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Galectin-9 in autoimmune hepatitis

Correlation between serum levels of galectin-9 and M2BPGi in patients with autoimmune hepatitis

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Abstract

Autoimmune hepatitis (AIH) is a disorder of unknown etiology in which immune-mediated liver damage progresses to cirrhosis or hepatocellular carcinoma (HCC). The mainstay therapy for AIH is steroids and other immunosuppressive treatments. Currently, there are no validated markers for monitoring immune-mediated hepatic inflammation. Galectin-9 has recently been identified as a potential biomarker in patients with chronic liver disease. The objective of this study was to determine whether Galectin-9 and other serum proteins are associated with active disease in AIH patients.

We enrolled 77 Japanese patients with well-documented AIH who were identified from the National Hospital Organization-AIH-liver-network database, as well as 32 patients with chronic hepatitis C (CHC), 27 patients with SLE, and 17 healthy control subjects. Serum levels of galectin-9, and markers of liver injury were measured and compared between groups.

Serum levels of galectin-9 were significantly higher in AIH patients than in CHC patients (13.8 ± 4.9 ng/mL vs 8.9 ± 3.0 ng/mL, $P < .001$) or healthy controls (13.8 ± 4.9 ng/mL vs 5.0 ± 1.3 ng/mL, $P < .001$). In AIH group, serum galectin-9 levels weakly correlated with alanine aminotransferase levels or total bilirubin (TB) and strongly correlated with C–X–C motif chemokine 10 (CXCL10) and Mac-2 binding protein glycosylation isomer (M2BPGi) levels, but did not correlate with the histological grade of liver fibrosis. Steroid treatment of AIH patients significantly reduced serum galectin-9 levels (14.1 ± 4.9 ng/mL vs 8.3 ± 3.8 ng/mL, $P < .001$). SLE patients exhibited higher galectin-9 levels, whereas the galectin-9 levels did not correlate with liver function tests such as alanine aminotransferase levels.

Serum galectin-9 correlated with disease status in AIH patients and could thus be useful biomarkers to detect hepatic autoimmunity. Because circulating galectin-9 reflects autoimmune-mediated inflammation, it may have additional utility as a biomarker for other autoimmune disorders.

Editor: Yan Li.

Supplemental Digital Content is available for this article.

Declarations: Ethical Approval and Consent to participate: The study was approved by the Ethics Committee of the NHO Central Internal Review Board and participating NHO liver-network hospitals (H30-0313001). Written informed consent was obtained from each individual.

Consent for publication: Not applicable.

Competing interests: KM has received research grants from Chugai, Pfizer, and AbbVie. Rest of the authors declares that they have no competing interests.

Funding: This work was supported by a grant from the National Hospital Organization.

The authors report no conflicts of interest.

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Medicine (2019) 98:35(e16924)

Received: 12 January 2019 / Received in final form: 8 July 2019 / Accepted: 26 July 2019

http://dx.doi.org/10.1097/MD.0000000000016924

Abbreviations: AIH =autoimmune hepatitis, ALT = alanine aminotransferase, CXCL10 = C-X-C motif chemokine 10, Gal-9 = Galectin-9, M2BPGi = Mac-2 binding protein glycosylation isomer, NHO = National Hospital Organization, SLE = systemic lupus erythematosus, Tim-3 = T-cell immunoglobulin and mucin domain 3.

Keywords: autoimmune hepatitis, CXCL10, cytokine, galectin-9, M2BPGi, systemic lupus erythematosus

1. Introduction

Galectin-9 (Gal-9) is a conserved S-type lectin that plays multiple immune modulatory roles in both innate and adaptive immune responses.[1] Gal-9 has been shown to reduce the abundance of Th1 and Th17 immune effector cells by binding to T-cell immunoglobulin and mucin domain 3 (Tim-3) and inducing programmed cell death.[2] Also, Gal-9 has therapeutic effects in animal models of autoimmune disease,[3] and suppresses experimental autoimmune arthritis by manipulating the functions of T cells and macrophages.[4]

Autoimmune hepatitis (AIH) is a progressive inflammatory liver disorder characterized by circulating autoantibodies and the presence of interface hepatitis histologically.[5] Gal-9 is highly expressed in the liver[1] and is involved in the hepatic inflammatory processes.[6–8] Because Gal-9 expression is elevated during chronic inflammation and fibrosis and its immunoregulatory functions appear to play a role in hepatic disorders,[9,10] circulating Gal-9 levels have been proposed to be a biomarker for chronic liver diseases. More recently, Gal-9 was also shown to be a novel biomarker of the interferon (IFN) signature in patients with systemic lupus erythematosus (SLE),[11] suggesting that its levels might correlate with a number of autoimmune conditions, including hepatic disorders.

The present study had three major aims: to examine the levels of Gal-9 and several additional inflammatory/immune mediators in serum samples from patients with AIH; to compare them with the levels in healthy subjects and patients with CHC; and to determine the correlations between circulating Gal-9 levels and other inflammatory/immune mediators, thereby elucidating the potential utility of Gal-9 as a serum biomarker for AIH.

2. Materials and methods

2.1. Study population

Patients with well-documented and untreated AIH were enrolled from the National Hospital Organization (NHO)-AIH-liver-network database, a multicenter registry for Japanese patients with AIH.[12] The diagnosis of AIH was made according to the diagnostic criteria defined by International Autoimmune Hepatitis Group,[13] including a raised IgG level and presence of organ-specific and non-organ-specific autoantibodies, presence of interface hepatitis and portal plasma cell infiltration on liver histology and the absence of other liver diseases of known etiology. Patients were excluded from the study if there was histological evidence of cholangitis or non-alcoholic steatohepatitis. Patients positive for hepatitis B virus surface antigen or HCV RNA were also excluded. Patients with other causes of liver disease, such as excess alcohol or drug use, were excluded based on reviews of their appropriate history and investigations. As controls, patients with CHC (untreated CHC, n=32; female/male=16/16; mean age, 56.2±7.8 years; aspartate aminotransferase [AST], 43.4±23.6 IU/L; alanine transaminase [ALT], 54.1 ±39.0 IU/L), TB, 0.8±0.4 mg/day were included. As controls, 31 healthy subjects (10 males, 21 females, mean age of 42.0±7.6 years) and as controls for autoimmune disease, SLE patients (n=27; female/male=21/6) satisfying American College of Rheumatology classification criteria were included. The baseline characteristics of SLE patients are listed in Supplementary file Table 1, http://links.lww.com/MD/D200. Mean age was 35.2± 13.4 years, mean age at diagnosis was 30.0±12.5 years. AST, 38.1±35.5 IU/L; ALT, 38.4±72.9 IU/L; TB, 0.8±0.8 mg/day. The study was approved by the Ethics Committee of the NHO Central Internal Review Board and participating NHO liver-network hospitals. Written informed consent was obtained from each individual.

2.2. Histological assessments

Liver biopsy and laboratory tests were obtained at baseline prior to treatment. In the histological diagnosis of AIH, each specimen was assessed for inflammatory grading including the degree of portal inflammation, presence of interface hepatitis, and the degree of parenchymal inflammation, as well as the stage of fibrosis (0, absent; 1, expansion of fibrosis to parenchyma; 2, portal–central or portal–portal bridging fibrosis; 3, presence of numerous fibrous septa; and 4, multi-nodular cirrhosis) according to the criteria of Desmet et al.[14] Cirrhosis was diagnosed histologically when a loss of normal lobular architecture, reconstruction of hepatic nodules, and the presence of regenerative nodules were observed.

2.3. Enzyme-linked immunosorbent assay for sICAM-1, CXCL-10, and Galectin-9

Serum concentrations of CXCL10 and Galectin-9 were measured using human enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instruction.

2.4. Measurement of M2BPGi

Serum M2BPGi level was directly measured with the HISCL M2BPGi reagent kit (Sysmex, Kobe, Japan) using an automatic immunoanalyzer HISCL-5000 (Sysmex, Hyogo, Japan). M2BPGi levels were indexed using the following equation: Cut-off Index (C.O.I.)=([M2BPGi]sample-[M2BPGi]NC)/([M2BPGi]PC)-[M2BPGi]NC), where [M2BPGi]sample represents the M2BPGi count of the serum sample, PC is positive control, and NC is negative control.[15]

2.5. Statistical analysis

Correlations between continuous variables were analyzed by the Pearson correlation test. Results for non-normally distributed continuous variables were summarized as mean or medians (interquartile ranges) and were compared by the Mann–Whitney *U* test. Paired data were analyzed by

Table 1

Baseline characteristics of 77 Japanese AIH type 1 patients.

Characteristics	n = 77
Female, n/total (%)	63 (81.8)
Age, year, mean ± SD	63.0 ± 12.3
Biochemistry	
AST, IU/L, median (IQR)	471.8 (11–2010)
ALT, IU/L, median (IQR)	607.5 (12–2426)
ALP, IU/L, median (IQR)	450.9 (123–1208)
Total Bilirubin, mg/dL, median (IQR)	4.5 (0.4–25.1)
Albumin, g/dL, median (IQR)	3.9 (2.4–5.0)
IgG, mg/dL, median (IQR)	2361.2 (923–4966)
Prothrombin time, %, median (IQR)	82.6 (37.8–122.7)
Platelets, $10^4/\mu L$, median (IQR)	18.9 (3.4–40.4)
Serology	
ANA ≧ 1:40, n/total (%)	52/77 (67.5)
ASMA ≧ 1:40, n/total (%)	43/77 (55.8)
Histology	
Cirrhosis, n/total (%)	9/77 (11.7)
IAIHG criteria	
Score, median (IQR)	16.4 (9–22)
Prednisolone use, n (%)	75 (97.4)
Immunosuppressant use, n (%)	11 (14.3)
Cirrhosis, n (%)	9 (11.7)
Complications of collagen diseases (%)	18 (11.0)
Fibrosis (N)F0/F1/F2/F3/F4	N = 74 3/33/14/20/4
Activity (N)A0/A1/A2/A3	N = 74 0/11/27/36

ALP = alkaline phosphate, ALT = alanine aminotransferase, ANA = anti-nuclear antibody, ASMA = anti-smooth muscle antibody, AST = aspartate aminotransferase, IAIHG = International Autoimmune Hepatitis Group, IgG = immunoglobulin G, IQR = interquartile range.

non-parametric tests using the Wilcoxon signed-rank test for the comparison of paired data.

3. Results

3.1. AIH patients

Table 1 shows the demographic data of the 77 AIH patients. Among the 77 patients with type-1 AIH, 52 (67.5%) were positive (>1:40) for anti-nuclear antibodies. Nine patients (11.7%) had liver cirrhosis at the time of diagnosis.

3.2. Serum levels of Gal-9 in patients and healthy subjects

We compared serum Gal-9 levels in AIH patients, CHC patients, and healthy subjects using specific ELISA kits. As shown in Fig. 1, serum Gal-9 levels were significantly higher in AIH patients compared with CHC patients or healthy subjects. There was no significant gender difference in serum Gal-9 levels in AIH patients (male 14.3 ± 3.9 ng/mL, female 13.7 ± 5.1 ng/mL), as well as CHC patients (male 9.3 ± 3.6 ng/mL, female 8.5 ± 2.3 ng/mL) or SLE patients (male 17.3 ± 9.9 ng/mL, female 17.4 ± 9.0 ng/mL).

3.3. Relationships between serum Gal-9 levels, liver function markers, and inflammatory mediators

To investigate the relationship between Gal-9 and clinical parameters, we examined correlations between serum levels of Gal-9 and several liver function markers and inflammatory mediators. Among the liver function markers tested, we detected weak but significant correlations between Gal-9 and alanine aminotransferase (ALT) or TB levels (Fig. 2). Whereas there was no significant correlation between Gal-9 and IgG (data not shown). Among the inflammatory mediators, serum levels of Mac-2 binding protein glycan isomer (M2BPGi) and C-X-C motif chemokine 10 (CXCL10) were significantly correlated with Gal-9 levels in AIH patients (Fig. 3). Most importantly, serum Gal-9 levels were strongly correlated with M2BPGi levels in the AIH patients (Fig. 3A).

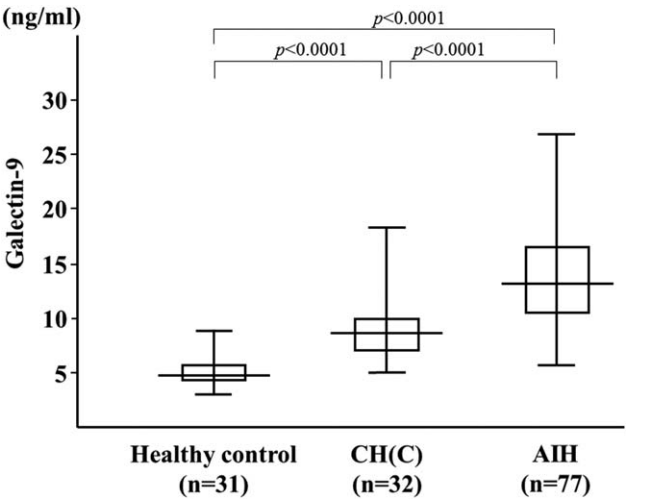

Figure 1. Serum levels of Gal-9 in AIH patients (n = 77), patients with chronic hepatitis C (HCV, n = 32) and healthy subjects (n = 18). The vertical lines indicate the range and the horizontal boundaries of the boxes represent the first and third quartiles. Results were compared by non-parametric Mann–Whitney *U* test.

3.4. Relationships between serum Gal-9 levels and liver fibrosis and necroinflammation scores

To evaluate whether serum Gal-9 correlated with liver histology, we grouped the AIH patients according to liver fibrosis stage (F0–F4). The mean serum concentrations of Gal-9 at each fibrosis stage were 13.4 ± 5.1 ng/mL for patients at F0–F1 stage, 13.3 ± 3.1 ng/mL for those at F2, and 15.6 ± 7.1 ng/mL for those at F3+F4; however, the differences in Gal-9 levels between liver fibrosis stages were not significantly different (Fig. 4A). Gal-9 values were also stratified by necroinflammatory grade (A1–A3). The mean serum concentrations of Gal-9 for necroinflammatory grades were 11.1 ± 4.5 ng/mL for A1, 15.1 ± 4.9 ng/mL for A2, and 14.3 ± 6.2 ng/mL for A3. Although the Gal-9 levels increased according to the necroinflammatory grade, the differences were not statistically significant (Fig. 4B).

3.5. Changes in Gal-9 by corticosteroid therapy

Circulating levels of Gal-9 were measured before and after corticosteroid therapy in paired serum samples from 57 AIH patients. Serum levels of Gal-9 were down-regulated by corticosteroid therapy and there was a significant difference in serum levels of Gal-9 before and after corticosteroid therapy in AIH patients (Fig. 5).

3.6. Association between serum Gal-9 levels and clinical parameters of SLE patients

Because Gal-9 was recently reported to be a novel biomarker of the IFN signature in patients with SLE,[11] we measured the levels of Gal-9, liver function markers, and inflammatory/immune mediators in SLE patients. As shown in Fig. 6, serum Gal-9 levels

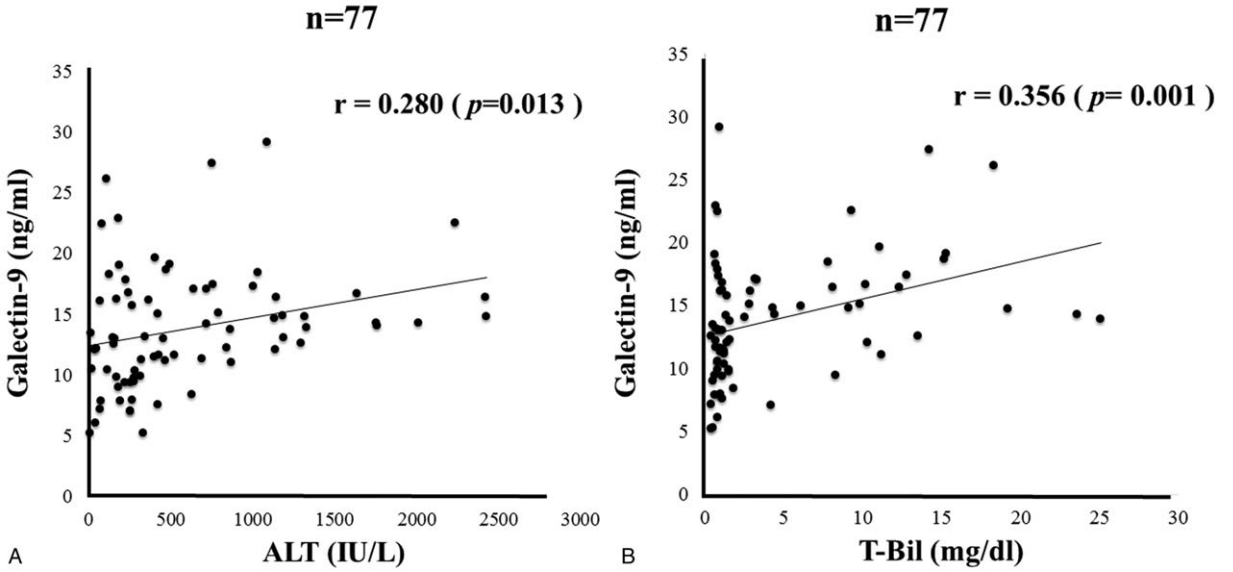

Figure 2. Correlations between serum levels of Gal-9 and ALT (A) or TB (B) levels in patients with AIH. Serum Gal-9 significantly correlated with serum ALT and TB level. Statistics and regression line are represented by the solid line. ALT = alanine aminotransferase, Gal-9 = galectin-9, T-Bil = total bilirubin.

were significantly higher in patients with SLE compared with the AIH patients. Moreover, Gal-9 levels in SLE patients were also positively correlated with M2BPGi levels (Fig. 7), whereas, they did not correlate with ALT (Fig. 8A) or TB levels (Fig. 8B), suggesting that the mechanisms underlying the increased concentrations of Gal-9 was different for SLE and AIH patients.

4. Discussion

Gal-9, a β-galactoside-specific lectin, is involved in liver inflammation, fibrosis, and tumorigenesis in patients with chronic liver disease.[1] Hepatic stellate cells, macrophages, and Kupffer cells are known to produce Gal-9 during liver fibrosis.[16] In addition, Gal-9 also has immunomodulatory properties through its interaction with T cells.[17] During viral hepatitis, Gal-9 modulates T cell immunity via binding to Tim-3, which induces T cell apoptosis.[18] Serum Gal-9 has been proposed to be a biomarker of fibrosis in patients with chronic liver diseases.[10] Also Gal-9 is known to plays crucial roles in tumor biology and is protective against HCC.[19]

Here, we showed not only that serum Gal-9 levels are elevated in AIH patients compared with CHC patients and healthy

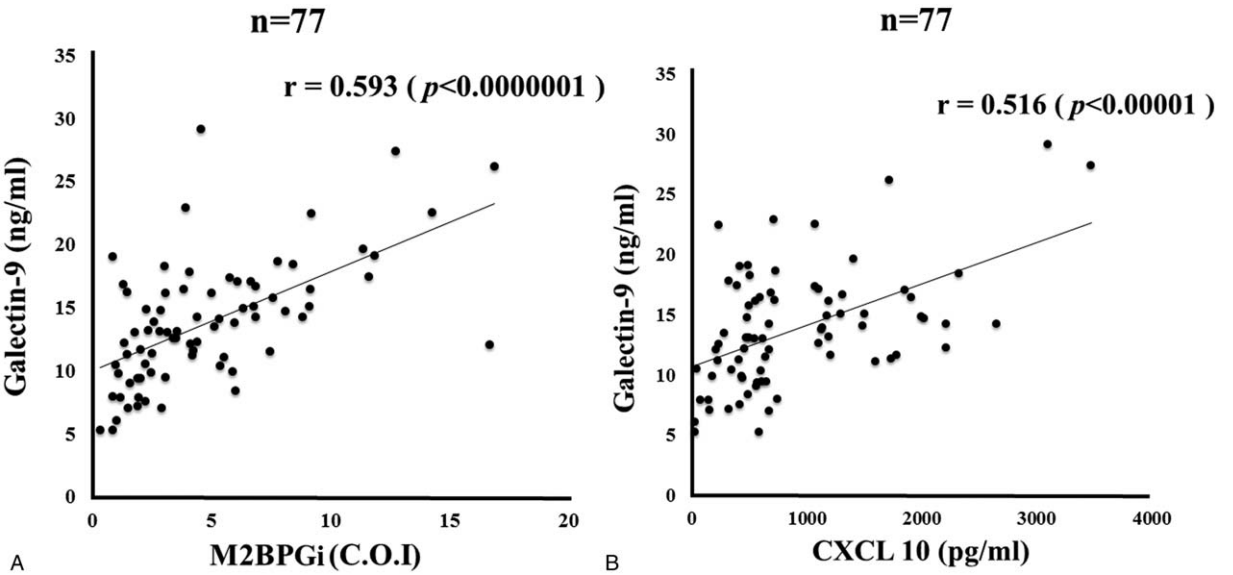

Figure 3. Correlations between serum levels of Gal-9 and M2BPGi (A) or CXCL 10 (B) in patients with AIH. Serum Gal-9 significantly correlated with serum levels of M2BPGi or CXCL-10. Statistics and regression line are represented by the solid line. Gal-9 = galectin-9, I CXCL10 = C-X-C motif chemokine 10, M2BPGi = Mac-2 binding protein glycosylation isomer.

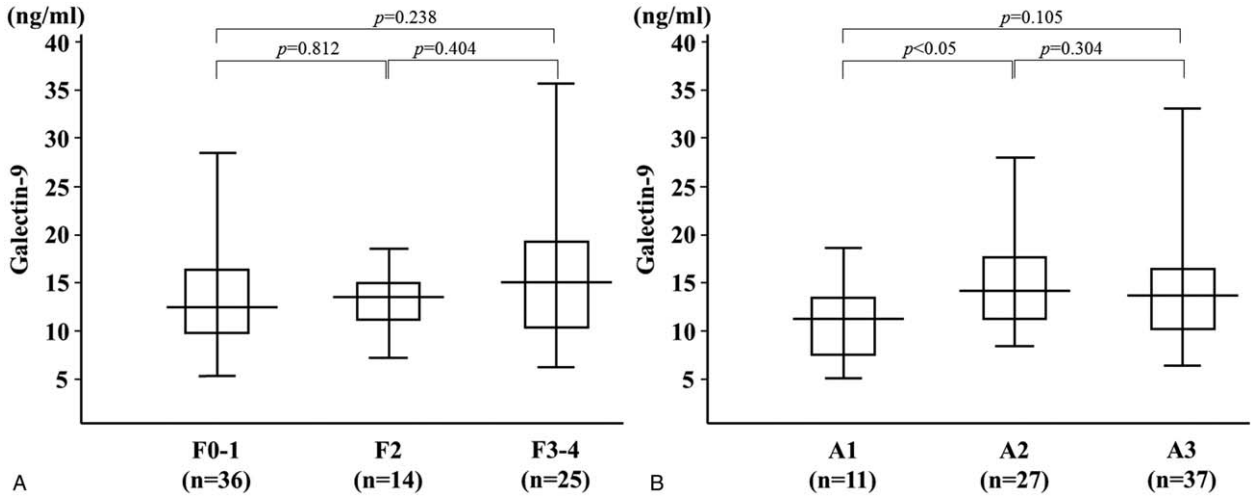

Figure 4. Serum levels of Gal-9 according to liver fibrosis stage (A) and liver inflammation grade (B). The vertical lines indicate the range and the horizontal boundaries of the boxes represent the first and third quartile. Results were compared by non-parametric Mann–Whitney *U* test. Gal-9 = galectin-9.

subjects but also that the Gal-9 levels in AIH patients correlated with the inflammatory mediators and liver injury markers, including M2BPGi, which is a known liver fibrosis marker.[20] We previously demonstrated the close relationship between serum levels of M2BPGi and immune-mediated hepatic injury in addition to liver fibrosis in AIH patients.[21] Serum Gal-9 levels increased in parallel with liver inflammation scores (grading) but not with liver fibrosis scores (staging). Moreover, serum Gal-9 levels were rapidly down-regulated by steroid therapy, suggesting that immune-mediated hepatic inflammation contributed to the elevated levels of Gal-9 in AIH patients. The function of Gal-9 is pleiotropic and varies with the pathophysiological context.[22] Thus, in AIH patients, circulating Gal-9 levels could be modulated by the acute phase of liver inflammation as well as by immunomodulatory factors such as cytokines or chemokines.

The cellular origin of serum Gal-9 in AIH patients is unknown, but is presumed to be hepatic stellate cells (HSCs), macrophages, and/or Kupffer cells, all of which have known roles in liver fibrosis.[23] Similar to other members of the galectin family, Gal-9 is thought to act as a bridge between cytokines and T cells.[24] In the present study, we detected significant correlations between serum Gal-9 and CXCL10 and M2BPGi levels in AIH patients, which raises the possibility that Gal-9 may be induced in autoimmune diseases. Mac-2BP (M2BP) was identified as a ligand of galectin-3 and this protein was also demonstrated to

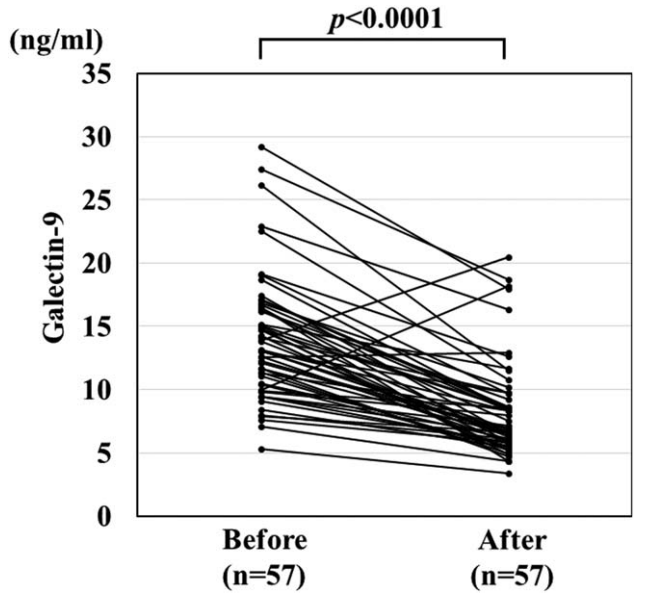

Figure 5. Changes in individual Gal-9 values before and after (4 weeks) steroid treatment in 57 AIH patients. Paired samples from the same subjects were compared by Wilcoxon signed-rank test. Gal-9 = galectin-9.

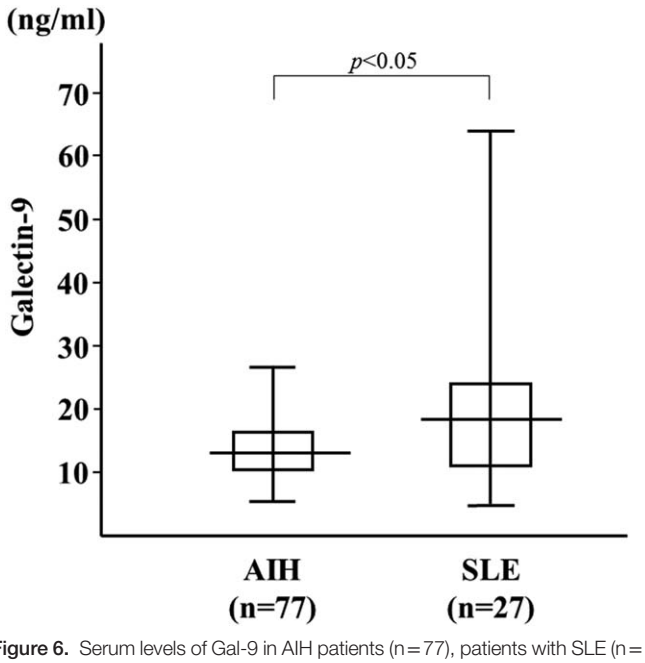

Figure 6. Serum levels of Gal-9 in AIH patients (n = 77), patients with SLE (n = 27). The vertical lines indicate the range and the horizontal boundaries of the boxes represent the first and third quartiles. Results were compared by non-parametric Mann–Whitney *U* test. Gal-9 = galectin-9.

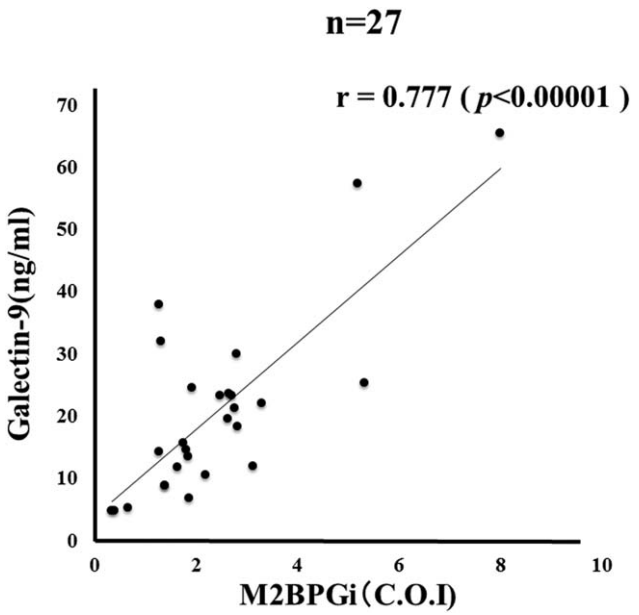

Figure 7. Correlations between serum levels of Gal-9 and serum M2BPGi levels in patients with SLE. Serum Gal-9 significantly correlated with serum M2BPGi levels. Statistics and regression line are represented by the solid line. Gal-9 = galectin-9, M2BPGi = Mac-2 binding protein glycosylation isomer.

bind other member of the galectin family, galectin-1, and galectin-7.[25] It is interesting to note a strong correlation between galectin-9 and M2BPGi in patients with AIH. In critical phase of viral infection, the levels of galectin-9 were demonstrated to be associated with IL-8 and CXCL-10.[26] Also galectin-9 and CXCL-10 are reported to be induced in viral infected endothelial cells.[27] It is possible that galectin-9 and M2BP could be linked in viral or autoimmune-mediated immunity via inflammatory cytokines. The detailed mechanism of galectin-9 and M2BP needs to be further determined in autoimmune disorders. Although the exact mechanism of autoimmune-associated Gal-9 production remains to be elucidated, different diseases may employ distinct immune mechanisms to elevate Gal-9 levels.

Recent work has demonstrated that Gal-9 is a biomarker of the IFN signature in SLE patients,[11] and, consistent with this, we found higher serum Gal-9 levels in SLE patients. In our study, we did not detect a correlation between serum Gal-9 levels and markers of liver injury (ALT and TB) in patients with SLE, suggesting that in the absence of liver injury, Gal-9 might be up-regulated in autoimmune diseases. In contrast, in patients with liver injury, Gal-9 may be derived from liver and could thus be a diagnostic biomarker for hepatic inflammation. Gal-9 has a variety of biological functions in innate and adaptive immunity.[28] Our data suggest the dichotomous role of Gal-9 in immune-mediated liver diseases, whereas elevated Gal-9 is evident in autoimmune diseases without liver injury. Further studies are needed to elucidate the pathophysiology of Gal-9 mediated immune-mediated disorders.

There are several limitations to this study. First, this is a retrospective study and AIH patients were enrolled from a limited number of hospitals (n = 9). Therefore, the impact of selection biases cannot be ignored. Second, the number of study participants was relatively small. Third, given the cross-sectional design of the study, the causal relationship between Gal-9 levels and the treatment responses or clinical outcomes of AIH patients could not be determined. Serum Galectin-3 concentrations were shown to be higher in women compared with men in the community.[29] Whereas there was no significant gender difference in Gal-9 concentrations in AIH patients as well as controls. Although our results could not demonstrate the gender difference in serum Gal-9 levels, our sample size is relatively

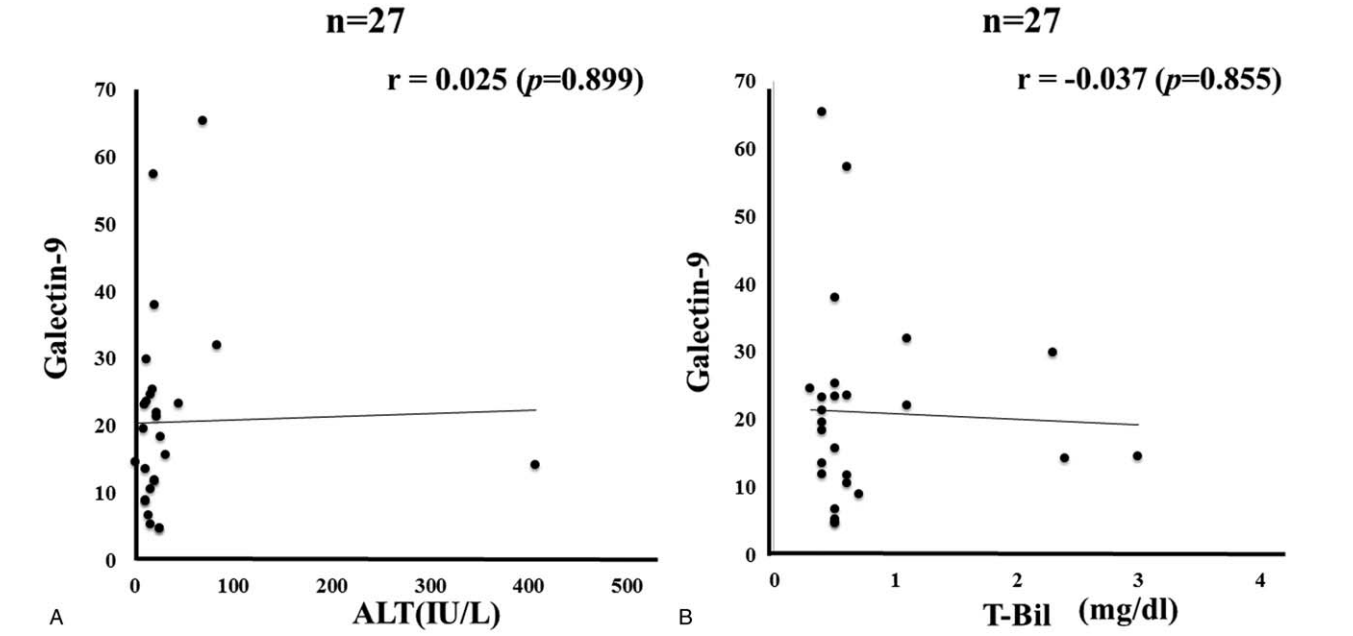

Figure 8. Correlations between serum levels of Gal-9 and ALT (A) or TB (B) levels in patients with SLE. Serum Gal-9 significantly correlated with serum ALT and TB level. Statistics and regression line are represented by the solid line. ALT = alanine aminotransferase, Gal-9 = galectin-9, T-Bil = total bilirubin.

small. Therefore, further studies utilizing a larger sample should be necessary.

5. Conclusion

In conclusion, we have identified a significant correlation between high serum levels of Gal-9 and M2BPGi in AIH patients. This finding provides further insights into the immunopathogenesis of AIH and suggests that Gal-9, in addition to M2BPGi, could be an important biomarker for monitoring hepatic inflammation. Our observations also shed light on the role of Gal-9 in immunity, which may at least partially explain the association between Gal-9 and cytokines involved in inflammation. Large-scale population-based studies will be needed to elucidate the mechanisms of action of Gal-9 in the pathogenesis of autoimmune diseases and to evaluate whether Gal-9 could be a novel biomarker for such diseases.

Author contributions

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Validation: Hiromasa Ohira.
Writing – original draft: Naoki Matsuoka.
Writing – review & editing: Kiyoshi Migita.

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