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Research Article

The Glycodiversity of HCV E2 Glycoprotein-Specific Antibodies as a Signature of Hepatic Damage and Virotherapy Efficacy

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Abstract

The HCV E2 glycoprotein-specific Abs (E2-Ab) is an important factor in the host resistance to hepatitis C virus (HCV) infection. There is evidence that the E2-Ab sialylation is associated with liver damage and virotherapy efficacy. The aim of this study was to further profile the E2-Ab glycosylation. The fucosylation and sialylation of E2-Ab in one hundred six (HCV)-infected patients were tested using the lectin-based ELISA platform. Data were analyzed by the stage of hepatic fibrosis, HCV genotype and the response to IF-RBV virotherapy. The changes in the E2–Ab glycosylation were also evaluated by the receiver operator characteristic curve (ROC) and multiple regression analysis.

The E2-Ab reactivity to fucose-specific Aleuria aurantia lectin (AAL) and sialo-specific Sambucus nigra agglutinin (SNA) was decreased in the advanced stages of liver fibrosis. The SNA binding analysis was more informative in the discrimination of patients with advanced fibrosis compared to those with earlier fibrosis stages or no fibrosis group. No significant correlation between the reactivities of SNA and AAL lectins was established irrespective of HCV genotype. The patients infected with HCV 3a genotype showed an increased E2-Ab fucosylation, a lower E2-SNA/E2-AAL ratio and a better response to virotherapy. The association of the E2-SNA/E2-AAL ratio with virotherapy outcome was observed in patients infected with HCV 1b genotype. A better response to IF-RBV therapy was found in patients with a higher fucosylated E2 Abs and a lower E2-SNA/E2-AAL ratio. Thus the significance of E2 antibodies in the course of HCV hepatitis was demonstrated to be dependent on their glycosylation, the sialylation/fucosylation ratio, as well as on HCV genotype.

Keywords: E2-specific antibody; Ab glycosylation; HCV hepatitis; HCV genotypes; Virotherapy efficacy; Liver damage; Non-invasive markers

Abbreviations

AAL: Aleuria Aurantia Lectin; ACC: Accuracy of ROC Analysis; E2-Ab: The Level of Antibodies to HCV E2 Glycoprotein; E2-AAL: The Level of AAL Binding to E2-Specific Antibodies; E2-SNA: The Level of SNA Binding to E2-Specific Antibodies; IF-RBV: Pegylated Interferon-α-2a Plus Ribavirin Therapy; NR: No Response; RL: Relapse; ROC: Receiver Operator Characteristics Curve Analysis; SNA: Sambucus Nigra Agglutinin; SNA/AAL ratio: E2-SNA Reactivity/E2-AAL Reactivity Ratio; SVR: Sustained Virologic Response; TF: The Thomsen--Friedenreich Antigen (Galα1-3Gal NAc-R); αGal: Xenogenic Alpha-Gal Glycotope (Galα1-3Galβ1-4GlcNAc-R)

Introduction

The E2 envelope glycoprotein of hepatitis C virus (HCV) acts as a receptor binding protein, being thus one of the main targets for broadly neutralizing antibodies against HCV [1-4]. The immune response to E2 is an important factor in the resistance to and a spontaneous cure of HCV infection [5-7].

The critical role of Fc-linked glycans for both the pro-inflammatory and anti-inflammatory effector functions of IgG is now well established [8-11]. The predominant carbohydrate structure found in the serum IgG is a complex-type, core fucosylated, biantennary N-glycan. An increase of the G0F (agalactosylated, asialylated and fucosylated) IgG glycoform is the most prominent change in a variety of chronic inflammatory and autoimmune diseases, thus promoting a proinflammatory state [8,9,12-14]. The core fucosylation of IgG N-glycans, in which fucose α1,6 is added to the protein-adjacent GlcNAc of N-glycans, directly impacts IgG ADCC activity [15,16]. The IgG glycovariants lacking branching fucose have an increased affinity for specific Fc gamma receptors.

Notable, when compared to the advances made in the glycoprofiling of total serum immunoglobulins, an analysis of disease-specific Abs, including E2-specific antibodies, has remained relatively understudied. We reported recently that the E2 Ab sialylation as defined by SNA lectin binding was significantly decreased in the terminal (F4) stages of hepatic fibrosis (F) [17], thus being a good marker of advanced liver fibrosis. Interestingly, HCV unrelated natural antiglycan (TF, αGal)-specific Abs demonstrated quite opposite fibrosis-related changes, i.e. an increased sialylation in stage F4, and the combination of E2-specific and natural antiglycan

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Abs using multiple regression analysis allowed us to get a very high accuracy degree (>90%) in the diagnosis of hepatic fibrosis progression [18].

In the present study, we investigated the fucosylation and sialylation of E2 Abs as well as their interrelationship (the E2-SNA/E2-AAL ratio) in HCV infected patients. The data were analyzed for HCV genotype (GT), stage of hepatic fibrosis (F) and treatment outcome and the clinical significance of the findings was estimated.

Material and Methods

Subjects

Serum samples were taken from 106 HCV infected treatment-naïve patients: men 66 and women 40 of median age of 39 years (range 19-68). The HCV genotype-based characteristics of patients and the effect of antiviral therapy are presented in Table 1. The investigation was carried out in accordance with the ICH GCP Standards and approved by Tallinn Medical Research Ethics Committee, Estonia. A written informed consent was obtained from all patients.

The diagnosis of HCV infection was based on the presence of anti-HCV antibodies in the patients sera, detection of serum HCV RNA, histologically verified fibrosis stage and clinical follow-up. The HCV genotype was determined by the hybridization technique using the VERSANT HCV genotype assay (LiPA) (Bayer HealthCare LLC, Tarrytown, NY). The range of fibrosis was classified according to the Metavir scoring system from F0 to F4 (cirrhosis). The serum samples were stored in aliquots at -40°C until use.

HCV infection treatment (pegylated interferon-α-2a plus ribavirin therapy) (IFN-RBV) was conducted according to the National Guidelines approved by the Estonian Society for Infectious Diseases in 2010. The response to therapy was evaluated as a sustained virological response (SVR), no response (NR) or relapse (RL) at 24 weeks following the end of treatment.

The E2 glycoprotein-specific IgG antibody assay

The level of anti-E2 and two antiglycan IgG antibodies was determined by the enzyme-linked immunosorbent assay (ELISA) as described earlier [17]. The plates (NUNC Maxisorp, Denmark) were coated with an E2 recombinant protein (ViroStat, ME, USA), in carbonate buffer, pH 9.6, 5μg/ml. After the overnight incubation, triple washing and blocking with a Superblock solution (Pierce, USA) for 30min at 25°C, the serum samples diluted 1:50 in PBS-0.05% Tween (Tw) were applied for 1.5 h at 25°C. After the subsequent washing with PBS-Tw, the level of bound Abs was determined using the alkaline phosphatase (AP) conjugated goat anti-human IgG (Sigma, USA) and p-nitrophenylphosphate disodium hexahydrate (pNPP, Sigma, USA). The absorbance values were read at 405nm (Tecan Reader, Austria). The optical density value (O.D) of control wells (no serum sample) was subtracted from that of Abs-coated wells and each sample was analyzed in duplicate.

The SNA and AAL lectin reactivity of E2-specific antibodies

The lectin reactivity of E2-specific antibodies was measured in a similar way, except that the binding of the neuraminic acid (sialic acid)-specific Sambucus nigra agglutinin (SNA) or fucose-specific Aleuria aurantia lectin (AAL) to the absorbed antibodies was determined. After triple washing the biotinylated SNA (Vector Laboratories, Inc., USA) in 10mmol/L Hepes, 0.15mol/L NaCl, 0.1mmol/L $CaCl_2$, pH 7.5 or biotinylated AAL (Vector Laboratories Inc., USA) in 10mmol/L HEPES, 0.15mol/L NaCl buffer, pH 7.5, were applied at a concentration of 5μg/mL for 1.5h at 25°C.

The bound lectin was detected with a streptavidin-AP conjugate (Dako, USA) and pNPP (Sigma, USA). The O.D of control wells (no serum sample) was subtracted from that of serum-coated wells to determine the lectin binding. Each sample was analyzed in duplicate. The value of the SNA binding to Abs and the ratio of SNA binding to IgG level (SNA/IgG ratio) were determined. In addition, the ratio of E2-Ab SNA reactivity (E2-SNA) to E2-Ab AAL binding (E2-AAL) was calculated.

Table 1a: The characteristics of patients under investigation. The distribution by HCV genotype and hepatic fibrosis stage.

HCV	Hepatic fibrosis stage				
Genotype	**0**	**1**	**2**	**3**	**4**
(n)	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**
1b (53)	26 (49)	9 (17)	5 (9.5)	3 (5.7)	10 (18.9)
3a (46)	21 (45.7)	14 (30)	6 (13)	1 (2.2)	4 (8.7)
1a+2a/2c (6)	4 (66.7)	1 (16.7)	1 (16.7)	0	0
All cases (105)	51 (48.6)	24 (22.9)	12 (11.4)	4 (3.8)	14 (13.3)

Table 1b: The characteristics of patients under investigation. The distribution by HCV genotype and response to virotherapy.

HCV	Response to treatment			
Genotype	**SVR**	**NR**	**RL**	**ST**
(n)	**n (%)**	**n (%)**	**n (%)**	**n (%)**
1b (50)	24 (48)	10 (20)	13 (26)	3 (6)
3a (45)	36 (80)	1 (2.2)	5 (11.1)	3 (6.7)
1a+2a/2c (6)	5 (83.3)	0	0	1 (16.7)
All cases (101)	65 (64.4)	11 (10.9)	18 (17.8)	7 (6.9)

Genotype 1a: One Patient and GT2a/2c: Four Patients. SVR: Sustained Virologic Response; NR: No Response; RL: Relapse. In selen patients treatment was stopped (ST) due to intolerance.

Statistical analysis

Comparison between the groups was performed using the nonparametric Mann–Whitney U test for unpaired data or Student's t-test where appropriate and $P \leq 0.05$ value was considered statistically significant. The differences in the SNA and AAL lectin reactivities were also analyzed by multiple regression and the receiver operator characteristic curve (ROC) analysis using the combination of parameters for hepatic fibrosis stages as well as therapy outcomes discrimination. All calculations were performed using the STATISTICA 10 and RStudio-1.1.463 software.

Results

The E2-specific IgG level was very similar in all stages of fibrosis or without it (Figure 1a). The AAL binding to E2 Abs as well as the E2-AAL/IgG ratio was significantly decreased in the terminal stages of fibrosis (Figures 1b and 1c). The HCV genotype had no significant impact on E2 Ab level (Figure 2a) but the E2-AAL reactivity and especially the E2-AAL/IgG ratio was decreased in patients infected with HCV 1b GT compared with 3a and other genotype groups

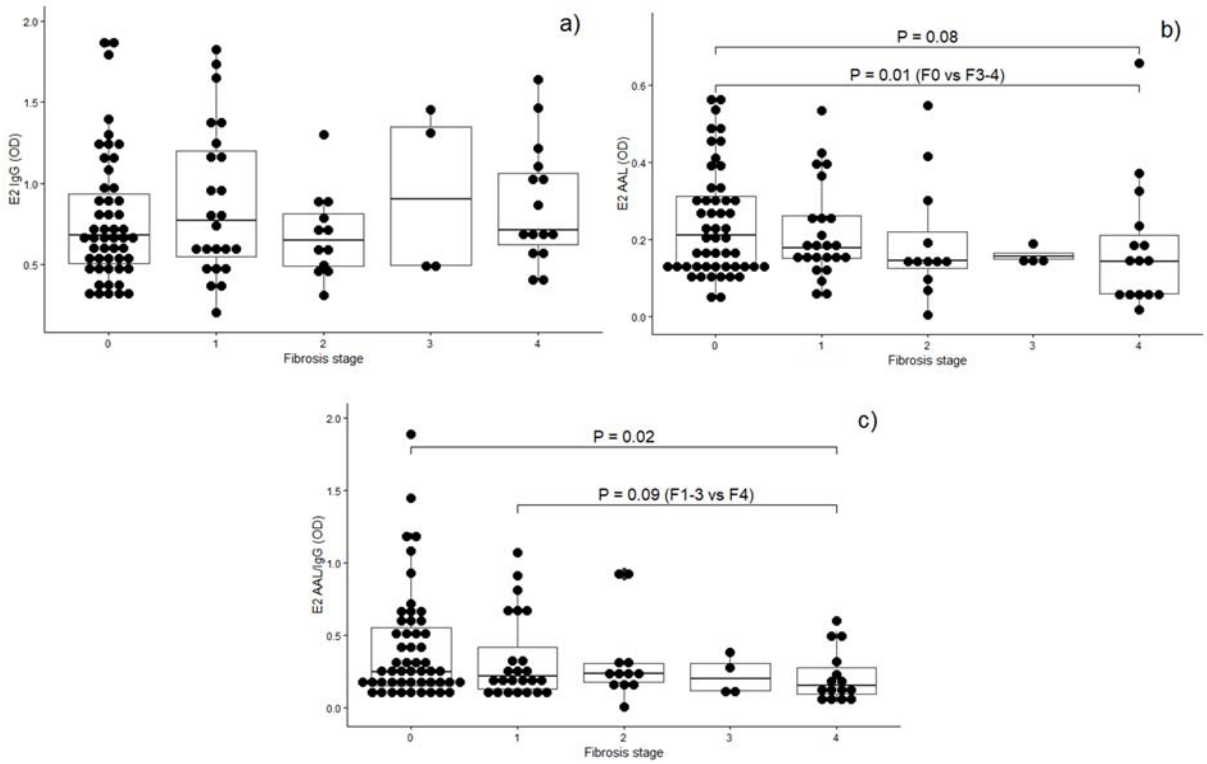

Figure 1: The E2-specific IgG antibody level and AAL reactivity by hepatic fibrosis stage: (a) E2-Ab level; (b) E2-Ab AAL reactivity; (c) E2-Ab AAL reactivity/E2-Ab IgG ratio.
Medians, ranges and quartiles are shown and P values are indicated for significant differences (P ≤0.05) and trends (P ≤0.1).

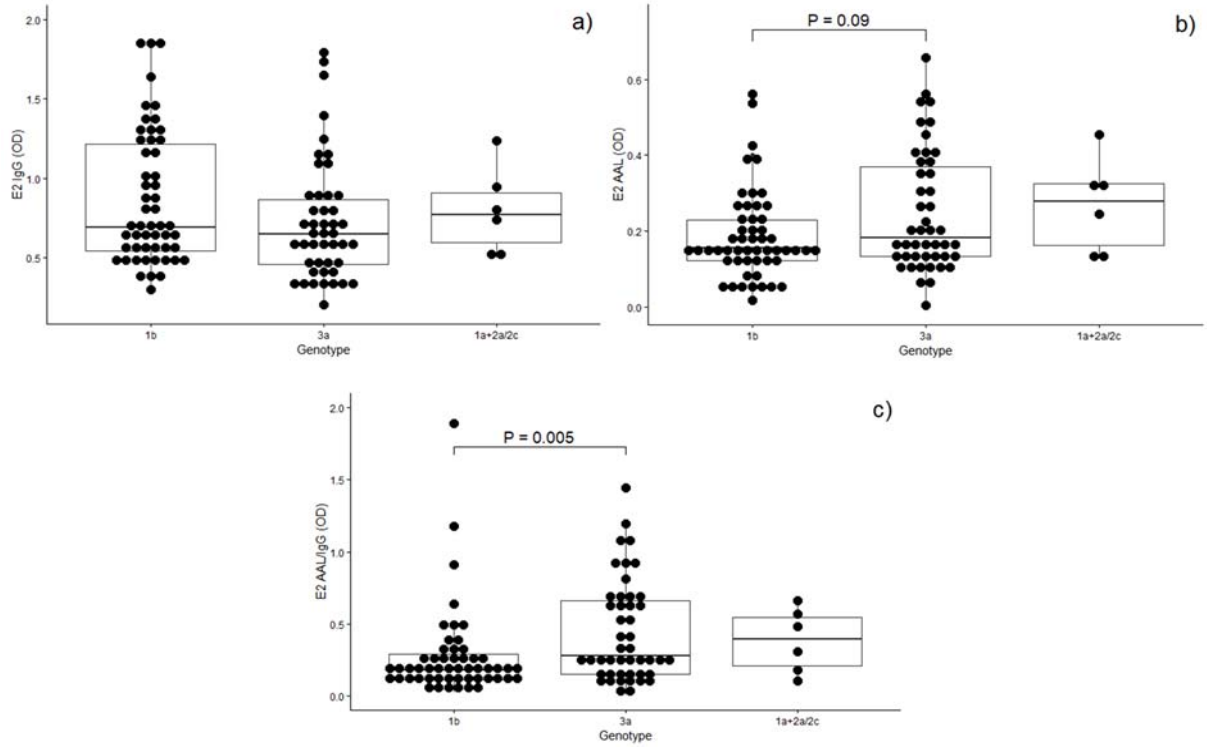

Figure 2: The E2-specific IgG antibody level and their AAL reactivity by HCV genotype: (a) E2-Ab level; (b) E2-Ab AAL reactivity; (c) E2-Ab level/E2-Ab AAL reactivity ratio.

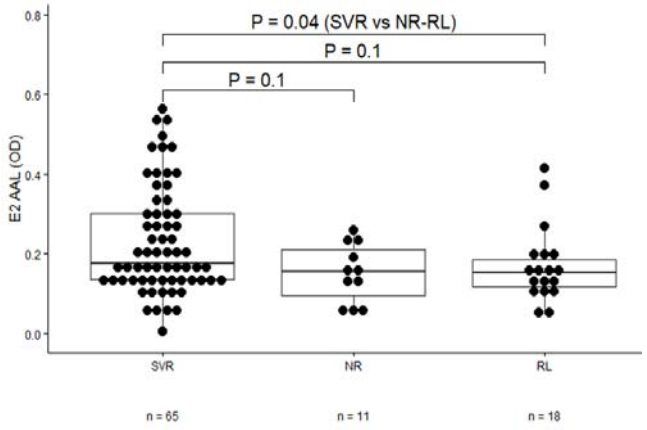

Figure 3: The E2-specific Abs AAL reactivity and IFN-RBV therapy efficacy. SVR: Sustained Virologic Response; NR: No Response; RL: Relapse. Medians, ranges and quartiles are shown and P values are indicated for significant differences.

(Figure 2b and 2c).

Patients with the sustained virological response to virotherapy showed a clear trend to a higher level of E2-AAL reactivity compared with NR and RL groups (Figure 3). Of note, regarding patients with the sustained virological response, the level of E2 Abs was significantly lower in patients with 3a genotype: OD = 0.99 + 0.46 (Mean + SD) *vs.* OD = 0.72+0.42 in 1b GT, P = 0.017.

The SNA reactivity of E2 Abs was also significantly lower in the terminal (F4) stage of liver fibrosis (Figures 4a and 4b) compared with both F0 or earlier (F1-3) fibrosis stages. The E2 antibody SNA reactivity was more informative in the discrimination of F4 from the F0 group than E2-AAL reactivity: ACC = 0.726, P = 0.02 and ACC = 0.597, P = 0.109, for SNA and AAL, respectively. The relationship between the E2-SNA and E2-AAL reactivities was analyzed by multiple regression analysis, however, it did not improve the discrimination between these groups (F4 *vs.* F0: ACC = 0.708, P = 0.025) probably due to a unidirectional change of both parameters. This was also true for comparison of other fibrosis stages where ACC values did not exceed 0.63 and the P values were higher than 0.23 in all comparisons (data not shown).

The interaction between the SNA and AAL reactivities was further evaluated using the E2-SNA/E2-AAL ratio. Higher ratio values were observed in the advanced stages of fibrosis (Figure 5a) and in patients infected with HCV 1b genotype (Figure 5b). The sustained viral responders revealed a lower E2-SNA/E2-AAL ratio compared with the NR group (P = 0.03) or the NR + RL combined group (P = 0.01) (Figure 5c).

Among patients infected with HCV 3a genotype who demonstrated the sustained virological response a higher E2-SNA/E2-AAL ratio was found in the F2-F4 stage of fibrosis compared with both F0 and F1 stages (P = 0.004-0.006) (Figure 6a). However, the small number of patients with the F2-4 stage of fibrosis (n = 6) did not allow a final conclusion to be drawn. The 1b genotype patients in this SVR group did not show such an increase or any association with the fibrosis stage (Figure 6b).

Compared with patients who showed no response (NR) to therapy or relapse (RL) (Table 1) the SVR response was more often observed in patients infected with HCV 3a genotype (36 of 42 *vs.* 24 of 47, $\chi^2 = 10.59$, df 1, P = 0.001). In contrast, patients with relapse were more often found in the 1b GT group: 26% versus 11.1% in patients of 3a GT ($\chi^2 = 4.54$, df-1, P = 0.033).

The receiver operator characteristic curve (ROC) analysis showed that the association of E2-SNA/E2-AAL ratio changes with the effect of virotherapy was detected mostly in patients with HCV 1b GT (Figure 7a and 8c). In this group, patients with a good response to therapy (SVR) significantly differed from non-responders and those with relapse (ACC = 0.66, P = 0.04), while in the 3a GT group no differences between SVR and NR + RL groups where established (ACC = 0.51) (Figure 7b and 8a). Thus, the decreased values of E2-SNA/ E2-AAL ratio in 1b GT patients (i.e. a higher E2 Ab sialylation) may predict the SVR response to (IF-RBV) virotherapy (Figure 7a and 8c).

Discussion

The various non-invasive biomarkers for liver fibrosis that mirror the changes in liver extracellular matrix turnover, including hepatocyte apoptosis, and the functional alterations in the liver are

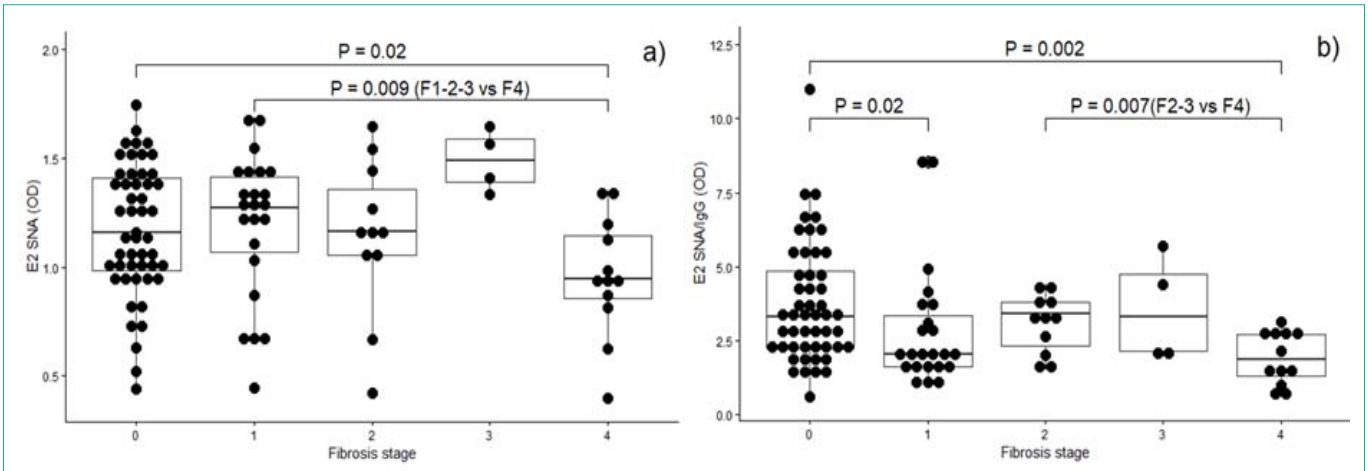

Figure 4: The E2-Ab SNA reactivity by hepatic fibrosis stage: (a) E2-SNA binding (OD); (b) E2-SNA binding/E2 IgG level ratio.

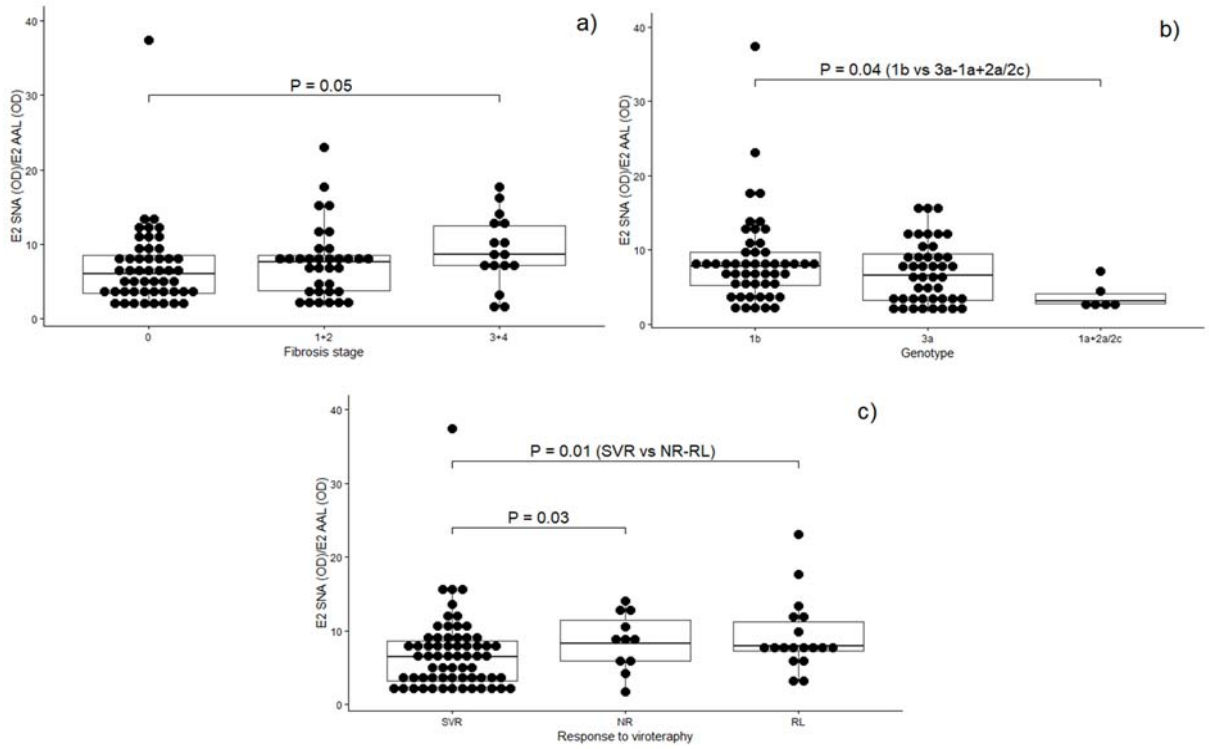

Figure 5: The ratio of E2-Ab SNA reactivity to E2-Ab AAL reactivity by: (a) hepatic fibrosis stage; (b) HCV genotype; (c) response to virotherapy.

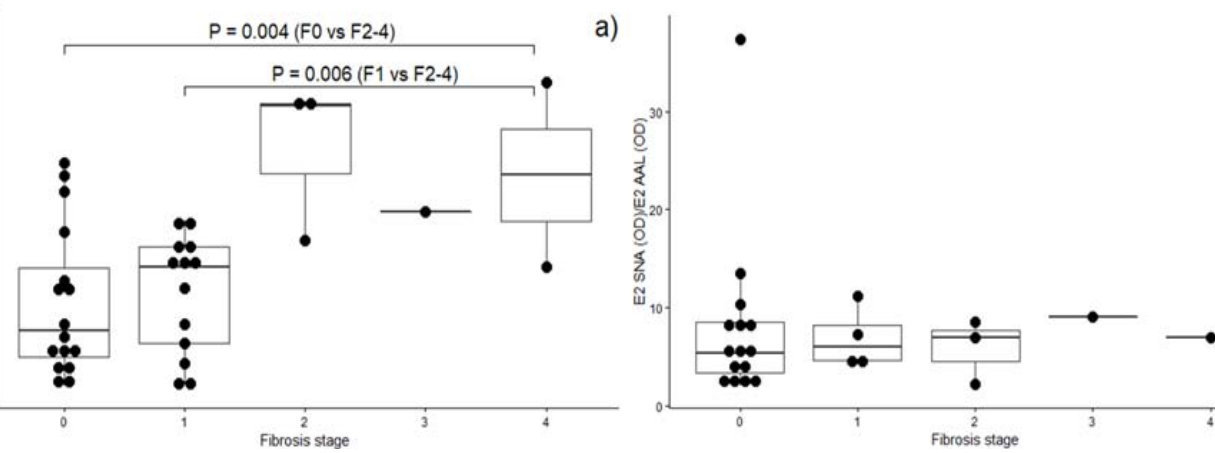

Figure 6: The ratio of E2-Ab SNA to E2-Ab AAL reactivity (E2-SNA/E2-AAL ratio) in the SVR group of patients infected with: (a) HCV 3a; (b) HCV 1b genotype, by hepatic fibrosis stage.

described and validated using liver biopsy as the reference standard [19-21]. However, in general, these markers show insufficient accuracy to discriminate among different stages of fibrosis.

The immune system-mediated inflammatory reactions are involved in the pathogenesis of liver injury [22,23]. The important role of anti-envelope Abs response in the resistance to HCV infection has been demonstrated [1,3,4-7]. It is to note, however, that the level of HCV neutralizing antibodies did not differ significantly between HCV infected patients with either no/mild fibrosis or cirrhosis [24]. In this study, the level of E2-Abs was also rather similar in patients with any stage of fibrosis or without it, irrespective of HCV genotype. We have shown recently that the E2 Ab sialylation is dramatically decreased in the late (F4) stage of fibrosis [17]. As far as we know, no data still exists about possible structural changes in other E2-Ab glycans during the liver damage progression. Thus it remains still rather unclear to what extent the glycodiversity of neutralizing E2-specific Abs may be related to the progression of liver damage as well as to virotherapy outcome.

The glycosylation profile of antibodies is a well-known factor of their functional activity [8,9,25,26]. The diverse clinical effect of

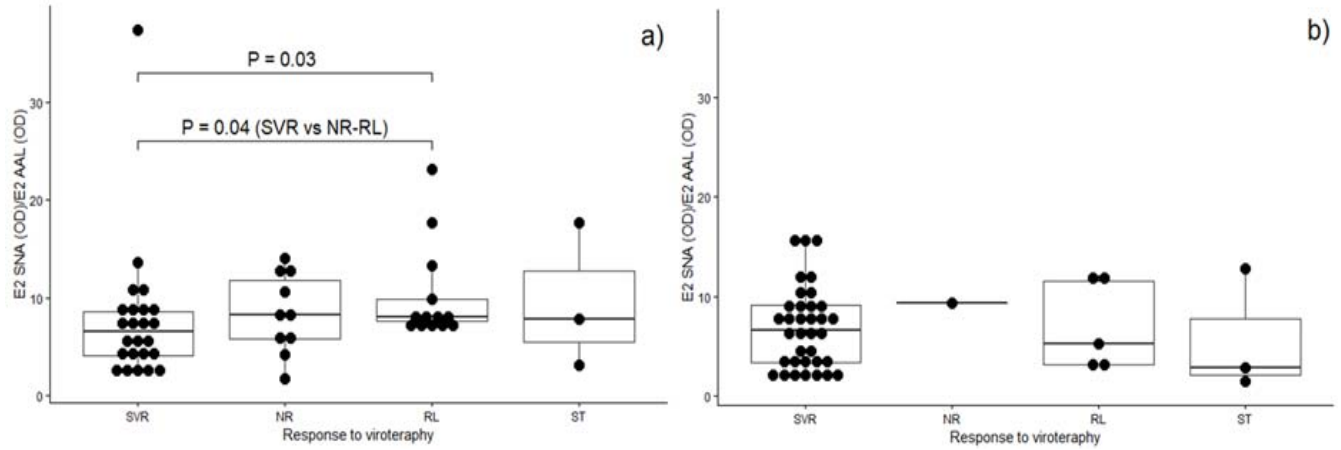

Figure 7: The ratio of E2-Ab SNA to E2-Ab AAL reactivity and response to virotherapy in patients infected with: (a) HCV 1b; (b) HCV 3a genotype.

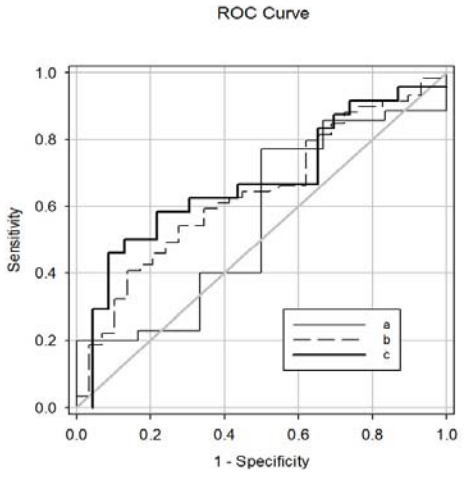

Figure 8: The accuracy of discrimination between the SVR and (NR + RL) groups based on E2-SNA/E2-AAL ratio and the ROC analysis by HCV genotype: (a) HCV 3a GT: sensitivity 0.51, specificity 0.50 ACC 0.51; (b) Combined HCV 1b + 3a GT group: sensitivity 0.61, specificity 0.62, ACC 0.61; (c) HCV 1b GT: sensitivity 0.63, specificity 0.70, ACC 0.66.
SVR: Sustained Virologic Response; NR: No Response; R: Relapse.

different Ab glyco-subsets has been demonstrated in autoimmunity, infections, and cancer [12,27-30]. We hypothesized that the profiling of two important glycosylation sites of E2 Abs, namely sialylation and core fucosylation, as well as their mutual relationship might improve the non-invasive evaluation of liver damage and virotherapy efficacy prediction.

The analysis of the E2 Ab reactivity to sialo- and fucose-specific lectins (SNA and AAL) showed that both the sialylation and fucosylation of E2 Abs was decreased in the late stages of liver fibrosis. However, despite this similarity, there was no significant correlation between the reactivities of both lectins, suggesting that the glycosylation changes do not coincide at an individual level and obviously occur independently. It appeared that the relative prevalence of E2 Abs sialylation, i. e. the higher SNA/AAL ratio, was characteristic of the late stages of fibrosis and was more often observed in patients with HCV 1b GT (Figures 5a and 5b). In contrast, the 3a genotype patients revealed a higher AAL reactivity and a lower SNA/AAL ratio, respectively. Besides, a majority of patients with the sustained virological response also belonged to the 3a GT group (Figure 7b). Notably, the level of E2 Abs was not dependent on HCV genotype (Figure 2a).

The SVR response was more often observed in patients with 3a genotype and mostly in those who had neither fibrosis nor its early (F1) stage (Figure 6). At the same time, patients with a good response to therapy showed a lower SNA/AAL ratio (i.e. a higher E2 Ab sialylation) compared with those with no response or relapse (Figure 5c).

We reported recently that some natural antiglycan Abs (TF, αGal) demonstrated changes that were quite opposite to those of E2 Ab glycosylation, and their combination could appreciably improve the discrimination between the early and late stages of hepatic fibrosis [19]. Therefore, we assumed that the multiple regression analysis using SNA and AAL reactivity data could be beneficial in assessing the stage of fibrosis. However, the combination of these two parameters in the present study did not improve the discrimination ability. At the same time, the ROC analysis using the SNA/AAL ratio data surprisingly showed an association with virotherapy outcome (Figure 7 and 8). Namely, better outcome (SVR response) should be expected in HCV 1b GT patients with a lower SNA/AAL ratio (Figure 7a). The differences between the SVR and NR+RL groups were observed mostly in HCV 1b GT patients (Figure 8c) who demonstrated a lower SNA/AAL ratio in the SVR subgroup (Figure 7a). The HCV 3a GT patients, who in general showed a higher proportion of SVR responders (Table 1), revealed a decreased SNA/AAL ratio compared with the 1b GT group (Figure 5b).

Thus, our findings give evidence of that the sialylation of E2 Ab is clearly associated with hepatic fibrosis progression whereas the sialylation/fucosylation balance (ratio) is related to IF-RBV virotherapy efficacy. Besides, these associations are also dependent on HCV genotype. We suggest that a decrease of the SNA reactivity of E2 Abs in the late stages of hepatic fibrosis reflects a higher degree of inflammation due to a pro-inflammatory activity of hypo-sialylated antibodies.

We conclude that based on the AAL and SNA reactivities of

E2 Abs as well as on their interrelationships the discrimination between the early and late stages of fibrosis is possible. The decrease of E2 Ab SNA reactivity is more informative in this respect, being a good marker of advanced liver fibrosis [18]. It turns out that not the level of E2 Abs per se but rather changes in their sialylation and fucosylation can be clinically useful for evaluation of liver damage and treatment outcome prediction. The infection with various HCV genotypes shows different E2 Ab glycosylation profiles as well as different responses to therapy. These findings can partially explain why the analysis of only the level of E2 antibodies does not always make it possible to predict the course of infection and the efficacy of treatment. This implies that when analyzing the role of E2 antibodies in the course of HCV infection, it is necessary to take into account the individual characteristics of their glycosylation, the sialylation/fucosylation ratio, as well as HCV genotype.

We suppose that a more detailed analysis of E2 Ab glycoforms may improve the non-invasive discrimination between the different stages of liver damage and prediction of HCV hepatitis therapy efficacy. The clinical significance of these findings will require further research, in particular, an experimental analysis of the neutralizing activity and protective effect of various E2-specific antibody glycosubsets. One of the future promising approaches could be the construction of E2-Ab glycoforms with the predicted potential to improve the immunotherapy of HCV infection.

Conclusion

In conclusion, in this paper, significant differences in the glycosylation of E2 antibodies in patients infected with two predominant HCV genotypes in Estonia (1b and 3a), as well as the association of these changes with the progression of liver fibrosis and the efficacy of IF-RBV virotherapy were found and dicussed.

Declaration

Data availability: The data that support the findings of this study are available on request from the corresponding author.

Conflicts of interest: The authors have approved this version of the manuscript and declare that there is neither conflict of interest nor financial gain regarding the publication of this paper.

Authors' contributions: OK proposed the research idea, analyzed and interpreted the data, and finalized the manuscript. JJ designed and performed the research, analyzed the data and wrote the manuscript. BS performed the statistical ROC analysis and multiple regression analysis. JG analyzed and discussed the data and revised the manuscript.

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References

1. Pestka JM, Zeisel MB, Bläser E, Schürmann P, Bartosch B, Cosset FL, et al. Rapid induction of virus-neutralizing antibodies and viral clearance in a single-source outbreak of hepatitis C. Proc Natl Acad Sci USA. 2007; 104: 6025-6030.
2. Ball JK, Tarr AW, McKeating JA. The past, present and future of neutralizing antibodies for hepatitis C virus. Antiviral Research. 2014; 105: 100-111.
3. Swann RE, Mandalou P, Robinson MW, et al. Anti-envelope antibody responses in individuals at high risk of hepatitis C virus who resist infection. Journal of Viral Hepatitis. 2016; 23: 873-880.
4. Eliyahu S, Sharabi O, Elmedvi S, Timor R, Davidovich A, Vigneault F, et al. "Antibody Repertoire Analysis of Hepatitis C Virus Infections Identifies Immune Signatures Associated With Spontaneous Clearance". Front Immunol. 2018; 9: 3004.
5. Wahid A, Dubuisson J. Virus-neutralizing antibodies to hepatitis C virus. Journal of Viral Hepatitis. 2013; 20: 369-376.
6. Olbrich A, Wardemann H, Böhm S, et al. Repertoire and Neutralizing Activity of antibodies Against Hepatitis C Virus E2 Peptide in Patients with Spontaneous Resolution of Hepatitis C. The Journal of Infectious Diseases. 2019; 220: 1209-1218.
7. Walker MR, Leung P, Eltahla AA, et al. Clearance of hepatitis C virus is associated with early and potent but narrowly-directed, Envelope-specific antibodies. Scientific Reports. 2019; 9: 13300.
8. Kaneko Y, Nimmerjahn F, Ravetch JV. Anti-inflammatory activity of immunoglobulin G resulting from Fc sialylation. Science. 2006; 313: 670-673.
9. Seeling M, Brückner C, Nimmerjahn F. Differential antibody glycosylation in autoimmunity: sweet biomarker or modulator of disease activity? Nature Reviews Rheumatology. 2017; 13: 621-630.
10. Markina YV, Gerasimova EV, Markin AM, Glanz VY, Wu WK, Sobenin IA, et al. Sialylated Immunoglobulins for the Treatment of Immuno-Inflammatory Diseases. Int J Mol Sci. 2020; 21: 5472.
11. Cobb BA. The history of IgG glycosylation and where we are now. Glycobiology. 2020; 30: 202-213.
12. Shade K-T, Anthony R. Antibody glycosylation and inflammation. Antibodies. 2013; 2: 392-414.
13. Knopf J, Biermann MHC, Munoz LE, Herrmann M. Antibody glycosylation as a potential biomarker for chronic inflammatory autoimmune diseases. AIMS Genetics. 2016; 3: 280-291.
14. Maverakis E, Kim K, Shimoda M, et al. Glycans in the immune system and the altered glycan theory of autoimmunity: a critical review. Journal of Autoimmunity. 2015; 57: 1-13.
15. Pereira NA, Chan KF, Lin PC, Song Z. The "less-is-more" in therapeutic antibodies: Afucosylated anti-cancer antibodies with enhanced antibody-dependent cellular cytotoxicity. MAbs. 2018; 10: 693-711.
16. Li C, Chong G, Zong G, Knorr DA, Bournazos S, Aytenfisu AH, et al. Site-Selective Chemoenzymatic Modification on the Core Fucose of an Antibody Enhances Its Fcγ Receptor Affinity and ADCC Activity. J Am Chem Soc. 2021; 143: 7828-7838.
17. Kurtenkov O, Jakovleva J, Sergejev B, Geller J. "Association of the Sialylation of Antibodies Specific to the HCV E2 Envelope Glycoprotein with Hepatic Fibrosis Progression and Antiviral Therapy Efficacy". Dis Markers. 2020: 8881279.
18. Kurtenkov O, Jakovleva J, Sergejev B, Geller J. Sialylation of HCV E2 glycoprotein-specific and natural anti-glycan (TFaGal) antibodies as signatures of livar gamage. J Hepat Res. 2021; 6: 1043.
19. Chin JL, Pavlides M, Moolla A, Ryan JD. Non-invasive Markers of Liver Fibrosis: Adjuncts or Alternatives to Liver Biopsy? Front Pharmacol. 2016; 7: 159.
20. Valva P, Ríos DA, De Matteo E, Preciado MV. Chronic hepatitis C virus infection: Serum biomarkers in predicting liver damage. World J Gastroenterol. 2016; 22: 1367-1381.
21. Loomba R, Adams LA. Advances in non-invasive assessment of hepatic fibrosis. Gut. 2020; 69: 1343-1352.
22. Mengshol JA, Golden-Mason L, Rosen HR. Mechanisms of Disease: HCV-

induced liver injury. Nat Clin Pract Gastroenterol Hepatol. 2007; 4: 622-634.

23. Irshad M, Gupta P, Irshad K. Immunopathogenesis of Liver Injury during Hepatitis C Virus Infection. Viral Immunol. 2019; 32: 112-120.

24. Pedersen J, Lundbo LF, Krarup H, Bukh J, Weis N. Neutralizing antibodies in patients with chronic hepatitis C and correlation to liver cirrhosis and estimated duration of infection. J Med Virol. 2016; 88: 1791-1803.

25. Böhm S, Schwab I, Lux A, Nimmerjahn F. The role of sialic acid as a modulator of the anti-inflammatory activity of IgG. Seminars in Immunopathology. 2012; 34: 443-453.

26. Plomp R, Ruhaak LR, Uh HW, et al. Subclass-specific IgG glycosylation is associated with markers of inflammation and metabolic health. Scientific Reports. 2017; 7: 12325.

27. Kodar K, Stadlmann J, Klaamas K, Sergeyev B, Kurtenkov O. Immunoglobulin G Fc N-glycan profiling in patients with gastric cancer by LC-ESI-MS: relation to tumor progression and survival. Glycoconjugate Journal. 2012; 29: 57-66.

28. Kurtenkov O, Izotova J, Klaamas K, Sergeyev B. Increased sialylation of anti-Thomsen-Friedenreich antigen (CD176) antibodies in patients with gastric cancer: a diagnostic and prognostic potential. Biomed Res Int. 2014; 2014: 830847.

29. Alter G, Ottenhoff THM, Joosten SA. Antibody glycosylation in inflammation, disease and vaccination. Seminars in Immunology. 2018; 39: 102-110.

30. Dekkers G, Rispens T, Vidarsson G. Novel concepts of altered immunoglobulin G galactosylation in autoimmune diseases. Frontiers in Immunology. 2018; 9.