Author's response to reviews

Title: Aplastic anaemia associated to interferon alpha 2 alpha in a patient with chronic hepatitis C virus infection: a case report

Authors:

Savvas Ioannou (savvasdoc@hotmail.com)
Gregorios Hatzis (grhatzis@med.uoa.gr)
Ioanna Vlahadami (ioannamd@yahoo.gr)
Michael Voulgarelis (mvoulgar@med.uoa.gr)

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

We would like to thank you for your thoughtful comments. Our manuscript has been revised according to the editorial requests and the reviewers’ comments. Please find below a point-by-point response to the concerns raised by the reviewers.

Editorial requests:

In order to protect the patient's identity, please remove the dates of treatment from the case presentation.

OUR REPLY: The dates of treatment have been removed. The first paragraph of the case presentation has been changed accordingly to: “A Greek 46-year-old man was diagnosed with HCV infection (genotype 4h) and a combination treatment of PEG-IFN-α 2a (180 µg, weekly) and ribavirin (1200 mg/day) was commenced for a forty-eight-week period. The treatment was well tolerated with a normalization of liver function tests. Four months later he was referred to our hospital with a bleeding tendency and unexplained fatigue of recent onset.” Additionally the first sentence of the last paragraph of the case presentation has been changed accordingly to: “Eight months after the first course of immunosuppressive treatment, the Hb was 10.6 g/dl, platelets 32000/mm³ and WBC 3590/mm³ with an absolute neutrophil count of 2261/mm³.”
Please include a conclusion section as the last section of the text. This should state what can be learnt from the case.

OUR REPLY: A conclusion section has been included in the revised manuscript: “We presented a case of a 46-year-old man who developed very severe AA whilst being treated with PEG-IFN-α 2a for chronic HCV infection. To the best of our knowledge, this is the first report of this complication associated to PEG-IFN-α 2a in the growing body of literature. As health care providers, physicians should be aware of this rare but life-threatening complication of PEG-IFN-α 2a.”

Reviewer: Dariusz Lebensztejn

Minor Essential Revisions:

There are not short conclusions in the end of manuscript. Conclusion is only included in abstract.

OUR REPLY: A conclusion has been added at the end of the text: “We presented a case of a 46-year-old man who developed very severe AA whilst being treated with PEG-IFN-α 2a for chronic HCV infection. To the best of our knowledge, this is the first report of this complication associated to PEG-IFN-α 2a in the growing body of literature. As health care providers, physicians should be aware of this rare but life-threatening complication of PEG-IFN-α 2a.”

In the title and in the abstract is: interferon alpha-2#; should be: interferon alpha

OUR REPLY: The abstract as well as the title of the text have been changed accordingly to: “As a syndrome of bone marrow failure, hepatitis-associated aplastic anaemia is not uncommon. However, hepatitis-associated aplastic anaemia which is an immune-
mediated disease does not appear to be caused by any of the known hepatitis viruses including hepatitis C virus. In addition no reported case of development of aplastic anaemia associated with pegylated interferon alpha 2a treatment of patients with chronic hepatitis C virus infection has been documented. We report the case of a Greek 46-year-old man who developed very severe aplastic anaemia whilst being treated with pegylated interferon alpha 2a for chronic hepatitis C virus infection. He presented with generalized purpura and bruising, and pallor of the skin and mucous membranes, whilst his blood tests showed pancytopenia. He finally underwent syngeneic allogeneic bone marrow transplantation upon completion of two courses of immunosuppressive therapy with antithymocyte globulin and cyclosporin A. The combination of a specific environmental precipitant represented by the hepatitis C virus infection, an altered metabolic detoxification pathway due to pegylated interferon alpha 2a and a facilitating genetic background such as polymorphism in metabolic detoxification pathways and specific human leukocyte antigen genes, conspired possibly synergistically in the development of aplastic anaemia in this patient. A review of the literature and underlying pathophysiologic mechanisms are discussed. Our case shows clearly that the causative role of pegylated interferon alpha 2a in aplastic anaemia development must not be ignored.”

TITLE: “Aplastic anaemia associated to interferon alpha 2a in a patient with chronic hepatitis C virus infection: a case report.”

Reviewer: Domingo Balderramo

Specific Comments:
Title: should include “... associated to pegylated interferon ...”

OUR REPLY: The title of the text has been changed accordingly to: “Aplastic anemia associated to pegylated interferon alpha 2a in a patient with chronic hepatitis C virus infection: a case report.”

Introduction:

- hepatitis-associated aplastic anaemia may be induced by hepatitis B virus and hepatitis C virus (HCV), and also by other viruses, such as human immunodeficiency virus, Epstein-Barr virus, transfusion-transmitted virus and echovirus (Gonzalez-Casas R et al. Anemia in chronic liver disease, World J Gastroenterol 2009 October 7; 15(37): 4653-4658). The sentence that mentions no association of HCV with HAA should be change.

OUR REPLY: This sentence in the text has been changed accordingly to: “Hepatitis-associated aplastic anaemia (HAA) may be induced by HCV and hepatitis B virus (HBV), and also by other viruses such as human immunodeficiency virus, Epstein-Barr virus, transfusion-transmitted virus and echovirus [2]”. Additionally a new reference (reference 2) has been added: “Gonzalez-Casas R, Jones EA, Moreno-Otero R. Spectrum of anemia associated with chronic liver disease. World J Gastroenterol 2009; 15:4653-4658.”

Case Presentation

- Was the patient in contact with benzene or pesticides?

OUR REPLY: The first paragraph of the case presentation has been changed accordingly to: “A Greek 46-year-old man was diagnosed with HCV infection (genotype 4h) and a combination treatment of PEG-IFN-α 2a (180 µg, weekly) and ribavirin (1200
mg/day) was commenced for a forty-eight-week period. His blood tests previous to combination treatment were normal with platelets 250000/mm$^3$, haemoglobin (Hb) 16.3 g/dl, and a white blood cell (WBC) count of 6300/mm$^3$. The treatment was well tolerated with a normalization of liver function tests. Four months later he was referred to our hospital with a bleeding tendency and unexplained fatigue of recent onset. No contact with benzene or pesticides was mentioned.”

- “Thyroid function and all biochemistry…” What is included in “all biochemistry”? A more clear description (renal function, sodium, calcium, etc) of the test performed should be state.

OUR REPLY: A more clear description of the tests performed is stated in the revised manuscript: “Further investigations showed the following: urea 20 mg/dl (normal range 17 – 50 mg/dl), creatinine 1.0 (normal range 0.7 – 1.4 mg/dl), sodium 139 mMol/L (normal range 136 – 145 mMol/L), potassium 3.8 mMol/L (normal range 3.5 – 5.0 mMol/L), glucose 99 mg/dl (normal range 74 – 115 mg/dl), calcium 8.8 mg/dl (normal range 8.6 – 10.2 mg/dl), amylase 48 U/L (normal range 20 – 104 U/L), creatine phosphokinase 200 U/L (normal range 20 – 190 U/L), lactate dehydrogenase 296 U/L (normal range 200 – 460 U/L), uric acid 4.6 mg/dl (normal range 3.5 – 7.2 mg/dl), erythrocyte sedimentation rate 34 mm in the first hour (normal range 0 – 20 mm), and C-reactive protein 37.4 mg/L (normal range 0 – 5 mg/L). Screening for several autoantibodies was negative. Thyroid function tests and complement serum levels were normal.”
- Values of platelets, haemoglobin, and WBC previous to PEG-INF treatment should be included. Liver biopsy and/or abdominal ultrasound pre-treatment should be mention if they were available.

**OUR REPLY:** Liver biopsy and abdominal ultrasound pre-treatment are not mentioned as they weren’t available. Pre-treatment blood values have been added in the first paragraph of the case presentation: “His blood tests previous to combination treatment were normal with platelets 250000/mm$^3$, haemoglobin (Hb) 16.3 g/dl, and a white blood cell (WBC) count of 6300/mm$^3$.”

- *Haematologic values previous to BMT are relatively normal except platelet count. Was the patient receiving blood or platelet transfusion at this moment? Was this the final indication of BMT?*

**OUR REPLY:** At that time patient continued to be blood and platelet transfusion dependent and sometimes he was receiving G-CSF as well. After two courses of immunosuppressive treatment failure, BMT was indicated as our last treatment option. Therefore the last paragraph of the case presentation has been changed accordingly to: “Eight months after the first course of immunosuppressive treatment, the Hb was 10.6 g/dl, platelets 32000/mm$^3$ and WBC 3590/mm$^3$ with an absolute neutrophil count of 2261/mm$^3$. At that time patient was still receiving blood and platelet transfusion.”

- *Since AA is a probable complication of PEG-INF therapy, evolution after BMT should be mention.*

**OUR REPLY:** The patient died of a hemorrhagic stroke during the recovery phase and therefore no evolution after BMT can be mentioned. A sentence has been added in the
last paragraph of the revised manuscript: “He suffered a haemorrhagic stroke due to prolonged thrombocytopenia and died during the recovery phase.”

- Antibiotic, antiviral (ganciclovir), and antifungal agent were administered during the period of aplasia? This should be mention as part of general management of the patient.

OUR REPLY: A new sentence has been added in the case presentation regarding the general management of the patient: “During the period of aplasia patient was persistently pyrexial and broad spectrum antibiotics in the forms of antipseudomonal penicillin (piperacillin/tazobactam) and carbapenem (meropenem) were administered consecutively, as well as antifungal agent (liposomal amphotericin B).”

Discussion

- The case presentation is an AA related temporally with PEG-ING therapy in a patient with chronic HCV infection. Description of HAA should not be mention (page 7) (from “As a syndrome..” to “… HAA are immuno-mediated [8]”.

OUR REPLY: The description of HAA has been added to introduction: “As a syndrome of bone marrow failure, hepatitis-associated aplastic anaemia (HAA) is not rare, with hepatitis documented in 2 to 5% of AA cases occurring in the West and 4 to 10% in the Far East. Characteristically, the HAA syndrome is more prevalent amongst young males, the hepatitis generally follows a benign course, but the onset of AA 2 to 3 months later can be explosive and is usually fatal if untreated. HAA may be induced by HCV and hepatitis B virus (HBV), and also by other viruses such as human immunodeficiency virus, Epstein-Barr virus, transfusion-transmitted virus and echovirus [2]. Most cases though are seronegative for the known hepatitis viruses, including hepatitis A, B, C, and G (GB virus C) [3]. The clinical features and moreover the response to
immunosuppressive treatment strongly indicate that the liver and marrow abnormalities in HAA are immune-mediated [4, 5].”

Additionally a new reference (reference 4) has been added as per your requirement:


- Characteristics of the anemia related to ribavirin should be mention in brief.

OUR REPLY: Characteristics of the anemia related to ribavirin have been added in the introduction section: “The primary observed serious adverse side-effect of ribavirin is haemolytic anaemia. Ribavirin is an antiviral nucleoside analogue; the mechanism of ribavirin-induced haemolytic anaemia has not been clearly established. Anaemia is likely related to extensive ribavirin accumulation in erythrocytes subsequent to active unidirectional transmembrane transport. Ribavirin exerts its toxicity through an inhibition of intracellular energy metabolism and oxidative membrane damage, leading to an accelerated extravascular haemolysis by the reticulo-endothelial system [8]. Lau et al explain that ribavirin, following uptake into cells, is phosphorylated and converts to ribavirin triphosphates, which then must be dephosphorylated for elimination from cells [9]. However, because red blood cells lack dephosphorylation enzymes, ribavirin accumulates in cells and destroys the cells, causing haemolytic anaemia. Severe anaemia develops in about 10% of treated patients, and requires close monitoring of haemoglobin and often ribavirin dose reduction, which may compromise sustained virologic response.”

Two new references (references 8, 9) have been added regarding the anaemia related to ribavirin: “Russmann S, Grattagliano I, Portincasa P, Palmieri VO, Palasciano G.

The authors mention an alteration of drug metabolic detoxification pathways associated to PEG-INF. As the patients did not receive other medications after starting PEG-INF therapy, alteration of detoxification pathways is not really important. PEG-INF by itself may be the cause of an immune-mediated damage of haematopoietic cells (drug-induced AA).

**OUR REPLY:** We believe that the development of AA in our case was the result of several facts which conspired synergistically, such as the specific environmental precipitant represented by the HCV infection, an aberrant expression of cellular proteins in the marrow cells caused by a disturbed PEG-IFN-α 2a-associated drug metabolic detoxification pathway as well as specific HLA genes. It is well known that interferon causes multiple adverse effects, with flu-like symptoms being the most disturbing at first. Such symptoms can be ameliorated by several medications, such as paracetamol, non-steroidal anti-inflammatory drugs or even steroids. In our opinion, taking into consideration that our patient may have received such medications after starting PEG-IFN-α 2a, it is important to mention the alteration of metabolic detoxification pathways.

**References:**

- The article of Gonzalez Casas et al. Alimnt Pharmacol Ther 2009; 30: 436 should be included in the references.
OUR REPLY: This reference has been added in the revised manuscript (reference 4) as mentioned above (description of HAA).