

Case Report

Youwen Tan*, Li Chen

Sustained false-positive results for hepatitis A virus immunoglobulin M: A case report and literature review

<https://doi.org/10.1515/med-2021-0336>
received May 30, 2021; accepted July 22, 2021

Abstract: Hepatitis A virus immunoglobulin M (HAV-IgM) is often used to diagnose acute hepatitis A virus (HAV) infection serologically. However, false-positive test results can interfere with the diagnosis. A 56-year-old woman was readmitted to the hospital owing to abnormal liver function tests for the last 18 months. She had been diagnosed with acute HAV and was hospitalized in isolation based on a positive HAV-IgM test 18 months ago. Regular follow-up after discharge showed abnormal liver function and an elevated level of antinuclear antibodies and immunoglobulin G. For the last 15 days, the patient had fatigue, decreased appetite, and yellow urine, signaling recrudescence. Liver function tests were also abnormal. Liver biopsy revealed histological changes consistent with typical autoimmune hepatitis. After 2 months of methylprednisolone treatment, liver function returned to normal, and HAV-IgM turned negative. The diagnosis of acute HAV in nonendemic areas requires a comprehensive analysis of epidemic history, clinical characteristics, etiology, etc.

Key words: immunoglobulin M, hepatitis A, false-positive

1 Introduction

Immunoglobulin M (IgM) is a marker for acute viral infection and has definitive clinical value. Therefore, physicians depend on the serum marker for the diagnosis of

active infection. However, an increased number of false-positive test results can lead to misdiagnosis and incorrect treatment. Relying solely on this test result further compounds the problem. Many healthcare professionals are still unaware of this issue. False-positive IgM results can occur with any pathogen. We report a case of HAV-IgM with persistently abnormal liver function tests that lasted for 18 months. The patient was incorrectly diagnosed with acute hepatitis A and, finally, with autoimmune hepatitis (AIH).

2 Case report

A 56-year-old woman was readmitted to the hospital with abnormal liver function tests, persisting for the last 18 months. Her hepatic markers were as follows, tested 18 months ago: alanine aminotransferase (ALT) 113 U/L, aspartate aminotransferase (AST) 104 U/L, alkaline phosphatase (ALP) 124 U/L, and glutamate aminotransferase (GGT) 78 U/L. This was attributed to a positive HAV-IgM (ELISA, OD value of attributed absorbance ≥ 2.1 , determined to be positive). She was diagnosed with acute hepatitis A and hospitalized in isolation for treatment. Abnormal symptoms, such as fever, fatigue, abdominal pain, or yellow urine, were not observed. She was treated with glutathione 0.9 g and glycyrrhetic acid 150 mg/day for 45 days. The liver function recovered, and the markers were as follows: ALT 56 U/L, AST 43 U/L, ALP 109 U/L, and GGT 65 U/L. However, HAV-IgM was still positive. During this time, anti-nuclear antibodies (ANA) 47 U/L (normal <10 U/L) were also detected. Tests for antimitochondrial antibodies (AMAs) and anti-liver kidney microsomal (LKM) antibodies, and anti-smooth muscle antibodies (SMAs) were negative. The immunoglobulin G (IgG) level was 18.3 g/L (normal range 6.2–16.1 g/L) and the IgM level was 2.71 g/L (normal range 0.98–2.04 g/L). Following discharge, regular monthly reexamination showed abnormal liver function with repeated fluctuations (Figure 1). The

* **Corresponding author: Youwen Tan**, Department of Hepatology, The Third Hospital of Zhenjiang Affiliated Jiangsu University, No. 300, Daijiamen, Runzhou District, Zhenjiang 212003, China, e-mail: tyw915@sina.com, tel: +86-13914567088, fax: +86-511-88970796

Li Chen: Department of Hepatology, The Third Hospital of Zhenjiang Affiliated Jiangsu University, Zhenjiang 212003, China

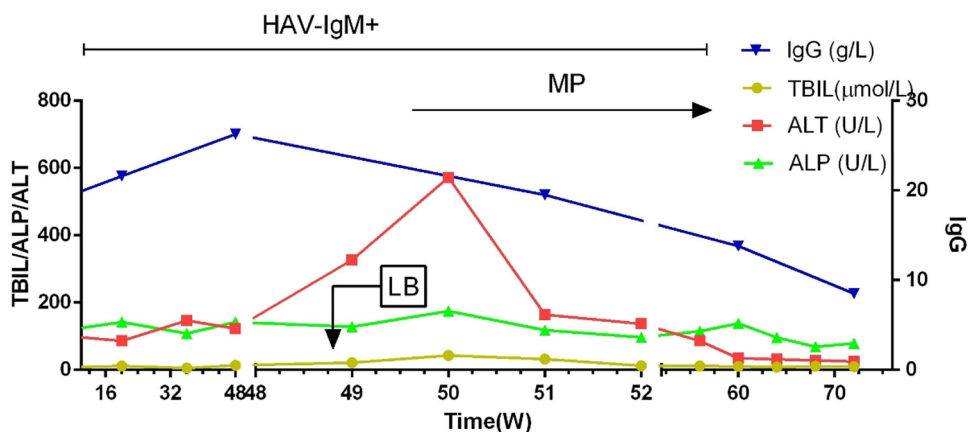


Figure 1: Changes in the liver function and immunological indexes (LB: liver biopsy; MP: methyl prednisolone).

patient developed fatigue, decreased appetite, and yellow urine for 15 days, thus indicating recrudescence. Liver function tests showed abnormal results [total bilirubin (TBIL) 43 mol/L, ALT 452 U/L, AST 254 U/L, ALP 175 U/L, GGT95 U/L]. Further testing revealed the ANA level of 102 U/L, IgG 26.3 g/L, IgM 2.71 g/L, and IgA 5.32 g/L (normal range: 0.76–3.9 g/L). The analysis of a liver biopsy specimen revealed histological changes consistent with typical autoimmune hepatitis. There was moderate to severe interfacial inflammation, numerous lympho-plasma cell infiltrates, lymphocytic penetration, and rosette-like hepatocytes (Figure 2). HAV-IgM was positive. To rule out the possibility of HAV infection, a reverse transcription-polymerase chain reaction (RT-PCR) was conducted to detect HAV RNA in the serum. The analysis of the current samples, as well as those from a year ago, was found to be negative. Clinical diagnosis of AIH was made with a false-positive HAV-IgM. Methylprednisolone 32 mg/day was administered, which was tapered to 20 mg/day after 1 month. The liver function tests, done daily, returned to normal after 2 months, following which, methylprednisolone was further tapered to

8 mg/day as a maintenance treatment. On reexamination, the patient was negative for HAV-IgM and HAV RNA. The tests for Epstein-Barr virus (EBV), cytomegalovirus (CMV), herpes simplex virus (HSV), varicella-zoster virus, and adenovirus were negative. There was also no drug-induced liver injury or history of alcohol addiction.

Ethics statement: Ethics Statement is not applicable for the case report according to the Medical Ethics Committee of the Third Hospital of Zhenjiang Affiliated Jiangsu University, but Informed consent was obtained from the patient for publication of this case report and accompanying images. The study was conducted in accordance with the Declaration of Helsinki.

3 Discussion

HAV is a globally prevalent infectious agent causing intestinal disease. It is mainly transmitted by the fecal-

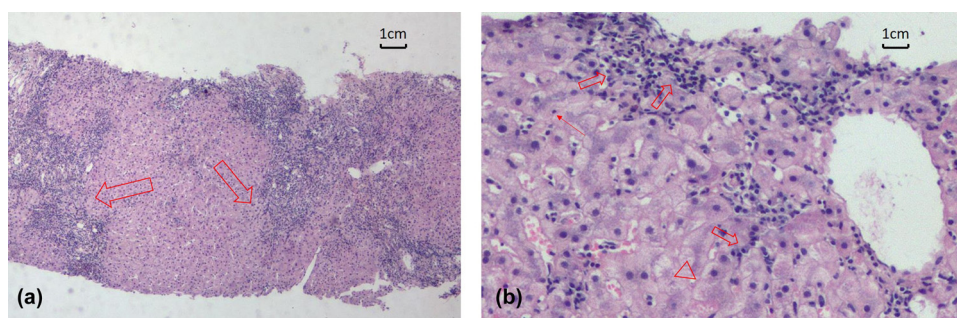


Figure 2: Pathological features of the liver (hematoxylin-eosin staining). (a) Severe interfacial inflammation (thick arrow), $\times 100$ and (b) lympho-plasma cell infiltration (thick arrow), lymphocytes penetrate into the hepatocytes (thin arrow), and rosette cluster hepatocytes (arrow), $\times 200$.

oral route, usually developing as an acute infection followed by self-recovery [1]. HAV-IgM is indicative evidence of an active HAV infection. It appears one week after HAV infection and generally lasts for 3–6 months. HAV-IgM has rarely been detected beyond 1 year [2,3]. Compared to HAV RNA detection, HAV-IgM detection is convenient and fast. It is the primary indicator of current infection with HAV, especially in endemic areas. The accuracy of anti-HAV IgM testing is comparable to HAV RNA detection in the diagnosis of acute hepatitis A [4]. In the absence of a hepatitis A outbreak, a disadvantage of the HAV RNA RT-PCR assay is its high cost as it is not a routine investigation in our country, nor in most other countries. Conversely, it has the advantage of being able to detect acute hepatitis A in its early stages, especially during a hepatitis A epidemic [5]. This will prove to be of great significance to control the further spread of the disease. Another limitation of the HAV RNA assay is that it is susceptible to environmental contamination and is not a genuine test for the HAV virus. However, HAV RNA can be present in the blood for a long time (mean: 79 days) and has also been reported to be present for more than 1 year [6].

The HAV vaccine was included in China's immunization program from 2008. With the universal vaccination of children, the reported incidence of HAV in China has decreased significantly, from 7.34/100,000 in 2004 to 1.66/100,000 in 2015, thus indicating that the prevalence of HAV has been controlled [7]. HAV-RNA is no longer used as a routine diagnostic test having been replaced by the more convenient and cheaper method of IgM antibody testing. In countries where HAV outbreaks are under control, real cases of HAV are rare. For example, in the United States, 10,735 HAV-IgM samples were collected from Kansas City, Missouri, from January 2007 to December 2010. Among them, 49 HAV-IgM positive test results were found in 35 patients. Finally, only 4 patients were detected with acute HAV (11%) [8]. In conclusion, in areas where HAV is controlled, the diagnosis of acute HAV infection should take into account a comprehensive analysis of epidemic history, clinical characteristics, and laboratory examination [9]. Testing HAV-IgM in individuals with asymptomatic infection or with no history of epidemic disease is not recommended [10]. An investigative report shows that the Department of Health and the Centers for Disease Control and Prevention in the United States conducted a survey on individuals with HAV-IgM positivity from 2002 to 2004 [6]. Those who did not meet the clinical criteria for the case were defined as HAV. The report shows that most of the positive test results did not represent a recent, acute HAV infection. In order to

improve the predictive value of positive HAV-IgM detection, clinicians should limit laboratory detection of acute HAV infection to those with typical clinical manifestations or those who have been exposed to suspected hepatitis infection in an environment favoring transmission.

The false-positive IgM mechanism is complex and is the result of comprehensive factors, such as polyclonal B cell activation [11,12], vigorous immune response [11,12], influenza vaccination [13], cross-reactive antibodies [14–16], autoimmune disease [17], heterologous reactions to similar viruses [18–20], subclinical reactivation of latent viruses [21], interfering substances (e.g., rheumatoid factor) [22,23], and naturally occurring biotin IgM antibodies [24]. In addition to the above, cutoff values being set too low [25,26], faulty reagents [27], technical errors (e.g., over-reading weakly reactive bands on immunoblots) [28], low pretest probability [8], and inappropriate testing [6,8] also affect the results.

Although a false-positive HAV-IgM should be common in areas where hepatitis A has been controlled, there is a lack of sizeable data based on population surveys. Our search on PubMed, EMBAS, Web of science, and other databases, using the keywords “False-positive HAV-IgM,” or “False-positive hepatitis A IgM,” revealed a total of 3 cases, with a false-positive HAV-IgM and related conditions [17,29,30]. Two cases of false-positive HAV-IgM were associated with AIH [17,29]; Table 1.

False positives for IgM usually occur under three circumstances. First, diseases with similar clinical presentation, such as infectious mononucleosis, CMV infection, EBV infection, human immunodeficiency virus infection [16,19,20], rubella, measles [31,32], hepatitis A, and hepatitis E viral infection [12,33,34]. However, false positives of HAV-IgM caused by CMV, EBV, and HIV are still reported in individual cases, and the mechanism is thought to be an immune cross-reaction. For example, EBV and HAV may induce autologous production of antibodies against triose-phosphate isomerase (IgM anti-TPI) [35]. Abnormal auto-antibody-induced immune damage can lead to hemolysis.

Secondly, when there is an overlap with the etiology of other diseases. Finally, IgM positivity with no corresponding clinical symptoms, biochemical indicators, or histological evidence.

False-positive IgM results can lead to a disproportionate response in the prevention and control of infectious disease, thus delaying the treatment of the original disease. This patient was diagnosed with acute HAV, and this attracted the attention of the disease control department. The residence was disinfected, and the patient was forcibly hospitalized in isolation. This inconvenienced both, her work and her life, in addition to mental trauma.

Table 1: False-positive HAV-IgM cases reported

Case	Country	Age	Sex	True etiology	LFT	Immunologic test	False HAV-IgM	Potential impact	Report
1	Australia	54	F	Idiopathic scleritis; DILI by infliximab	ALT, 829 U/L; AST, 554 U/L	IgG, 18.4 g/L; IgM, 5.71 g/L; ANA), 1:640	Measured on 2 occasions	Infliximab treatment was discontinued, biochemical improvement within 11 weeks	Elaine Tennant [17]
1	USA	78	F	Congestive heart failure	Mildly abnormal liver enzyme levels, which resolved as her CHF was treated	N/A	Low index, twice the cutoff	Public health investigation, exclusion from adult daycare center	Marie Louise Landry [30]
1	Switzerland	38	F	Autoimmune hepatitis and EBV infection	ALT, 424 U/L; AST, 244 U/L	ANA \geq 1:320; SMA \geq 1:80	Three times were detected, HAVIgM (+, +, -); HAVIgG (+, -, -)	N/A	Marika Valota [29]

Simultaneously, the treatment of AIH was also delayed to a certain extent. The same confusion was seen in other case reports. A 76-year-old woman with congestive heart failure was diagnosed as having HAV by a community doctor in view of a positive HAV-IgM test, which resulted in her being barred from living in the community [30]. False-positive HAV-IgM leads to misdiagnosis of the original condition, leading to the failure of timely and correct diagnosis and treatment of the primary disease. For example, in this case, corticosteroid therapy was used to control the disease until AIH was confirmed by liver histological examination. In China, if liver function is abnormal, especially if liver enzyme levels are elevated, hepatologists often prescribe dicyclol and other drugs, which have been proven to be beneficial for liver inflammation repair, improvement of fibrosis, and alleviation of liver enzymes [36,37]. Although these drugs are yet to be validated in prospective, randomized, controlled, multicenter studies, no significant side effects have been found with these drugs. Some patients use Chinese herbs, including Schisandra fruit and Chuangcao, to protect the liver [38,39]. These herbs have also been shown to be beneficial for treating liver diseases.

Therefore, even though a positive HAV-IgM test forms the basis for the diagnosis of acute HAV, it should not be accepted as the only confirmatory test, especially when results persist, the patient's condition is severe enough to require hospitalization, or when there are no epidemiological findings. Instead, wherever possible, the diagnosis should be confirmed by other means.

Funding information: No funding was received.

Author contributions: Y.W. Tan designed the research; L. Chen collected and analyzed the data, and drafted the manuscript; Y.W. Tan wrote and revised the manuscript; all authors have read and approved the final version to be published.

Conflict of interest: The authors declare that they have no conflict of interest.

Data availability statement: The data of the study are available from the corresponding author on reasonable request.

References

- [1] Lemon SM, Ott JJ, Van Damme P, Shouval D. Type A viral hepatitis: a summary and update on the molecular virology,

- epidemiology, pathogenesis and prevention. *J Hepatol.* 2017 Sep 5;68:167–84.
- [2] Bower WA, Nainan OV, Han X, Margolis HS. Duration of viremia in hepatitis A virus infection. *J Infect Dis.* 2000 Jul;182(1):12–7.
- [3] Cohen JL, Feinstone S, Purcell RH. Hepatitis A virus infection in a chimpanzee: duration of viremia and detection of virus in saliva and throat swabs. *J Infect Dis.* 1989 Nov;160(5):887–90.
- [4] Lemon SM. Type A viral hepatitis: epidemiology, diagnosis, and prevention. *Clin Chem.* 1997 Aug;43(8 Pt 2):1494–9.
- [5] Cuthbert JA. Hepatitis A: old and new. *Clin Microbiol Rev.* 2001 Jan;14(1):38–58.
- [6] Centers for Disease C, Prevention. Positive test results for acute hepatitis A virus infection among persons with no recent history of acute hepatitis – United States, 2002–2004. *Morb Mortal Wkly Rep.* 2005 May 13;54(18):453–6.
- [7] Sun X, Wang F, Zheng H, Miao N, Yuan Q, Cui F, et al. The impact of expanded program on immunization with live attenuated and inactivated Hepatitis A vaccines in China, 2004–2016. *Vaccine.* 2018 Feb 28;36(10):1279–84.
- [8] Alatoon A, Ansari MQ, Cuthbert J. Multiple factors contribute to positive results for hepatitis A virus immunoglobulin M antibody. *Arch Pathol Lab Med.* 2013 Jan;137(1):90–5.
- [9] Nainan OV, Xia G, Vaughan G, Margolis HS. Diagnosis of hepatitis a virus infection: a molecular approach. *Clin Microbiol Rev.* 2006 Jan;19(1):63–79.
- [10] Desai AN, Kim AY. Management of hepatitis A in 2020–2021. *JAMA.* 2020 Jul 28;324(4):383–84.
- [11] Woods CR. False-positive results for immunoglobulin M serologic results: explanations and examples. *J Pediatric Infect Dis Soc.* 2013 Mar;2(1):87–90.
- [12] Sakiani S, Koh C, Heller T. Understanding the presence of false-positive antibodies in acute hepatitis. *J Infect Dis.* 2014 Dec 15;210(12):1886–9.
- [13] Mac Kenzie WR, Davis JP, Peterson DE, Hibbard AJ, Becker G, Zarvan BS. Multiple false-positive serologic tests for HIV, HTLV-1, and hepatitis C following influenza vaccination, 1991. *JAMA.* 1992 Aug 26;268(8):1015–7.
- [14] Asnis DS, Conetta R, Teixeira AA, Waldman G, Sampson BA. The West Nile Virus outbreak of 1999 in New York: the Flushing Hospital experience. *Clin Infect Dis.* 2000 Mar;30(3):413–8.
- [15] Centers for Disease C, Prevention. Human Jamestown canyon virus infection – Montana, 2009. *Morb Mortal Wkly Rep.* 2011 May 27;60(20):652–5.
- [16] Hyams C, Mabayoje DA, Copping R, Maranao D, Patel M, Labbett W, et al. Serological cross reactivity to CMV and EBV causes problems in the diagnosis of acute hepatitis E virus infection. *J Med Virol.* 2014 Mar;86(3):478–83.
- [17] Tennant E, Post JJ. Production of false-positive immunoglobulin M antibodies to hepatitis A virus in autoimmune events. *J Infect Dis.* 2016 Jan 15;213(2):324–5.
- [18] Sohn MJ, Cho JM, Moon JS, Ko JS, Yang HREBV. VCA IgM and cytomegalovirus IgM dual positivity is a false positive finding related to age and hepatic involvement of primary Epstein-Barr virus infection in children. *Medicine (Baltimore).* 2018 Sep;97(38):e12380.
- [19] Post JJ, Chan MK, Whybin LR, Shi Q, Rawlinson WD, Cunningham P, et al. Positive Epstein-Barr virus and cytomegalovirus IgM assays in primary HIV infection. *J Med Virol.* 2011 Aug;83(8):1406–9.
- [20] Just-Nubling G, Korn S, Ludwig B, Stephan C, Doerr HW, Preiser W. Primary cytomegalovirus infection in an outpatient setting—laboratory markers and clinical aspects. *Infection.* 2003 Oct;31(5):318–23.
- [21] Aalto SM, Linnavuori K, Peltola H, Vuori E, Weissbrich B, Schubert J, et al. Immunoreactivation of Epstein-Barr virus due to cytomegalovirus primary infection. *J Med Virol.* 1998 Nov;56(3):186–91.
- [22] Meurman OH, Ziola BR. IgM-class rheumatoid factor interference in the solid-phase radioimmunoassay of rubella-specific IgM antibodies. *J Clin Pathol.* 1978 May;31(5):483–7.
- [23] Bartels EM, Ribbel-Madsen S. Cytokine measurements and possible interference from heterophilic antibodies—problems and solutions experienced with rheumatoid factor. *Methods.* 2013 May 15;61(1):18–22.
- [24] Chen T, Hedman L, Mattila PS, Jartti L, Jartti T, Ruuskanen O, et al. Biotin IgM antibodies in human blood: a previously unknown factor eliciting false results in biotinylation-based immunoassays. *PLoS One.* 2012;7(8):e42376.
- [25] Kamata A, Obinata K, Niizuma T, Kinoshita K, Shimizu T. The validity of the criteria for primary infection of Chlamydia pneumoniae in children by measuring ELISA IgM antibodies. *J Infect Chemother.* 2012 Jun;18(3):308–12.
- [26] Prince HE, Lieberman JM. Impact of the Yosemite hantavirus outbreak on hantavirus antibody testing at a national reference laboratory. *Clin Vaccine Immunol.* 2013 Aug;20(8):1213–6.
- [27] Centers for Disease C, Prevention. False-positive results with a commercially available West Nile virus immunoglobulin m assay – United States, 2008. *Morb Mortal Wkly Rep.* 2009 May 8;58(17):458–60.
- [28] Seriburi V, Ndukwe N, Chang Z, Cox ME, Wormser GP. High frequency of false positive IgM immunoblots for *Borrelia burgdorferi* in clinical practice. *Clin Microbiol Infect.* 2012 Dec;18(12):1236–40.
- [29] 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 May 10;12:5.
- [30] Landry ML. Immunoglobulin M for Acute Infection: True or False. *Clin Vaccine Immunol.* 2016 Jul;23(7):540–5.
- [31] Jenkerson SA, Beller M, Middaugh JP, Erdman DD. False positive rubeola IgM tests. *N Engl J Med.* 1995 Apr 20;332(16):1103–4.
- [32] Costa E, Tormo N, Clari MA, Bravo D, Munoz-Cobo B, Navarro D. Performance of the Epstein-Barr virus and herpes simplex virus immunoglobulin m assays on the liaison platform with sera from patients displaying acute parvovirus B19 infection. *Clin Vaccine Immunol.* 2009 Aug;16(8):1247–8.
- [33] Lanciotti RS, Kerst AJ, Nasci RS, Godsey MS, Mitchell CJ, Savage HM, et al. Rapid detection of west nile virus from human clinical specimens, field-collected mosquitoes, and avian samples by a TaqMan reverse transcriptase-PCR assay. *J Clin Microbiol.* 2000 Nov;38(11):4066–71.
- [34] Jang JH, Jung YM, Kim JS, Lee SH, Kim JW, Hwang SG, et al. Coexistence of IgM antihepatitis A virus and IgM antihepatitis E virus in acute viral hepatitis: a prospective, multicentre study in Korea. *J Viral Hepat.* 2011 Oct;18(10):e408–14.
- [35] Ritter S, Schroder S, Uy A, Ritter K. Haemolysis in hepatitis A virus infections coinciding with the occurrence of autoantibodies against triosephosphate isomerase and the reactivation

- of latent persistent Epstein-Barr virus infection. *J Med Virol.* 1996 Nov;50(3):272–5.
- [36] Hassan A, Fontana RJ. The diagnosis and management of idiosyncratic drug-induced liver injury. *Liver Int.* 2019 Jan;39(1):31–41.
- [37] Katarey D, Verma S. Drug-induced liver injury. *Clin Med (Lond).* 2016 Dec;16(Suppl 6):s104–9.
- [38] Zhu P, Li J, Fu X, Yu Z. Schisandra fruits for the management of drug-induced liver injury in China: a review. *Phytomedicine.* 2019 Jun;59:152760.
- [39] Li Z, He X, Liu F, Wang J, Feng J. A review of polysaccharides from *Schisandra chinensis* and *Schisandra sphenanthera*: Properties, functions and applications. *Carbohydr Polym.* 2018 Mar 15;184:178–90.

Appendix

Table A1: Changes of main indexes of liver function and immunoglobulin G

Time (w)	TBIL (mol/L)	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)	IgG (g/L)
0	8.3	56	45	109	65	18.3
1	12.7	131.2	89	97	49	
4	7.8	104	77	115	64	
20	11.7	86	48	142	90	21.6
36	5.4	147	112	108	57	
48	13.5	123	94	142	59	26.3
49	21.7	327	227	128	86	
50	43	572	554	175	112	
51	32.5	164.4	107	118	89	19.5
52	12.6	137.1	84	97	57	
56	11.7	86.4	56	115	49	
60	10.6	35.2	28	138	63	13.8
64	8.5	31.6	22	96	38	
68	10.7	28.6	18	69	36	
72	9.4	25.2	23	79	47	8.5

W: week; TIL: total bilirubin; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; GGT: glutamine transpeptidase.