have given full consideration to all concerns and comments raised by the Reviewer, and accordingly we have done our best effort for better discussing and supporting the causal role of indomethacin in the present case of PVT.

**Report to Referee 2**

**Query 1**
The authors present a very interesting case of PVT. However the fail to discuss in depth all the potential implications regarding the pathogenesis of this condition. It is very important to mention the weight of the patient as obesity is a major risk factor of fatal and non-fatal thrombotic events including pulmonary embolism (obesity-related vs non-obesity related).

It is recommended for the authors to consult the following papers:


Therefore, the authors should state clearly if their patient was obese, overweight or of normal weight.

**Reply 1**
Please note that the present patient had a normal weight (BMI: 25). This information has been introduced into the Case presentation section in the revised manuscript (see page 2, line 24). In addition, the role of obesity as a risk factor for thrombotic events has been now mentioned in the Introduction of the revised manuscript (see page 2, line 10). The suggested references have been quoted in the Introduction of the revised manuscript (see page 2, line 11, references 4-6)
Query 2
Also, it is not mentioned if the patient has been investigated for a possible cancer. It is known that various thrombotic events may be promoted by occult cancers.

Reply 2
On day 5 from admission, the patient underwent a CT-scan of both chest and abdomen, which did not show signs of cancer or suspected lesions in any district. This information has been introduced into the Case presentation section of the revised manuscript (see page 4, lines 6-7). Moreover, since the patient is chronically managed at our Hospital for his liver disease, we can confirm the absence of any sign of cancer both before his admission for PVT and after his discharge. Finally, it is noteworthy that he recovered promptly after indomethacin discontinuation and the start of adequate anticoagulant therapy, and that he has not experienced any further thrombotic event throughout the post-discharge follow-up (see page 4, lines 23-24).

Query 3
The authors should also mention if the patient was a smoker or not, the alcohol intake and if he had diabetes mellitus.

Reply 3
The present patient did not present any other risk factor such as smoking, alcohol intake and diabetes mellitus. These information have been introduced into the Case presentation section of the revised manuscript (see page 2, lines 24-25).

Query 4 - Minor Essential Revisions
Please provide a diagram reiterating the possible mechanism for the pro-thrombotic action of indomethacin.

Reply 4
As requested, a diagram (Figure 1) has been introduced into the revised manuscript. This diagram has been quoted in the text at page 7 lines 11 and page 8 lines 1 and 12.

Query 5
Also, the authors may wish to include the following papers in their presentation:

Reply 5
We have performed an evaluation of the papers suggested by the reviewer #2, and decided to quote the three most appropriate ones (Biere-Rafi et al., 2011 quoted as reference 13 at page 7, line 4; Rebordosa et al., 2010, quoted as reference 14 at page 7 line 8; and Eizayaga et al., 2006, quoted as reference 16 at page 7, line 24) in the text and to introduce them into the reference list. The study by Brune et al., (2009) has not been quoted in an attempt of avoiding excessive redundancy of information concerning the detrimental actions of indomethacin and NSAIDs on both the gastrointestinal tract and vascular system.

Query 9 - Discretionary Revisions
If possible US, CT and endoscopy images should be included in the paper

Reply 9 We decided of not including the images of US, CT and endoscopy because the case report would become too extensive.