Author's response to reviews

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

Authors:

Stefania Mantarro (stefaniamantarro@gmail.com)
Marco Tuccori (marco.tuccori@gmail.com)
Giuseppe Pasqualetti (giuseppe.pasqualetti@gmail.com)
Sara Tognini (pharmacovigilace.pisa@gmail.com)
Sabrina Montagnani (sbn.mt@tiscal.it)
Fabio Monzani (fabio.monzani@med.unipi.it)
Corrado Blandizzi (c.blandizzi@gmail.com)

Version: 3 Date: 10 October 2012

Author's response to reviews: see over
Dear Sir,

Please find, herewith enclosed, the revision version of the manuscript entitled “Acute portal vein thrombosis precipitated by indomethacin in a HCV-positive elderly patient” by Mantarro S. et al., for possible publication in *BMC Geriatrics* as a case report.

We have made our best efforts to provide a complete reply for each query reported by the two reviewers. On the basis of the referees requests, we introduced some changes in the manuscript which are highlighted in red in the Revised Manuscript - revision highlighted - enclosed as supplementary file. The changes are listed below:

1. **Query 1** by referee 1. In order to better substantiate our contention that indomethacin acted as the precipitating factor of PVT in the context of a patient with a complex clinical picture, we have made further efforts of reviewing with more detail the patient 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 11-14), and the Discussion has been integrated (see page 5, lines 3-16).

2. **Query 2** by referee 1. We better explained the 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, it is worth noting the following points: a) 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 8 lines 2-15). b) 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 20-24 and page 9, lines 1-2). c) 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 8 lines 2-15; see also references no. 2, 3, 24, 25 in the revised manuscript). d) 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 16-19 and reference 11)

3. Query 3 by referee 1. 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 22-23). 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).
4. **Query 4 by referee 1.** We discussed the role of a short aPTT as a possible risk factor for hypercoagulability. As mentioned above, the aPTT value, recorded about 2 months before the PVT presentation, was within the normal range. However, we cannot 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 Referee 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 5-9, and reference no. 10).

5. **Query 5 by referee 1.** As a short aPTT is a marker of an increase in thrombin generation, the referee 1 requested if the prothrombin fragment F1+2 levels were assayed. We reported that, 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).

6. **Query 6 by referee 1.** We explained the mechanism of a possible activation of extrinsic pathway of blood coagulation. 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 section at page 2, lines 21-23; page 3, lines 11-14; 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 4-9).

7. **Query 1 and 3 by referee 2.** We introduced the obesity among risk factors of portal vein thrombosis (PVT) in the Introduction of the revised manuscript (see page 2, line 10) and reported into the Case presentation section (see page 2, lines 23-24) that the patient had a normal weight (BMI: 25), and did not present any other risk factors for thrombosis, such as smoking, alcohol intake or diabetes mellitus. The references suggested by referee 2 have been quoted in the Introduction of the revised manuscript (see page 2, line 11, references 4-6).

8. **Query 2 by referee 2.** Since thrombotic events may be promoted by occult cancers, we specified that the findings of a CT-scan of both chest and abdomen, performed on day 5, 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 22-23).

9. **Query 4 by referee 2.** In order to facilitate understanding of the proposed mechanisms linking indomethacin to PVT, a diagram (Figure 1) has been introduced. This diagram has been quoted in the text at page 7 line 12 and page 8 lines 2 and 13.

10. **Query 5 by referee 2.** We have performed an evaluation of the papers suggested by the referee 2, and decided to quote the three most appropriate ones (Biere-Rafi et al., 2011 quoted as reference 13 at page 7, line 5; Rebordosa et al., 2010, quoted as reference 14 at page 7 line 9; 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.

11. **Query 6 by referee 2.** We decided of not including the images of ultrasonography, CT and endoscopy, as requested by referee 2, because we would prefer to avoid the attachment of these pictures to limit the length of the manuscript. Nevertheless, if the Editor will decide to enclose them, we are ready to provide them at any time.

If I can be of any assistance, please do not hesitate to contact me at the address reported below.

Thanking you for your time and kind attention, I look forward to your reply, and I remain,

Sincerely yours,

Dr. Marco Tuccori, PharmD

Tuscan Regional Centre for Pharmacovigilance
Unit of Pharmacology
University Hospital of Pisa
Via Roma 55, 56126, Pisa, Italy
Phone: +39 050 2218641; Mobile: +39 346 1699937
Fax: +39 050 2218758; e-mail: marco.tuccori@gmail.com