Anti-rods/rings autoantibody seropositivity does not affect response to telaprevir treatment for chronic hepatitis C infection

S. John Calise¹ · Nicola Bizzaro² · Thuy Nguyen¹ · Danila Bassetti³ · Brunetta Porcelli³ · Paolo Almi⁵ · Giuseppina Barberio⁶ · Giampaola Pesce⁷ · Minoru Satoh⁸ · Edward K. L. Chan¹

Received: 29 September 2016 / Accepted: 19 October 2016 / Published online: 14 November 2016 © The Author(s) 2016. This article is published with open access at Springerlink.com

Abstract

Purpose Autoantibodies to intracellular ‘rods and rings’ structures (anti-rods/rings or anti-RR) are strongly associated with hepatitis C (HCV) patients treated with interferon-α/ribavirin (IFN/RBV) and are linked with non-responsiveness to IFN/RBV or relapse, especially in Italian patients. This is the first study to determine whether there is any correlation of anti-RR with non-responsiveness to IFN/RBV treatment in patients also treated with telaprevir (TPV), one of several new therapies for chronic HCV recently implemented.

Methods From 2013 to 2014, 52 HCV-infected patients were treated with IFN/RBV and TPV at five Italian clinics. Patient sera were collected and analyzed by indirect immunofluorescence for the presence of anti-RR antibodies. Patients were classified as anti-RR positive or anti-RR negative, and then various biological and clinical variables were analyzed to compare the two groups, including gender, age, HCV genotype, previous IFN/RBV treatment, and IFN/RBV/TPV treatment outcome.

Results Of these 52 HCV patients treated with IFN/RBV/TPV, 10/32 (31%) who previously received IFN/RBV were anti-RR positive, compared to 0 of 20 treatment-naïve patients. Anti-RR-positive patients relapsed more than anti-RR-negative patients (3/10, 30% vs. 2/42, 5%; p < 0.05). However, zero anti-RR-positive patients were non-responsive, and frequencies of sustained virological response were similar (anti-RR positive: 7/10, 70% vs. anti-RR negative: 33/42, 79%).

Conclusions Overall, the data suggest that anti-RR seropositivity is not associated with resistance to TPV treatment in this patient cohort, but monitoring anti-RR-positive patients for relapse within the first 6 months after treatment may be useful.

Keywords Direct-acting antivirals · Inosine monophosphate dehydrogenase · Interferon-α · Ribavirin · Rods and rings · Telaprevir

Abbreviations

ANA Antinuclear antibody
anti-RR Anti-rods/rings autoantibody
DAAs Direct-acting antivirals
HCV Hepatitis C
IFN Interferon-α
IFN/RBV Interferon-α and ribavirin therapy
IMPDH Inosine 5’-monophosphate dehydrogenase
RBV Ribavirin
RRs Rods and rings
SVR Sustained virological response
TPV Telaprevir
Introduction

Chronic hepatitis C (HCV) infection is associated with the production of autoantibodies, including organ-specific autoantibodies directed against targets in the thyroid, adrenal cortex, pancreatic islet cells, and gastric parietal cells, and non-organ-specific autoantibodies such as antinuclear, anti-smooth muscle, anti-mitochondrial, anti-liver/kidney microsomal, and anti-neutrophil cytoplasmic antibodies [1–8]. Recent studies have also demonstrated a link between HCV and the production of autoantibodies targeting intracellular filamentous structures termed ‘rods and rings’ (RRs) [9–16]. In most studies, anti-rods/rings (anti-RR) seropositivity appears to be almost exclusive to HCV patients treated with interferon-α and ribavirin combination therapy (IFN/RBV) and is rarely seen in treatment-naïve HCV patients or other disease groups. However, anti-RR has been observed in one hepatitis B patient [11], one systemic lupus erythematosus patient [17], and healthy individuals with no previous IFN/RBV treatment [17]. In cultured cells, RRs are composed of inosine 5'-monophosphate dehydrogenase (IMPDH), and/or cytidine 5'-triphosphate synthase under certain conditions [9, 18, 19]. RRs tend to assemble when de novo purine biosynthesis is inhibited and guanine nucleotide levels become depleted [20–25]. Many patients with anti-RR react with IMPDH, which is inhibited by direct binding to RBV and appears to be the major autoantigen in RRs [9, 26, 27]. Although no mechanistic evidence suggesting that anti-RR autoantibody contributes to resistance to IFN/RBV therapy has yet been reported, previous studies showed that anti-RR antibodies were more prevalent in patients who did not respond to therapy or relapsed, when compared to sustained responders [10, 15]. Additionally, non-responsive or relapsing patients had higher anti-RR titers, suggesting that anti-RR positivity may be indicative of poor treatment outcomes [28]. In recent years, direct-acting antivirals (DAAs), such as telaprevir (TPV), have been developed for chronic HCV infection in an effort to reduce therapy duration and increase drug tolerability, while also improving patient outcomes. Currently, TPV is included with IFN/RBV as a triple therapy. Here, we examine the relationship between anti-RR and treatment outcomes in a cohort of Italian patients treated with IFN/RBV and telaprevir.

Methods

Patient and treatment information

From 2013 to 2014, 52 HCV-infected patients were treated with IFN/RBV and TPV at five Italian clinics located at the (1) Ospedale San Antonio, Tolmezzo, (2) Ospedale Santa Chiara, Trento, (3) Università degli Studi di Siena, (4) Ospedale Regionale, Treviso, and (5) Università degli Studi di Genova. Dosages depended on patient weight (75 kg discriminating weight) and were typically administered as follows: 80–180 lug weekly pegylated interferon-α, 600–1400 mg daily ribavirin, and 2250 mg daily TPV. Patients were classified as: not responsive to therapy (HCV RNA still detectable at week 24 of therapy), relapsed (HCV RNA detectable after the end of treatment in patients with previous virological response), or responsive to therapy (HCV RNA not detectable in the 24 weeks after the completion of therapy). The study conforms to the Institutional Review Board requirements in all institutions.

Informed consent

Informed consent was obtained from all individual participants included in the study. All patients provided written informed consent to receive IFN/RBV/TPV and permission for use of their medical records for this study.

Antinuclear antibody indirect immunofluorescence assay (ANA-IIF)

Anti-rods/rings in patient sera were detected by indirect immunofluorescence, using NOVA Lite Hep-2 ANA substrate (INOVA Diagnostics, San Diego, CA: 508100) as previously described [10]. Staining patterns of test sera were compared to staining of human prototype anti-RR serum It2006 used in previous studies [9, 10]. It2006 and all anti-RR-positive sera described in this study correctly recognize the rods and rings ANA pattern, designated as pattern AC-23 by the International Consensus on ANA Patterns (ICAP) [29]. All sera were tested at a dilution of 1:80 in PBS as previously described [30]. For anti-RR-positive patients who also had serial samples available, anti-RR end point titers were determined using twofold serial dilution of sera in PBS, with a starting dilution of 1:80 and ending dilution of 1:1280. Anti-RR positivity and titers were independently validated by two trained individuals (S.J.C. and T.N.). End point titer was defined by more than 50% of cells containing detectable RR staining. Donkey anti-human IgG conjugated to DyLight 488 (Jackson ImmunoResearch, West Grove, PA) diluted 1:100 in PBS was used to detect autoantibody staining. Fluorescent images were captured with a Zeiss Axiovert 200 M microscope fitted with a Zeiss AxioCam MRm camera using a 40× (0.75 NA) objective (Carl Zeiss Microscopy, Jena, Germany).

Statistical analysis

Biological and clinical variables analyzed for statistical significance include: gender, age, HCV genotype, previous treatment with IFN/RBV (prior to beginning of TPV...
regimen), and treatment outcome (see Table 1). Mann–Whitney $U$ test was used to compare different groups containing continuous data, and Fisher’s exact test or the Fisher–Freeman–Halton exact test was used for categorical data. Differences were considered statistically significant if $p < 0.05$. Mann–Whitney $U$ test and Fisher’s exact test were performed using GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA). Fisher–Freeman–Halton test was performed using StatXact 10 (Cytel, Cambridge, MA).

**Results and discussion**

The goal of this study was to probe for an association between the presence of anti-RR autoantibody and treatment outcome in a cohort of 52 Italian HCV patients treated with the new DAA telaprevir. This is the first study to examine anti-RR in patients treated with any of the recently approved DAAs. It must be pointed out that, in general, the availability of patients for studies of anti-RR antibody is naturally limited by the low prevalence of this autoantibody response. Although it has been reported that 20–40% of HCV patients treated with IFN/RBV produce anti-RR autoantibodies, this response is very rarely observed in treatment-naïve HCV patients or other disease groups [10, 11, 14–16, 31].

A total of 52 HCV-infected patients were treated with IFN/RBV and TPV. Thirty-six patients (69%) were male and 16 (31%) were female, with a mean age of 54 ± 9 years. Twelve patients (23%) had genotype 1a, 39 patients (75%) had genotype 1b, and one patient (2%) had genotype 3b. Thirty-two patients (62%) had been previously treated with IFN/RBV prior to being put on TPV. Patient demographics are included in Table 1. All 52 patients were assayed for the presence of anti-RR according to standard antinuclear antibody indirect immunofluorescence (ANA-IIF) protocols using HEp-2 cells as a substrate. ANA-IIF analysis revealed that 10 out of 52 patients (19%) were positive for anti-RR at a dilution of 1:80. Figure 1 displays the images of the ‘rods and rings’ staining pattern from all ten anti-RR-positive patients (patient codes C1TN, FV1S, TBN1S, VA1S, TG1TO, SL1G, VG1G, MS1G, RC1G, and CB1G). Anti-RR-negative patients CD2T and E1TN are also shown for comparison. Twenty-one out of 52 patients had serial collections of sera available (104 total sera for 52 patients), representing multiple visits to the clinic over time periods ranging from 2 weeks to 13 months. Anti-RR status did not change over time in any of these 21 patients (i.e., anti-RR-positive patients remained positive and negative patients remained negative) and anti-RR titers did not significantly change in positive patients. Accordingly, for purposes of statistical analysis, patients were simply considered either positive or negative, and serial collections were not taken into consideration.

Patients were divided into two main groups, anti-RR-positive ($n = 10$) and anti-RR-negative ($n = 42$), for subsequent statistical analysis. These groups were

| Parameters                          | Total patients ($n = 52$) | Anti-RR-positive patients ($n = 10$) | Anti-RR-negative patients ($n = 42$) | $p$ value |
|-------------------------------------|---------------------------|-------------------------------------|-------------------------------------|-----------|
| Male                                | 36 (69%)                  | 7 (70%)                             | 29 (69%)                            | NS        |
| Female                              | 16 (31%)                  | 3 (30%)                             | 13 (31%)                            | NS        |
| Age (years) ± SD                    | 54 ± 9                    | 53 ± 14                             | 54 ± 8                              | NS        |
| Genotype 1a$^a$                      | 12 (23%)                  | 3 (30%)                             | 9 (21%)                             | NS        |
| Genotype 1b$^a$                      | 39 (75%)                  | 7 (70%)                             | 32 (76%)                            | NS        |
| Previous IFN/RBV                    | 32 (62%)                  | 10 (100%)                           | 22 (52%)                            | <0.01     |
| Treatment outcome                   |                           |                                     |                                     |           |
| SVR, no side effects                | 35 (67%)                  | 6 (60%)                             | 29 (69%)                            | NS        |
| SVR, but side effects               | 5 (10%)                   | 1 (10%)                             | 4 (10%)                             | NS        |
| SVR, combined                       | 40 (77%)                  | 7 (70%)                             | 33 (79%)                            | NS        |
| Relapse                             | 5 (10%)                   | 3 (30%)                             | 2 (5%)                              | <0.05     |
| No response                         | 7 (13%)                   | 0 (0%)                              | 7 (17%)                             | NS        |
| Fisher–Freeman–Halton exact test    |                           |                                     |                                     |           |
| Anti-RR positive vs. anti-RR negative, with SVR separated |                           |                                     | NS        |
| Anti-RR positive vs. anti-RR negative, with SVR combined |                           |                                     | <0.05     |

Values presented as $n$ (%) unless otherwise indicated

Anti-RR anti-rods/rings autoantibody, IFN/RBV interferon-α/ribavirin therapy, NS not statistically significant ($p > 0.05$), SD standard deviation, SVR sustained virological response

$^a$ One patient without anti-RR was genotype 3b
compared based on several demographic, clinical, and serological parameters to determine any differences between anti-RR-positive and anti-RR-negative patients (Table 1). There was no significant difference observed between the two groups when comparing gender, age, or HCV genotype. Both patient groups were also categorized into four different treatment outcome groups: (1) sustained virological response (SVR) with no side effects, (2) initial SVR but therapy was discontinued due to side effects, (3) relapse within 6 months of treatment, and (4) no response to treatment. SVR patients with or without side effects were all determined to be SVR at the same time point (after 1 month, according to international guidelines). When the Fisher–Freeman–Halton exact test was performed to compare anti-RR-positive vs. anti-RR-negative patient groups based on all four treatment outcome parameters, no significant difference was observed. However, additional Fisher–Freeman–Halton analysis with all SVR patients combined (regardless of side effects) resulted in a statistically significant p value <0.05 (Table 1, bottom row).

Thus, our data indicate that anti-RR-positive and anti-RR-negative patients in this cohort appear to differ with regard to distribution of treatment outcomes. The most notable difference is that anti-RR-positive patients were more likely to relapse than anti-RR-negative patients (p < 0.05). Despite the increase in relapse, 0 of the 10 anti-RR-positive patients were non-responsive to therapy, compared to 7 of the 42 (17%) anti-RR-negative patients (p = 0.32). Additionally, there was no significant difference in SVR rates between both groups. SVR rates were compared with patients who experienced side effects and those who did not separated as different outcomes ("SVR, but side effects" and "SVR, no side effects") or with all SVR patients combined ("SVR, combined"), but both
analyses showed no significant difference between anti-RR-positive and anti-RR-negative patients.

We also discovered that anti-RR can be detected years after treatment with IFN/RBV. Two of the ten anti-RR-positive patients had serum collected both prior to and after IFN/RBV/TPV therapy was initiated. All samples from both patients were positive for anti-RR, initially suggesting that these patients may have been positive with no previous IFN/RBV treatment. However, after careful examination of medical records, it was determined that both patients had received IFN/RBV more than a decade prior to treatment with TPV. Patient TBN1S was diagnosed with liver cirrhosis in 2000 and treated with IFN/RBV from 2000 to 2002, but treatment was eventually discontinued due to side effects. In 2014, TBN1S began receiving IFN/RBV again with addition of TPV, but relapsed within 6 months. Patient VA1S previously received IFN/RBV for 6 months in 2003. Eleven years later, in 2014, the patient began receiving IFN/RBV again with addition of TPV, but therapy was discontinued due to side effects. Despite the lack of exposure to IFN/RBV for more than 10 years, both patients remained positive for anti-RR autoantibody, and VA1S even remained positive down to 1:1280 dilution. We speculate that long-lived plasma cells might be responsible for the long-term presence of anti-RR antibody in these patients. Previous studies have suggested that anti-RR titer increases throughout the duration of therapy, but declines upon cessation of treatment [11, 12, 15, 31]. To our knowledge, this is the first report of long-lived anti-RR autoantibody.

Previous studies established a strong association between anti-RR and IFN/RBV therapy [10–12, 14, 15], such that we previously described anti-RR as a drug-induced autoantibody [13]. Additionally, prolonged exposure to IFN/RBV increases the likelihood of anti-RR autoantibody production [11, 12, 15]. Our data support these findings, considering that 10 out of 32 (31%) patients previously treated with IFN/RBV were anti-RR positive, compared to 0 out of 20 patients who had not previously received IFN/RBV. In terms of treatment outcome, previous reports have indicated a link between anti-RR and non-responsiveness or relapse in American and Italian HCV patient cohorts [10, 15, 28]. In the current study with a new cohort of Italian patients, we again found that anti-RR seropositivity was associated with increased frequency of relapse. Interestingly, the frequency of non-responsiveness appeared to be decreased in anti-RR-positive patients (0/10, 0%) compared to anti-RR-negative patients (7/42, 17%), despite the opposite trend in relapse. When patients with no previous IFN/RBV treatment are removed from analysis, the trend remains similar, with non-responsiveness occurring in 0/10 (0%) of anti-RR-positive patients compared to 6/16 (27%) of anti-RR-negative patients ($p = 0.14$). Overall, 19% (10/52) of HCV patients in this study were positive for anti-RR, which is similar to the rates observed in previous studies [10, 11, 14–16]. Importantly, the addition of TPV to the IFN/RBV regimen did not induce a new anti-RR response in any patients; anti-RR-negative patients previously treated with IFN/RBV did not become positive after TPV was included in the regimen. Likewise, anti-RR titers did not significantly change after addition of TPV in anti-RR-positive patients with serial samples available. In all, our data suggest that inclusion of TPV in the IFN/RBV regimen for the treatment of chronic HCV does not alter the production of anti-RR autoantibody. However, based on the statistically significant increase in relapse rate, it may be useful to carefully monitor anti-RR-positive patients during and for 6 months after IFN/RBV/TPV therapy. While our study is limited by the number of available patients, the data indicate that anti-RR seropositivity does not affect the response to TPV treatment for chronic HCV.

Acknowledgements S. John Calise is supported by the National Institute of Dental and Craniofacial Research of the National Institutes of Health under Award Number 2T90DE021990-06. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

Informed consent Informed consent was obtained from all individual participants included in the study. All patients provided written informed consent to receive IFN/RBV/TPV and permission for use of their medical records for this study.

Human and animal rights The study conforms to the Institutional Review Board requirements in all institutions. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Animals were not used in this study.

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

1. Abuaf N, Lunel F, Giral P, Borotto E, Laprécéhe S, Poupon R, Opolon P, Huraux JM, Homberg JC (1993) Non-organ specific autoantibodies associated with chronic C virus hepatitis. J Hepatol 18:359–364
2. Prestiati D, La Rosa L, Covini G, Marcelli R, Ressalli S, Persani L, Del Ninno E, Meroni PL, Colombo M, Beck-Peccoz P (1995) Autoimmunity and thyroid function in patients with chronic active hepatitis treated with recombinant interferon alpha-2a. Eur J Endocrinol 132:587–593
17. Calise SJ, Carcamo WC, Ceribelli A, Dominguez Y, Satoh M, Chan EK (2014) Assembly of IMPDH2-based, CTPS-based, and mixed rod/ring structures is dependent on cell type and conditions of induction. J Genet Genomics 42:287–299

18. Keppkeke GD, Calise SJ, Chan EK, Andrade LE (2015) Assembly of IMPDH2-based, CTPS-based, and mixed rod/ring structures is dependent on cell type and conditions of induction. J Genet Genomics 42:287–299

19. Chang CC, Lin WC, Pai LM, Lee HS, Wu SC, Ding ST, Liu JL, Sung LY (2015) Cytoophidium assembly reflects upregulation of IMPDH activity. J Cell Sci 128:3550–3555

20. Ji Y, Gu J, Makho AM, Griffith JD, Mitchell BS (2006) Regulation of the interaction of inosine monophosphate dehydrogenase with myophenolic Acid by GTP. J Biol Chem 281:206–212

21. Gunter JH, Thomas EC, Lengefeld N, Kruger SJ, Worton L, Gardiner EM, Jones A, Barnett NL, Whitehead JP (2008) Characterization of inosine monophosphate dehydrogenase expression during retinal development: differences between variants and isoforms. Int J Biochem Cell Biol 40:1716–1728

22. Thomas EC, Gunter JH, Webster JA, Schieber NL, Oorschot V, Porton RG, Whitehead JP (2012) Different characteristics and nucleotide binding properties of inosine monophosphate dehydrogenase (IMPDH) isoforms. PLoS One 7:e51096

23. Gou KM, Chang CC, Shen QJ, Sung LY, Liu JL (2014) CTP synthase forms cytoophidia in the cytoplasm and nucleus. Exp Cell Res 323:242–253

24. Calise SJ, Carcamo WC, Krueger C, Yin JD, Purich DL, Chan EKL (2014) Glutamine deprivation initiates reversible assembly of mammalian rods and rings. Cell Mol Life Sci 71:2963–2973

25. Calise SJ, Purich DL, Nguyen T, Saleem DA, Krueger C, Yin JD, Chan EKL (2016) ‘Rod and ring’ formation from IMP dehydrogenase is regulated through the one-carbon metabolic pathway. J Cell Sci 129:3042–3052

26. Seelig HP, Appelhans H, Bauer O, Bluthner M, Hartung K, Schranz P, Schulze D, Seelig CA, Volkmann M (2011) Autoantibodies against inosine-5′-monophosphate dehydrogenase 2–characteristics and prevalence in patients with HCV-infection. Clin Lab 57:753–765

27. Probst C, Radzimski C, Blocker IM, Teegen B, Bogdanos DP, Stocker W, Komorowski L (2013) Development of a recombinant cell-based indirect immunofluorescence assay (RC-IFA) for the determination of autoantibodies against “rings and rods”-associated inosine-5′-monophosphate dehydrogenase 2 in viral hepatitis C. Clin Chim Acta 418:91–96

28. Carcamo WC, Ceribelli A, Calise SJ, Krueger C, Liu C, Davies M, Villalta D, Bizzaro N, Satoh M, Chan EKL (2013) Differential reactivity to IMPDH2 by anti-rods/ rings autoantibodies and unresponsiveness to pegylated interferon-alpha/ribavirin therapy in US and Italian HCV patients. J Clin Immunol 33:420–426

29. Chan EK, Danzoleaux J, Carballo OG, Conrad K, de Melo Cruvinel W, Francesantonio PL, Fritzler MJ, Garcia-De La Torre I, Herold M, Mimori T, Satoh M, von Muhlen CA, Andrade LE (2015) Report of the first international consensus on standardized nomenclature of antinuclear antibody HEP-2 cell patterns 2014–2015. Front Immunol 6:412

30. Satoh M, Chan EKL, Ho LA, Rose KM, Parks CG, Cohn RD, Jusko TA, Walker NJ, Germolec DR, Whitt IZ, Crockett PW, Pauley BA, Chan JY, Ross SJ, Birnbaum LS, Zeldin DC, Miller FW (2012) Prevalence and sociodemographic correlates of antinuclear antibodies in the United States. Arthritis Rheum 64:2319–2327

31. Keppkeke GD, Calise SJ, Chan EK, Andrade LE (2016) Anti-rods/rings autoantibody generation in hepatitis C patients during interferon-alpha/ribavirin therapy. World J Gastroenterol 22:1966–1974