Reduced Circulating Soluble Receptor For Advanced Glycation End-products in Chronic Hepatitis B Are Associated with Hepatic Necroinflammation

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Abstract

The diagnosis and disease management of Chronic hepatitis B (CHB) remain challenging due to the elusive assessment of disease severity. Recently, soluble receptor for advanced glycation end-products (sRAGE) has been shown to be implicated in the inflammatory-immune response initiated by liver injury. Nonetheless, its natural behavior and clinical importance in CHB remain elusive. One hundred and twenty CHB patients and forty healthy controls (HCs) were enrolled, and the serum sRAGE as well as RAGE expressin in biopsy specimens from these subjects was analyzed, and correlation of sRAGE with clinical features as well as its potential predictive value for monitoring the CHB was also evaluated. Reduced serum sRAGE levels and decreased tissular RAGE expression were observed in CHB patients. sRAGE and RAGE were inversely correlated with gradually increased grades of hepatic necroinflammation as well as the routine indicator ALT. Furthermore, receiver operating characteristic (ROC) analysis showed that combination of ALT and sRAGE exerted better predictive power (area under the ROC curve (AUC) of 0.86) for hepatic necroinflammation than that of ALT (AUC of 0.82), sRAGE (AUC of 0.81) or sRAGE-to-ALT ratio (sRAGE/ALT) (AUC of 0.85) alone. More importantly, circulating sRAGE alone exerted valuable predictive power for hepatic moderate-to-severe necroinflammation in CHB patients but with normal ALT (AUC of 0.81) or minimally elevated ALT (AUC of 0.85). In conclusion, reduced serum sRAGE levels may imply an increased severity for necroinflammation, and it may serve as a potential alternative biomarker for monitoring hepatic necroinflammation in CHB.

Introduction

Persistent HBV infection leads to adverse outcomes including acute and chronic hepatitis B (CHB), hepatitis B associated liver cirrhosis (HBLC) and hepatocellular carcinoma (HCC), which remain a public concern that causes considerable liver-related morbidity and mortality especially in China[1, 2]. Since active hepatic necroinflammation is the dominant risk factor for driving liver cirrhosis and HCC in CHB patients, a precise evaluation during the initial stage of hepatic inflammation is vital for subsequent treatment and surveillance, representing a high priority and growing medical need.

Presently, wide arrays of tests such as invasive or noninvasive procedures, including liver biopsy or serum biomarker such as alanine aminotransferase (ALT), have been used to assess disease activity[3]. Since a liver biopsy is an invasive and painful procedure and carries a risk of potential complications, its application has been limited to mass screening[4]. Serum biomarkers such as ALT are extensively used due to their simple operation but have several limitations due to their poor specificity, low sensitivity or unsatisfactory accuracy, which are easily affected by environmental and human factors[5]. The clinical diagnosis for active CHB as well as the time points for antiviral treatment are predominantly dependent on persistent or intermittent elevated values of ALT[6]. Nevertheless, it is unfeasible for patients to receive therapy when they have a high viral load but with normal or minimal ALT levels. Therefore, there is still a requirement for more reliable, noninvasive, and cost-effective biomarkers for CHB disease activity interpretation.

The receptor for advanced glycation end products (RAGE), a multiligand pattern recognition receptor, can be stimulated by a variety of damage-associated molecular patterns (DAMPs), activating downstream cascades and triggering an inflammatory-immune response implicated in host defense against infections, inflammation and cardiometabolic disorders[7–9]. RAGE-ligand interactions also contribute to the progression of numerous types of hepatic disorders by inducing oxidative stress and subsequently evoking inflammation, including liver injury, steatosis, fibrosis and even hepatocarcinogenesis[10]. These reports suggest that blocking the RAGE-ligand interactions as well as downstream cascades could be a novel therapeutic target for various liver diseases.

Soluble RAGE (sRAGE), a splicing variant of the full-length receptor that contains only the extracellular domain of RAGE formed by proteolytic cleavage, counteracts RAGE-ligand interactions by sequestering and eliminating RAGE ligands thereby reducing chronic inflammatory stresses and aggravating tissue injury[7]. A mouse model study showed that treatment with sRAGE could attenuate hepatic injury and inflammation, in parallel with increased expression of pro-regenerative cytokines[11]. Therefore, sRAGE can function as a “decoy” by binding to RAGE ligands and preventing their ligation, which attenuates the inflammatory-immune response. Several investigations have indicated that sRAGE levels are reduced in some disease states, and are negativly associated with disease risk and adverse outcomes, including coronary artery disease[12], systemic lupus erythematosus[13], axonal Guillain-Barré syndrome[14], chronic obstructive lung disease[15] and cancer[16, 17]. CHB is also a chronic disease with hepatic necroinflammation caused by HBV-induced immune cell death. However, the serum levels of sRAGE in patients with CHB and their underlying relationship with disease progression remain elusive.

In this study, we determined sRAGE levels in CHB and investigated their correlation with clinical parameters in a well-defined cohort of CHB patients, aiming to explore whether sRAGE can be used as a potential disease biomarker during CHB progression.

Material And Method

Patients

A total of one hundred and twenty treatment-naive CHB patients were enrolled in present study between May 2016 and May 2020 at the Second Affiliated Hospital of Chongqing Medical University. Diagnosis was primarily established by histology, serology, imagiology and medical history. Patients were not included if they were detected with other disease entities, including infection with hepatitis virus C, A, D, and E, autoimmune liver disease and alcoholic liver disease. Clinical characteristics of patients were recorded at the time of CHB diagnosis. CHB patients were consisted of four subgroups [HBeAg(−) inactive HBV carrier, HBeAg(−) immune reactivation phase, HBeAg(+) immune-tolerant phase and HBeAg(+) immune-active phase] based on the natural course of chronic HBV infection according to the American Association for the Study of Liver Diseases (AASLD) [18]. The obtained serum and biopsy specimens are stored at -80°C until further processing. In addition, forty serum samples from age and gender-matched healthy volunteers were enrolled as healthy controls (HCs). Five normal biopsy specimens form HCs that

underwent liver biopsy to exclude liver disease were also performed and stored until use. Informed written consent was obtained from all patients and the study was approved by the Institutional Ethics Committee for human studies at the Second hospital affiliated to Chongqing Medical University, Chongqing, China. All procedures were in accordance with the declaration of Helsinki. Patient characteristics are summarized in Table 1.

Table 1
The characteristics of enrolled individuals.

	CHB (n = 120)				HCs (n = 40)	*p*-value
Parameter	HBeAg(-)		HBeAg(+)			CHB vs HCs
	IC (n = 32)	IR (n = 31)	IT (n = 12)	IA(n = 45)		
Gender (male, %)	16 (50.0%)	19 (61.2%)	6 (50.0%)	34 (53.3%)	22 (55.0%)	> 0.05
Age (years)	38.5 (15.5)	39.5 (16)	35 (14)	39 (21)	37 (16)	> 0.05
ALT (U/L)	25 (15.75)	183 (182)	22 (16.75)	123 (173.5)	19 (24)	< 0.01
HBV DNA (log_{10} IU/ml)	2.46 (0.61)	4.6 (2.56)	7.01 (0.85)	5.43 (1.36)	N/A	
Grading of Necroinflammation(n) G0/G1/G2/G3/G4	4/16/5/7/0	1/11/8/9/2	1/2/0/5/4	0/5/3/20/17	N/A	

For age, ALT, HBV DNA titres, data are presented as median (interquartile range). IC, inactive carrier; IR, immune reactivation. IT, immune-tolerant; IA, immune-active. *p*-values < 0.05 are considered as significant. N/A, not available.

Immunohistochemical (IHC)

The expression of RAGE in liver biopsy specimens was examined by IHC. The sections from the formalin fixed, paraffin-embedded tissues were deparaffinized and dehydrated. Then, the sections were boiled for 10 min in a 0.01 M citrate buffer and incubated with 0.3% hydrogen peroxide in methanol for 15 min to block endogenous peroxidase. The sections were then incubated with the anti-RAGE B2513; Santa Cruz Biotechnology, USA), overnight at 4°C, following incubation with secondary antibody tagged with the peroxidase enzyme (SP-9000, Zhongshan Golden Bridge, China) for 30 min at room temperature and were visualized with 0.05% 3,3-diamino-benzidine tetrachloride till the desired brown reaction product was obtained. The sections were finally counterstained with hematoxylin. All the slides were observed under a BLYMPUS Microscope, and representative photographs were taken.

Hepatic biochemical and serological indexs

Hepatic biochemical indexs such as alanine aminotransferase (ALT) were measured by HITACHI 7600 (HITACHI, Japan). HBsAg, HBeAg, and antibodies against HBsAg (anti-HBs), HBeAg (anti-HBe) and hepatitis B core antigen (anti-HBc) were determined using the Abbatt i2000 Immunoassay Analyzer. HBV DNA was quantified by ABI 7500 PCR Analyzer.

Enzyme-linked immunosorbent assay

Serum samples were analyzed by commercial human sRAGE enzyme-linked immunosorbent assay kits (JYM, China) used according to the manufacturer's instructions. Samples were run in duplicate.

Statistical analysis

Data were analyzed using SPSS 17.0. Mann-Whitney or Kruskal-Wallis test was performed to determine significance of sRAGE in CHB patients with various clinical and biochemical features. Correlation coefficients (r) were calculated using spearman correlation. Receiver operating characteristic (ROC) curves were generated to classify patients in different groups, as well as for the evaluation of predicting power for serum sRAGE, ALT and sRAGE /ALT via calculation of the area under the ROC curve (AUC), sensitivity and specificity according to standard formulas. Multiple linear regression analysis was performed to determine independent determinants for levels of sRAGE. All the data represents the median and interquartile range (IQR). A p value < 0.05 was considered statistically significant.

Results

Serum levels of sRAGE in CHB

We detected and analyzed the serum levels of sRAGE in CHB patients to explore whether its levels are abnormally altered in CHB patients. CHB patients exhibited markedly lower serum sRAGE levels than HCs (Fig. 1a). We then detected RAGE expression in tissue sections from biopsy specimens in patients with CHB using IHC analysis. The results showed an attenuated signal of RAGE staining in patients with CHB, and its staining signal was not mainly diffused in the cell membrane but also in the nuclei of hepatocyte (Fig. 1b). According to AASLD guidelines, CHB patients were classified to four subgroups: HBeAg(-) inactive CHB phase (IC), HBeAg(-) immune reactivation phase (IR), HBeAg(+) immune-tolerant phase (IT) and HBeAg(+) immune-active phase (IA). We then assessed sRAGE levels in the four subgroups. In the two CHB with HBeAg(-) subgroups, the HBeAg(-) IR phase showed significantly lower sRAGE levels than the HBeAg(-) IC phase (Fig. 1b). In the two HBeAg(+) subgroups, the HBeAg(+) IA phase showed significantly lower sRAGE levels than the HBeAg(+) IA phase (Fig. 1c).

Determinants linked with sRAGE in CHB patients

Univariate analysis was performed to find the determinants of sRAGE. Among the studied variables, phases of CHB, necroinflammation grades (G) and necroinflammation parameter ALT were significantly linked to sRAGE (Table 2). To determine the independent determinants of sRAGE, multiple linear

regression analysis was performed. We found that necroinflammation G and ALT was independently related to serum levels of sRAGE (Table 2).

Table 2
Significant factors associated with sRAGE by step forward multiple linear regression analysis in CHB patients.

Variables	Univariate*			Multivariate+		
	ß	95% CI	*p* value	ß	95% CI	*p* value
Age (Years)	-3.95	-7.95, 0.06	0.054			
Gender						
Male[a]						
Female	-107.80	-197.05, -18.56	0.058			
CHB phases						
IC[a]						
IR	-110.276	-224.702, -4.149	0.049	8.713	-98.614-116.04	0.873
IT	76.766	-76.932, -333.28	0.325			
IA	-228.991	-333.28, -123.288	< 0.001	-72.323	-175.88-31.233	0.169
ALT (U/L)	-0.696	-0.914, -479	< 0.001	-0.568	-0.81–0.326	< 0.001
HBV DNA (log10 IU/ml)	-22.02	-47.08, -3.039	0.084			
Grading (G) of Necroinflammation						
G (0–1)[a]						
G (2–4)	-217.991	-306.338, -129.644	< 0.001	-148.719	-232.757–64.681	0.001
Fibrosis						
Present[a]						
Absent	-18.194	-112.9, -76.512	0.704			

a, reference group; ß, regression coefficient; *, univariate regression analysis; +, a stepwise multivariate regression analysis; IC, inactive carrier; IR, immune reactivation; IT, immune-tolerant; IA, immune-active. *p* value < 0.05 was considered statistically significant.

Distribution of serum sRAGE in CHB patients with different viral loads

We explored the distribution of serum sRAGE levels in CHB patients with different viral loads, including high HBV DNA levels (≥ 7 $\log_{10}$ IU/mL), intermediate HBV DNA levels (≥ 5–7 $\log_{10}$ IUml) and low HBV DNA levels (< 5 $\log_{10}$ IU/mL). No significant difference was found among the three subgroups (Fig. 2a). We further analyzed the correlation between serum sRAGE levels and HBV DNA levels in CHB patients. sRAGE levels were not found to be associated with HBV DNA levels in CHB patients (Fig. 2b). Since high viral loads mainly existed in the three subgroups of the HBeAg(-) IR phase, the HBeAg(+) IT phase and HBeAg(+) IA phase, the correlation between serum sRAGE levels and HBV DNA levels were also analyzed in the three subgroups. We found that there was no correlation between serum sRAGE levels and viral loads in all these three subgroups (Fig. 2c, d, e).

Correlation of serum sRAGE with hepatic necroinflammation in CHB

We observed lower sRAGE levels in patients with moderate-to-severe hepatic necroinflammation (G2-4) than those in patients with no or minimal hepatic necroinflammation (G0-1) (Fig. 3a). Serum sRAGE was inversely correlated with necroinflammation grades in patients with CHB (Fig. 3b). We then analyzed the association between serum sRAGE levels and the hepatic necroinflammation parameter ALT. An inverse correlation between serum sRAGE and ALT was verified in patients with CHB (Fig. 3c). Since elevated levels of ALT mainly existed in subgroups of the HBeAg(-) IR and the HBeAg(+) IA, the correlation between serum sRAGE and ALT levels in these two phases was also analyzed. A negative correlation of sRAGE with ALT levels was verified in these two subgroups (Fig. 3d, e). Moreover, RAGE expression was also analyzed in biopsy specimens from CHB patients with various grades of necroinflammation. We found that RAGE staining was gradually attenuated along with the increased hepatic necroinflammation grades (G0-G4) (Fig. 3f).

The clinical predictive power of sRAGE for hepatic necroinflammation in CHB

ROC curve analysis revealed that ALT, sRAGE and the sRAGE-to-ALT ratio (sRAGE/ALT) yielded AUC of 0.82 (95% CI, 0.75 to 0.88), 0.81 (95% CI, 0.74 to 0.89) and 0.85 (95% CI, 0.80 to 0.92) for predicting hepatic necroinflammation, respectively. Specifically, combination of ALT and sRAGE (ALT + sRAGE) was better than that of ALT, sRAGE or sRAGE/ALT alone, which yielded an AUC of 0.86 with 85% sensitivity, 72.5% specificity and 78.75% accuracy (Fig. 4a). indicating that combination of ALT and sRAGE could efficiently predicting hepatic necroinflammation. We further evaluated the potential predictive values of these indicators for necroinflammation severity. ROC curve analysis showed that none of these had obvious diagnostic value for identifying hepatic necroinflammation (G2-4) from necroinflammation (G0-1), which yielded AUCs of 0.68, 0.71, 0.72 and 0.72, respectively (Fig. 4b). Clinically, some patients with

normal or minimally increased ALT may have severe necroinflammation (G2-4) that may not be recognized without a liver biopsy. We then evaluated whether sRAGE can distinguish these misdiagnostic and neglected patients. In patients (G2-4) with normal ALT levels (ALT < upper limit of normal (ULN)), the AUC of sRAGE for predicting necroinflammation was 0.81 with 71.43% sensitivity and 80% specificity and 75.72% accuracy (Fig. 4c). Additionally, the AUC of sRAGE for identifying patients (G2-4) with minimally increased ALT (ALT < 2 ULN) was 0.83 with 87.9% sensitivity, 77.78% specificity and 82.84% accuracy (Fig. 4c), suggesting that serum sRAGE may be superior to ALT and be an alternative indicator for predicting moderate-to-severe hepatic necroinflammation in patients with normal or minimally elevated ALT.

Discussion

CHB is a dynamic liver disease characterized by hepatic necroinflammation that is influenced by host and virological factors. Elucidation of the host inflammatory-immune response involved in CHB pathogenesis provides new perspectives for the identification of biomarkers and therapeutic targets. RAGE functions as a crucial transducer in the inflammatory immune response by interacting with its ligand and activating downstream cascades. sRAGE, a circulating soluble isoform of RAGE, has recently gained interest due to its potential to ameliorate inflammation by competing with cell surface RAGE for ligand binding under pathological conditions. sRAGE can be detected in human serum and has been implicated in liver diseases, including nonalcoholic fatty liver disease (NAFLD)[19], autoimmune hepatitis[20] and HCC[21]. Nevertheless, its natural behavior and relationship with disease progression as well as its clinical predictive values in CHB development remain elusive. Here, we assessed the serum levels of sRAGE and its association with hepatic necroinflammation parameters during the dynamic course of CHB, aiming to determine its potential clinical significance for clinical judgment and surveillance of CHB.

The presence of HBV replication is closely associated with persistent hepatitis activity or intermittent hepatitis flares and subsequent disease progression, including hepatic decompensation, liver cirrhosis or HCC[22]. Generally, the HBV life cycle is heavily dependent upon and regulated by multiple host functions including intrinsic host innate defensive factors and gene products that provide functions necessary for the virus to complete its life cycle[23]. The present study demonstrated that there was no significant difference among CHB patients with different viral loads, which was confirmed in the three subgroups with high viral loads HBeAg(-) IR phase, HBeAg(+) IT phase and HBeAg(+) IA phase. These findings indicate that sRAGE may not be a factor of the host innate defense system that participates in HBV replication, consistent with a previous report showing that serum sRAGE levels were linked to the chronic inflammation in lung disease rather than pathogen infection[24].

The present study demonstrated that CHB patients in a well-defined cohort exhibited reduced sRAGE levels, implying that sRAGE may exert opposite functions in regulating CHB pathogenesis. This finding supports those of previous studies showing low sRAGE levels in other inflammatory-immune disorders[25–27]. It has been reported that sRAGE is mainly generated from the proteolytic cleavage of membrane RAGE by metalloproteases such as MMP9 and ADAM1[8, 28]. MMP9 and ADAM1 were also

reported to be in turn regulated by RAGE expression[29]. Based on the current finding of reduced levels of RAGE and sRAGE in CHB patients, we cannot exclude the possibility that decreased levels of RAGE along with possible reduced levels of metalloproteases together contributed to the reduced circulatory sRAGE levels.

The assessment of sRAGE levels in the CHB subgroups revealed that the HBeAg(-) IR and HBeAg(+) IA phases exhibited lower sRAGE levels than the HBeAg(-) IC and HBeAg(+) IA phases, respectively, suggesting an inverse correlation between sRAGE and hepatic necroinflammation. Since active hepatic necroinflammation in CHB patients represents one of the important risk factors for developing liver fibrosis and even HCC, an evaluation of necroinflammation severity as well as its early control is imperative to determine the need for surveillance and treatment in patients with CHB[30]. Here, patients with moderate-to-severe necroinflammation ($G \geq 2$) had lower sRAGE levels than those with no or minimal hepatic necroinflammation (G0-1). Serum sRAGE levels were inversely correlated with ALT levels, a classic indicator of hepatic necroinflammation and prior CHB treatment. Similar results were also observed in the HBeAg (-) IR and HBeAg (+) IA phases, which exhibited hepatic necroinflammation. These observations suggest that reduced sRAGE levels may be identical to elevated ALT, reflecting inflammatory activity. Furthermore, RAGE expression in biopsy specimens from CHB patients was reduced along with hepatic necroinflammation grades. While RAGE binds to its ligands, leading to the activation of downstream cascades, and maintains persistent inflammation[9]. With the inconsistence of reduced RAGE but its supporting role in inflammation, we hepothesize that persistent inflammation elicited by RAGE-ligand systems may be mainly dependent on RAGE's ligands, but this hypothesis stll needs to be clarified in future research.

Many investigators have been trying to develop noninvasive tests for predicting liver inflammation. To date, ALT remains widely used as a routine indicator for hepatic necroinflammation or therapeutic judgment for antiviral therapy[31]. Nonetheless, growing evidence has revealed that ALT alone is not as good as as an indicator as the grade of hepatic necroinflammation. One statistical study in China revealed that nearly half of CHB patients with ALT < 2 ULN showed moderate-to-severe necroinflammation ($G \geq 2$) [32]. Another biopsy report in the USA similarly showed that one-third of CHB patients with normal ALT levels showed severe inflammation[33]. Therefore, CHB patients may exhibit normal ALT levels, leading to an inaccurate assessment of disease severity or even misdiagnosis. The present data revealed that combination of sRAGE and ALT value was superior to ALT, sRAGE or sRAGE/ALT alone for predicting hepatic necroinflammation. Therefore, sRAGE combining with ALT is necessary and may be used for patients who lack clear-cut indications for treatment. We also found that sRAGE has predictive power for CHB patients ($G \geq 2$) with normal and minimally elevated ALT levels. This finding is meaningful that a CHB patient with a low serum sRAGE level may have significant hepatic necroinflammation even with a normal or minimally elevated ALT. However, further work on larger samples should be performed to substantiate the findings.

Nonetheless, several limitations to the present study exist. First, we did not assess the specificity of serum sRAGE in CHB compared to those with other liver disease entities such as chronic hepatitis C,

nonalcoholic fatty liver disease and alcoholic liver disease using the same set of analyses. Second, we investigated the sRAGE levels during the chronic phase of HBV infection, and it is insufficient to elucidate dynamic changes of sRAGE during the different phase of HBV infection from acute hepatitis, CHB, hepatic fibrosis, and HBLC to HCC. Last, the detailed molecular mechanism regarding underlying the role of sRAGE in RAGE signaling activation in CHB pathogenesis and whether sRAGE can serve as a therapeutic target still need further clarification.

Conclusion

In conclusion, the present study demonstrated that CHB patients exhibited reduced levels of sRAGE, and its levels were inversely correlated with hepatic necroinflammation. Furthermore, combination of ALT and RAGE exerted valuable predictive power for hepatic necroinflammation in CHB patients. More importantly, circulating sRAGE alone exerted valuable predictive power for hepatic moderate-to-severe necroinflammation in CHB patients but with normal or minimally elevated ALT levels. These data suggest that monitoring sRAGE and (or) its combination with ALT may facilitate decision-making regarding prophylaxis and treatment, ultimately leading to the prevention of CHB progression.

Declarations

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Not Applicable

AUTHOR CONTRIBUTION

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Xiuyu Zhang, Yan You, Qiao Liu, Xiaoyu Sun, Weixian Chen, Liang Duan. The first draft of the manuscript was written by Liang Duan and all authors commented on previous versions of the manuscript. All authors reviewed and approved the final manuscript.

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DATA AVAILABILITY

The authors confirm that the data supporting the findings of this study are available within the article and material.

Ethics Approval

All procedures were in accordance with the declaration of Helsinki. Informed written consent was obtained from all patients and the study was approved by the Institutional Ethics Committee for human studies at the Second hospital affiliated to Chongqing Medical University, Chongqing, China.

Consent for Publication. Written informed consent for publication was obtained from all participants.

Conflict of Interest.

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Figures

Figure 1

The expression levels sRAGE in patients with CHB. a ELISA analysis of sRAGE levels in serum samples from patients with CHB as well as healthy controls (HCs). CHB, n=120; HCs: n=40. b Representative images for RAGE staining by IHC in patients with CHB and HC. Black scale bars, 50 μm; Red scale bars, 100 μm; Blue scale bars, 200 μm. c ELISA analysis of sRAGE levels in serum samples from patients with CHB with different subgroups (HBeAg(-) IC, n=32; HBeAg(-) IR, n=31; HBeAg(+) IT, n=12; HBeAg(+)IA, n=45). Data represent the median (IQR). n, number. *p < 0.05, ***p < 0.001.

Figure 2

Correlations of serum sRAGE levels with HBV DNA in patients with CHB. a ELISA analysis of sRAGE levels in serum samples from CHB patients with different viral load (< 5, ≥ 5 to < 7, ≥ 7 $\log_{10}$ IU/ml). b Correlation between serum sRAGE levels and HBV DNA levels in CHB patients. c-e Correlation between serum sRAGE levels and HBV DNA levels in CHB patients with different subgroups HBeAg(-) IR(c) , HBeAg(+) IT (d) and HBeAg(+) IA (e). Data represents the median (IQR). Ns, no statistical significance.

Figure 3

Relationship between serum sRAGE levels and hepatic necroinflammation. a ELISA analysis of sRAGE levels in serum samples from CHB patients with moderate-to-severe necroinflammation (G2-4) and with no or minimal hepatic necroinflammation (G0-1). b Correlation between serum sRAGE levels and hepatic necroinflammation grades in CHB patients. c-e Correlation between serum sRAGE levels and ALT levels in CHB patients (c), subgroup of HBeAg(-) IR CHB patients (d) and HBeAg(+) IA CHB patients (e). f Representative images for RAGE staining by IHC in CHB patients with different necroinflammation grades (G0-G4). Black scale bars, 50 μm; Red scale bars, 100 μm; Blue scale bars, 200 μm. Data represents the median (IQR). ***p < 0.001.

Figure 4

Differentiating power of serum sRAGE for hepatic necroinflammation in CHB patients. a ROC curves of serum sRAGE, ALT, sRAGE/ALT, and combination of sRAGE and ALT (ALT +sRAGE) for predicting necroinflammation in CHB patients. b ROC curve of sRAGE, ALT, sRAGE/ALT and ALT+sRAGE for identifying liver moderate-to-severe hepatic necroinflammation (G2-4) from no or minimal hepatic necroinflammation grade (G0-1). c ROC curves of serum sRAGE for discriminating patients with moderate-to-severe necroinflammation (G ≥2) but ALT< ULN or ALT ≤2 ULN.