**Name of Journal:** *World Journal of Clinical Cases*

**Manuscript NO:** 66378

**Manuscript Type:** CASE REPORT

**False positive anti-hepatitis A virus immunoglobulin M in autoimmune hepatitis/primary biliary cholangitis overlap syndrome: A case report**

Yan J *et al*. False-positive of anti-IgM-HAV in AIH-PBC

Jun Yan, Yan-Sha He, Yi Song, Xin-Yu Chen, Hua-Bao Liu, Chun-Yan Rao

**Jun Yan, Yan-Sha He, Yi Song, Xin-Yu Chen, Hua-Bao Liu, Chun-Yan Rao,** Department of Hepatology, Chongqing Hospital of Traditional Chinese Medicine, Chongqing 400021, China

**Author contributions:** Yan J, Rao CY contributed to concept and design of the manuscript; Rao CY, He YS and Song Y reviewed the literature and drafted the manuscript; Chen XY and Liu HB were responsible for the revision of the manuscript; all authors issued the final approval for the version to be submitted.

**Supported by** Natural Science Foundation of Chongqing, China, No. cstc2020jcyj-msxmX0630; and Traditional Chinese Medicine United Foundation of Health Commission and Science & Technology Bureau of Chongqing, China, No. 2019ZY3202.

**Corresponding author: Chun-Yan Rao, MS, Doctor,** Department of Hepatology, Chongqing Hospital of Traditional Chinese Medicine, No. 6 Seventh Branch Road in Panxi, Jiangbei District, Chongqing 400021, China. chunyanrao@126.com

**Received:** April 10, 2021

**Revised:** April 30, 2021

**Accepted:** May 24, 2021

**Published online:**

**Abstract**

BACKGROUND

Autoimmune hepatitis (AIH) is an immune-mediated liver disease affecting all age groups. Associations between hepatitis A virus (HAV) and AIH have been described for many years. Herein, we report a case of an AIH/primary biliary cholangitis (PBC) overlap syndrome with anti-HAV immunoglobulin M (IgM) false positivity.

CASE SUMMARY

A 55-year-old man was admitted with manifestations of anorexia and jaundice along with weakness. He had marked transaminitis and hyperbilirubinemia. Viral serology was positive for HAV IgM and negative for others. Autoantibody screening was positive for anti-mitochondria antibody but negative for others. Abdominal ultrasound imaging was normal. He was diagnosed with acute hepatitis A. After symptomatic treatment, liver function tests gradually recovered. Several months later, his anti-HAV IgM positivity persisted and transaminase and bilirubin levels were also more than 10 times above of the upper limit of normal. Liver histology was prominent, and HAV RNA was negative. Therefore, AIH/primary biliary cholangitis (PBC) overlap syndrome diagnosis was made based on the “Paris Criteria”. The patient was successfully treated by immunosuppression.

CONCLUSION

This case highlights that autoimmune diseases or chronic or acute infections, may cause a false-positive anti-HAV IgM result because of cross-reacting antibodies. Therefore, the detection of IgM should not be the only method for the diagnosis of acute HAV infection. HAV nucleic acid amplification tests should be employed to confirm the diagnosis.

**Key Words:** Autoimmune hepatitis; Primary biliary cholangitis; Hepatitis A virus; Case report

Yan J, He YS, Song Y, Chen XY, Liu HB, Rao CY. False positive anti-hepatitis A virus immunoglobulin M in autoimmune hepatitis/primary biliary cholangitis overlap syndrome: A case report. *World J Clin Cases* 2021; In press

**Core Tip:** Autoimmune hepatitis (AIH)/primary biliary cholangitis (PBC) overlap syndrome is the specific clinical manifestation of AIH, which is an immune-mediated liver disease. Environmental factors including viral infections have been documented to externally trigger AIH. The association between hepatitis A virus (HAV) and AIH has been described for many years. But relying solely on anti- HAV immunoglobulin M (IgM) to diagnose acute HAV infection is not adequate. This case highlights that false-positive anti-HAV IgM might be caused by the cross-reaction of antibodies in individuals with autoimmune diseases or chronic or acute infections. HAV nucleic acid amplification can be used more broadly during the diagnosis workup to confirm HAV infection, especially in patients testing positive for anti-HAV IgM with a low cutoff value.

**INTRODUCTION**

The pathogenesis of autoimmune hepatitis (AIH) requires the interaction of epigenetic, environmental, and immunologic factors[1]. The shape of the immune repertoire plays an important role in the program of AIH. Environmental exposures, such as viral infections, are considered a potential trigger for AIH[2]. Some reported cases indicate that hepatitis A virus (HAV) infection is among the triggers of AIH[3-6]. However, if the diagnosis of acute HAV infection is solely based on Anti-HAV immunoglobulin (Ig)M, then it may be suspect. We describe herein a case of AIH/primary biliary cholangitis (PBC) overlap syndrome with anti-HAV IgM false positivity.

**CASE PRESENTATION**

***Chief complaints***

A 55-year-old man presented to the hepatology clinic of our hospital complaining of manifestations of anorexia and jaundice along with weakness.

***History of present illness***

The patient’s symptoms started 10 d previously with manifestations of anorexia, jaundice, and weakness, and had worsened over the last 2 d.

***History of past illness***

The patient had no past medical history.

***Personal and family history***

The patient did not abuse alcohol or substances. There was no family history of liver disease.

***Physical examination***

The clinical examination revealed that the skin and sclera were jaundiced.

***Laboratory examinations***

Blood samples revealed (Figure 1) alanine aminotransferase (ALT) 893 U/L, serum aspartate aminotransferase (AST) 831 U/L, γ-glutamyl-transpeptidase (γ-GGT) 423 U/L, alkaline phosphatase (ALP) 150 U/L, and total bilirubin 342 μmol/L. Thyroid-stimulating hormone, blood count, triiodothyronine, prothrombin time, and thyroxine were all normal. Serum anti-HAV IgM (1.93) was positive. Other viral serology viral tests were negative. Antibody screening found positive anti-mitochondria antibody (AMA) but negative anti-smooth muscle antibody (ASMA) and anti-liver kidney microsome type 1 (LKM 1). IgA, IgM and IgG levels were normal.

***Imaging examinations***

Abdominal ultrasound imaging was normal.

***Diagnosis procedure***

Based on the patient's medical history and evaluation, he was diagnosed with acute hepatitis A (Hep A); PBC could not be excluded. After symptomatic treatment, we discharged the patient on the 20th day of hospitalization with AST 36 U/L, γ-GGT 323 U/L, total bilirubin 62 μmol/L, ALT 50 U/L, and normal ALP and prothrombin time. The patient continued to take ursodeoxycholic acid after discharge.

Two months after discharge, his aminotransferase levels began to increase. In the subsequent months, repeated blood examinations revealed ALT 927 U/L, AST 864 U/L, γ-GGT 476 U/L, ALP 133 U/L, and total bilirubin 185 μmol/L. The patient’s serum anti-HAV IgM (3.09) remained positive. Antinuclear antibody (1:100) and AMA were positive while ASMA and anti-LKM were negative. Liver histology showed interface hepatitis accompanied by plasma cell infiltration. Moreover, we observed florid bile duct damage with lymphocytic cholecystitis as shown in Figure 2. Histologic lesions were graded G4S2-3 as per the modified Scheuer score. We also assayed HAV RNA, which was negative.

**FINAL DIAGNOSIS**

On the basis of the “Paris Criteria”, AIH/PBC overlap syndrome diagnosis was made, but not acute Hep A.

**TREATMENT**

Treatment with prednisone (30 mg/d) along with ursodeoxycholic acid (15 mg/kg/d), in combination with azathioprine (50 mg/d) after 2 wk was prescribed.

**OUTCOME AND FOLLOW-UP**

Liver function tests normalized within 50 d. On the 6th month of follow-up, anti-HAV IgM became negative.

**DISCUSSION**

HAV infection is commonly self-limiting, with patients completely recovering after about 3 mo. The diagnosis of acute hepatitis A primarily involves serological testing of anti-HAV IgM, which is highly specific and sensitive without testing for the pathogen itself. However, other factors can result in anti-HAV IgM seropositivity in the clinical evaluation[7], potentially leading to an incorrect diagnosis of acute hepatitis A. A Hep A false-positive result might also be caused by the cross-reaction of antibodies in individuals with autoimmune diseases or chronic or acute infections. Polyclonal activation of B lymphocytes can trigger the generation of anti-HAV IgM seropositivity[8]. Therefore, in our case, AIH/PBC overlap syndrome was an immune-triggered inflammatory liver disease that may have caused a false-positive anti-HAV IgM result because of cross-reacting antibodies. There is a report of a false positive hepatitis A serology result in a patient with an acute Epstein-Barr virus infection[9].

In our patient, the anti-HAV IgM result is closely related to the duration from the peak-ALT value to the day of testing[10]. Besides, true positive anti-HAV IgM tests have values that are often 9 to 10 times above the acute HAV cutoff, yet less than four times the cutoff is considered as a false positive result[11]. Our patient had persistent anti-HAV IgM positivity that had a low index of two or three times the cutoff when the ALT values peaked. Therefore, we had to consider that it was a false positive.

An HAV nucleic acid amplification test (NAAT) would be an ideal method to confirm positive results when a low anti-HAV IgM level is obtained. Although the time since the manifestation of clinical symptoms can affect the HAV assay result, previous NAAT experience documents that the viremic phase duration is frequently prolonged by more than 2 mo after the initial symptoms of infection[12], and that there is a positive association between HAV RNA and ALT[13]. Unfortunately, there was also no HAV RNA assay to confirm HAV infection in our patient even though ALT had been elevated to 20 times the upper limit of normal. A Hep A diagnosis can be ruled out in the absence of detectable HAV RNA in the serum[14].

Although viral infections can serve as environmental triggers resulting in the loss of self-tolerance to autoantigens in individuals genetically predisposed to AIH[2], and numerous case reports have documented a strong relationship of HAV with the onset of AIH. Anti-HAV IgM testing has proven valuable in the diagnosis of acute HAV infection. However, anti-HAV IgM false positives can result in misdiagnosis and inappropriate treatment, therefore the detection of IgM should not be the only method for the diagnosis of acute HAV infection. Other methods, such as NAATs should be employed to confirm the diagnosis, particularly in patients who test positive for anti-HAV IgM with a low cutoff value.

**CONCLUSION**

False anti-HAV IgM serological results can lead to misdiagnosis or premature termination of diagnostic tests. Relying solely on anti-HAV IgM to diagnose acute HAV infection is not sufficient. HAV nucleic acid tests can be used more broadly, especially in patients who test positive for anti-HAV IgM with a low cutoff value.

**REFERENCES**

1 **Mack CL**, Adams D, Assis DN, Kerkar N, Manns MP, Mayo MJ, Vierling JM, Alsawas M, Murad MH, Czaja AJ. Diagnosis and Management of Autoimmune Hepatitis in Adults and Children: 2019 Practice Guidance and Guidelines From the American Association for the Study of Liver Diseases. *Hepatology* 2020; **72**: 671-722 [PMID: 31863477 DOI: 10.1002/hep.31065]

2 **Taubert R**, Diestelhorst J, Junge N, Kirstein MM, Pischke S, Vogel A, Bantel H, Baumann U, Manns MP, Wedemeyer H, Jaeckel E. Increased seroprevalence of HAV and parvovirus B19 in children and of HEV in adults at diagnosis of autoimmune hepatitis. *Sci Rep* 2018; **8**: 17452 [PMID: 30487523 DOI: 10.1038/s41598-018-35882-7]

3 **Kim YD**, Kim KA, Rou WS, Lee JS, Song TJ, Bae WK, Kim NH. [A case of autoimmune hepatitis following acute hepatitis A]. *Korean J Gastroenterol* 2011; **57**: 315-318 [PMID: 21623141 DOI: 10.4166/kjg.2011.57.5.315]

4 **Tabak F**, Ozdemir F, Tabak O, Erer B, Tahan V, Ozaras R. Autoimmune hepatitis induced by the prolonged hepatitis A virus infection. *Ann Hepatol* 2008; **7**: 177-179 [PMID: 18626439 DOI: 10.1016/S1665-2681(19)31878-2]

5 **Singh G**, Palaniappan S, Rotimi O, Hamlin PJ. Autoimmune hepatitis triggered by hepatitis A. *Gut* 2007; **56**: 304 [PMID: 17303607 DOI: 10.1136/gut.2006.111864]

6 **Vento S**, Garofano T, Di Perri G, Dolci L, Concia E, Bassetti D. Identification of hepatitis A virus as a trigger for autoimmune chronic hepatitis type 1 in susceptible individuals. *Lancet* 1991; **337**: 1183-1187 [PMID: 1673738 DOI: 10.1016/0140-6736(91)92858-Y]

7 **Alatoom A**, Ansari MQ, Cuthbert J. Multiple factors contribute to positive results for hepatitis A virus immunoglobulin M antibody. *Arch Pathol Lab Med* 2013; **137**: 90-95 [PMID: 23276180 DOI: 10.5858/arpa.2011-0693-OA]

8 **Desbois D**, Grangeot-Keros L, Roquebert B, Roque-Afonso AM, Mackiewicz V, Poveda JD, Dussaix E. Usefulness of specific IgG avidity for diagnosis of hepatitis A infection. *Gastroenterol Clin Biol* 2005; **29**: 573-576 [PMID: 15980754 DOI: 10.1016/S0399-8320(05)82132-3]

9 **Valota M**, Thienemann F, Misselwitz B. False-positive serologies for acute hepatitis A and autoimmune hepatitis in a patient with acute Epstein-Barr virus infection. *BMJ Case Rep* 2019; **12** [PMID: 31079040 DOI: 10.1136/bcr-2018-228356]

10 **Hyun JJ**, Seo YS, An H, Yim SY, Seo MH, Kim HS, Kim CH, Kim JH, Keum B, Kim YS, Yim HJ, Lee HS, Um SH, Kim CD, Ryu HS. Optimal time for repeating the IgM anti-hepatitis A virus antibody test in acute hepatitis A patients with a negative initial test. *Korean J Hepatol* 2012; **18**: 56-62 [PMID: 22511904 DOI: 10.3350/kjhep.2012.18.1.56]

11 **Landry ML**. Immunoglobulin M for Acute Infection: True or False? *Clin Vaccine Immunol* 2016; **23**: 540-545 [PMID: 27193039 DOI: 10.1128/CVI.00211-16]

12 **Fujiwara K**, Yokosuka O, Ehata T, Imazeki F, Saisho H, Miki M, Omata M. Frequent detection of hepatitis A viral RNA in serum during the early convalescent phase of acute hepatitis A. *Hepatology* 1997; **26**: 1634-1639 [PMID: 9398009 DOI: 10.1053/jhep.1997.v26.pm0009398009]

13 **Lee MJ**, Sayers AE, Drake TM, Singh P, Bradburn M, Wilson TR, Murugananthan A, Walsh CJ, Fearnhead NS; NASBO Steering Group and NASBO Collaborators. Malnutrition, nutritional interventions and clinical outcomes of patients with acute small bowel obstruction: results from a national, multicentre, prospective audit. *BMJ Open* 2019; **9**: e029235 [PMID: 31352419 DOI: 10.1136/bmjopen-2019-029235]

14 **Tram J**, Le Baccon-Sollier P, Bolloré K, Ducos J, Mondain AM, Pastor P, Pageaux GP, Makinson A, de Perre PV, Tuaillon E. RNA testing for the diagnosis of acute hepatitis A during the 2017 outbreak in France. *J Viral Hepat* 2020; **27**: 540-543 [PMID: 31895489 DOI: 10.1111/jvh.13255]

**Footnotes**

**Informed consent statement:** Informed written consent was obtained from the patient for publication of this report and any accompanying images.

**Conflict-of-interest statement:** The authors declare that they have no competing interests.

**CARE Checklist (2016) statement:** The authors have read the CARE Checklist (2016), and the manuscript was prepared and revised according to the CARE Checklist (2016).

**Open-Access:** This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/Licenses/by-nc/4.0/

**Manuscript source:** Unsolicited manuscript

**Peer-review started:** April 10, 2021

**First decision:** April 23, 2021

**Article in press:**

**Specialty type:** Gastroenterology and hepatology

**Country/Territory of origin:** China

**Peer-review report’s scientific quality classification**

Grade A (Excellent): A

Grade B (Very good): B

Grade C (Good): 0

Grade D (Fair): 0

Grade E (Poor): 0

**P-Reviewer:** Hsu YC, Ogundipe OA **S-Editor:** Yan JP **L-Editor:** Filipodia **P-Editor:**

**Figure Legends**



**Figure 1 Time course of liver function tests and anti-hepatitis A virus immunoglobulin M, autoantibodies, immunoglobulin levels.** Total bilirubin: Upper limit of normal (ULN) < 28 μmol/L, aspartate aminotransferase ULN < 40 IU/L, alanine aminotransferase ULN < 45 IU/L, γ-glutamyl transferase ULN < 50 IU/L, alkaline phosphatase ULN < 105 IU/L. ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; ANA: Antinuclear antibody; ASMA: Anti-smooth muscle antibodies; AST: Aspartate aminotransferase; GGT: γ-glutamyl transferase; HAV: Hepatitis A virus; IgG: Immunoglobulin G; IgM: Immunoglobulin M; LKM 1: Anti-liver kidney microsome type 1.



**Figure 2 Hematoxylin-eosin staining.** A: Liver biopsy tissue shows chronic hepatitis with moderate interface hepatitis accompanied by plasma cell infiltration (hematoxylin-eosin staining; magnification: × 200); B: Image of the box shown in A at 2 × magnification. Showing plasma cell infiltration (white arrow) and bile duct lesions (black arrow).