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doi: 10.53388/ghr2020-12-024

ARTICLE

The relationship between viral response to Peg-IFNα-2a and the expression intensity of Hepatitis B core antigen in chronic hepatitis B patients

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Abstract: Hepatitis B core antigen (HBcAg) reflects viral replication and is the target of T cells which can reflect the progression of liver disease. We aim to evaluate the relationship between Peginterferon alfa-2a (Peg-IFNα-2a) and the expression intensity of hepatitis B core antigen in chronic hepatitis B (CHB) patients in this study. 207 patients were enrolled with HBeAg-positive CHB and performed liver biopsy to determine the expression intensity of HBcAg detected by immunocytochemistry. All patients received 180μg of Peg-IFNα-2a once weekly for 48 weeks. We evaluated therapy response after 48 weeks of Peg-IFNα-2a therapy. All patients were divided into four groups (0 point group, 1 point group,2 point group,3 point group)by the expression intensity of HBcAg. Statistics on 207 patients, 118 (57.00%) had over 66% HBcAg expression (3 point group), 30 (14.49%) had 34%-66% HBcAg expression (2 point group), 45(21.74%) cases had 5%-33% HBcAg expression (1 point group), while only 14 (6.76%) had less than 5% HBcAg (0 point group). 0 point group has the lowest baseline HBV DNA among the four groups ($P < 0.01$). The degrees of baseline Liver tissue inflammation and fibrosis among the four groups have no difference($P > 0.05$). The combination response was significantly higher in the 0 point group than in the 3 point group (57.1 % and 18.6 %, $P < 0.01$) after 48 weeks of Peg-IFNα-2a therapy. In conclusion, CHB patients at lower expression intensity of HBcAg have a better response to Peg-IFNα-2a therapy than at higher expression intensity of HBcAg.

Key words: Chronic hepatitis B, Hepatitis B core antigen, expression intensity, histology, Peginterferon alfa-2a

***Acknowledgments*:** Supported by the National Natural Science Foundation of China (No.81673660, No.81873242), Fujian Province health and family planning medical innovation research talent training project（No. 2018-CXB-28）,Xiamen Science and Technology Program Grant (No.3502Z20174028),the joint research of Xiamen important and major disease project (No.3502Z20179047).

***Authors' Contributions*:** Conceptualization: Hui-Qing Liang, Shao-Dong Chen; methodology: Hui-Qing Liang; formal analysis: Hui-Qing Liang; Shao-Dong Chen; investigation: Hui-Qing Liang, Jin-Mo Tang, Jia-En Yang; resources: Qian-Guo Mao, Hui-Qing Liang; writing—original draft preparation: Qian-Guo Mao, Hui-Qing Liang; writing—review and editing: Qian-Guo Mao, Hui-Qing Liang.

***Abbreviations*:** HBcAg, Hepatitis B core antigen; Peg-IFNα-2a, Peginterferon alfa-2a; CHB, chronic hepatitis B; HBeAg, hepatitis B envelope antigen; Peg-IFNα-2a, Peginterferon alfa-2a; HBsAg, hepatitis B surface antigen; CR, combination response; PR, partial response; NR, no response; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HBV, hepatitis B virus; OR, odds ratios; CI, confidence interval;

***Competing interests*:** The authors declare that there are no financial conflicts of interest.

***Citation*:** Liang HQ, Liu YY, Xiao QX, et al. The relationship between viral response to Peg-IFNα-2a and the expression intensity of Hepatitis B core antigen in chronic hepatitis B patients. *Gastroenterol Hepatol Res*. 2020;2(4):112-118. doi: 10.12032/ghr2020-12-024.

***Executive Editor*:** Xin Cheng.

***Submitted*:** 18 October 2020, ***Accepted*:** 06 December 2020, ***Published*:** 12 December 2020

TMR
doi: 10.53388/ghr2020-12-024

Introduction

HBcAg exists at the core of Dane particles, which is the structural protein of HBV [1]. HBcAg is known to be important markers for the proliferation of HBV and a major target for virus-specific T cells [2]. HBV is a type of noncytopathic virus which cause various degrees of damage to liver tissue, by host immune response. The immune response to HBcAg plays an important role in HBV clearance. Immunodetection of HBcAg in hepatocytes is uselful to evaluate the status of HBV replication, histological activity and diagnose CHB [3,4]. The expression intensity of HBcAg can be classified into four types by immunochemical staining, which are 3 points, 2 points, 1 point, and 0 point. 3 points show over 66% HBcAg positive cells in the hepatocytes, is the highest HBcAg expression intensity among the four types; 2 point and 1 point shows 34%-66% and 5%-33% HBcAg positive cells in the hepatocytes are respectively. But less than 5% HBcAg positive cells was detected in the hepatocytes is 0 point (Figure 1). The distribution of HBcAg and HBVDNA in the liver is usually consistent. There has a proportionality relationship between the content of HBVDNA and the expression intensity of HBcAg in the hepatocyte [4]. In the immunetolerance stage, There was no obvious inflammattion and fibrosis in the liver and had high serum HBV-DNA load , high expression intensity of HBcAg in the hepatocyte. However, in the viral clearance phase was found severe injury in the liver and low expression intensity of HBcAg and low serum HBV-DNA [5]. It was revealed that from lamivudine and interferon alfa treatment, the absence or low intensity of baseline HBcAg expression may be an important predictor in the response to hepatitis B e antigen (HBeAg)-negative patients [6]. There is no data in the HBeAg-positive patients. HBcAg lower expression staining was showed results from more active host immune T-cell response against the major viral target. Antiviral response by interferon alfa was depended on inhibiting viral replication and on modulating the immune response. Low expression intensity of HBcAg in the hepatocyte may be in the viral clearance phase and may have good viral response to Peginterferon alfa-2a (Peg-IFNα-2a). The purpose of this study is to evaluate and predict the relationship between viral response to Peg-IFNα-2a in HBeAg-positive CHB patients and the HBcAg expression intensity in hepatocytes.

Materials and methods

Patients

207 patients with CHB was enrolled during at January 2013 to January 2015, they were consecutively enrolled from Hepatology Unit of Xiamen Hospital of Traditional Chinese Medicine. The study protocol was approved by the ethics committee of Xiamen Hospital of Traditional Chinese Medicine (2012-Y003-02). Each recruited patient was agreed with the written informed consent and obtained before doing the questionnaire survey. All patients were hepatitis B surface antigen (HBsAg)-positive, hepatitis B envelope antigen (HBeAg)-positive for at least 6 months and had a serum HBVDNA level greater than 20000 IU/mL and elevated ALT levels were defined as ranging betwee n 2 upper limit of normal (ULN, 40 U/L)to 10 ×ULN. Patients with the following conditions were excluded from the study: evidence of concomitant etiologies including chronic hepatitis A, C or D coinfection or superinfection, autoimune hepatitis, alcoholic liver disease, non-alcoholic fatty liver disease, Wilson's disease, and drug-induced liver injury; or HIV coinfection or evidence of immune. All patients received 180 μg of Peg-IFNα-2a once a week after liver biopsy, and we checked HBV DNA level at baseline, week 12 or 24, and during antiviral therapy at 48 weeks (end of treatment). We defined viral response in serum HBV DNA was decreasing to an undetectable level (<500 IU/mL) run by PCR assays (COBAS TaqMan HBV test; Roche Diagnostics, Meylan, France), and viral showed no response as a nondecrease to undetectable level.

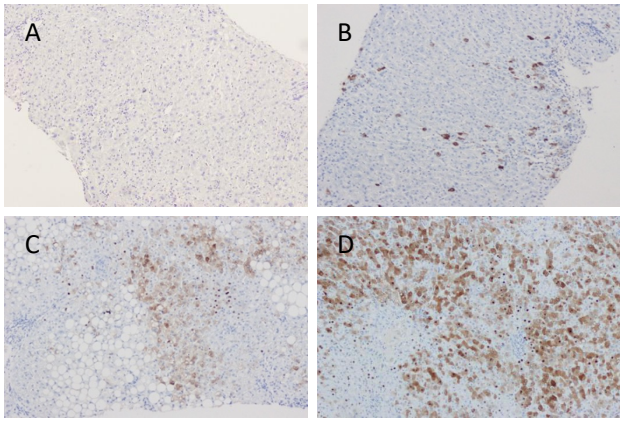

Figure 1. The different degrees of HBcAg expression intensity in the hepatocytes of CHB patient. The expression intensity of HBcAg in the hepatocytes of CHB patient was classified into four types in this study. **A.** 0 point group showed less than 5% HBcAg expression (immunohistochemical stain for HBcAg, × 200). **B.** 1 point group showed 5% - 33% HBcAg expression (immunohistochemical stain for HBcAg, × 200). **C.** 2 point group showed 34% - 66% HBcAg expression (immunohistochemical stain for HBcAg, × 200). **D.** 3 point group showed over 66% HBcAg expression (immunohistochemical stain for HBcAg, × 200).

Evaluation of liver biopsy specimens

All patients were agreed informed consents to the liver biopsy procedure. Liver biopsy was obtained using 16G biopsy needles guided by ultrasonography. A

qualified biopsy specimen was either a minimum 1.5 cm long or displayed 6 or more portal tracts. Scheuer's scoring system was used to determine the histological necroinflammation (G0,G1,G2,G3,G4) and fibrosis stages (S0,S1,S2,S3, S4)by the same pathologist, who was blinded to the characteristics of the patients. One serial section were stained with hematoxylin-eosin-safran to determine the histological necroinflammation and the other serial section were stained with Masson's trichrome to determine the fibrosis stages. The expression intensity of HBcAg in hepatocytes are detected by immunohistochemical staining.

Viral response

We set different response criterias to determine the effect of antiviral agents. Four different types of responses to Peg-IFNα-2a therapy were classified as combination response (CR), partial response (partial response, PR), non-response (no response, NR), and HBeAg seroconversion. CR is defined by ALT level less than ULN and HBV DNA less than 500 IU/ml and disappearance of HBeAg or HBeAg seroconversion. PR is defined by ALT level returned to normal or reduction of HBV DNA level by more than 2 log10 IU/mL or disappearance of HBeAg or HBeAg seroconversion.

NR is defined by no improvement of any marker mentioned above.

Statistics

The data were analysed using SPSS 13.0 software (IBM Co., Armonk, NY, USA); Continuous variables were presented as mean ± standard deviation or median (interquartile spacing). The Mann–Whitney U-test for continuous variables and chi-squared test or Fisher's exact test for categorical variables were used in the analyses as appropriate. Spearman rank correlation analysis was used to evalute the correlation between grouping and histopathology, Viral response. Multivariate logistic regression was used to determine the independent predictors of Combination response to Peg-IFNα. A two-sided P value < 0.05 was considered as statistically significant.

Results

Expression intensity of HBcAg in hepatocytes

The baseline features of the patients are listed in Table 1. 118 (57.00%) had over 66% hepatitis B core antigen expression (3 point group), 30 (14.49%) had 34%-66% hepatitis B core antigen expression (2 point group), 45(21.74%) cases had 5%-33% hepatitis B core antigen expression (1 point group), while only 14 (6.76%) had less than 5% hepatitis B core antigen expression (0 point group). 0 point group has the lowest baseline HBV DNA among the four groups (P < 0.01), while other characteristics such as age, sex, and ALT has no differences (P > 0.05).

Correlation between histologic necroinflammation, fibrosis stages of hepatitis and the expression intensity of HBcAg

From Table 2, Table 3, there was no correlation between histologic necroinflammation, fibrosis stages and HBcAg expression intensity pretreatment (P > 0.05) by the Spearman rank correlation analysis. But in a subgroup analysis, 0 point group and 1 point group reached G3/G4 (respectively 50.00%, 40.00%), while 2 point group and 3 point group reached G3/G4 (respectively 43.33%, 28.81%). 0 point group and 1 point group reached S3/S4 (respectively 21.43%, 11.11%), while 2 point group and 3 point group reached S3/S4 (respectively 13.33%, 4.24%).

HBcAg expression intensity and ALT, AST and HBV DNA level to 48 weeks of Peg-IFNα-2a treatment

After 48 weeks of Peg-IFNα-2a treatment, the ALT, AST and HBV DNA level in the four groups were significantly decreased compared to pretreatment in Figure 2.

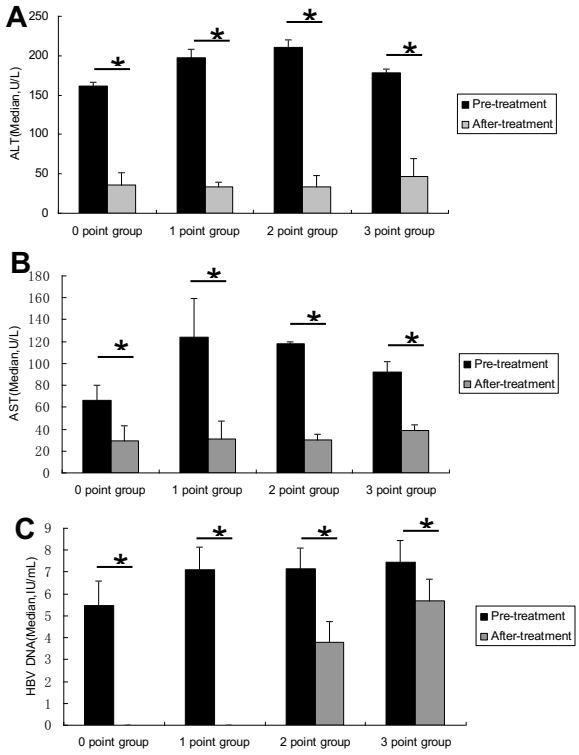

Figure 2. HBcAg expression intensity and ALT, AST and HBV DNA level to 48 weeks of Peg-IFNα-2a treatment. A. ALT level significantly decreased in four groups after treatment (P < 0.01). **B.** AST level significantly decreased in four groups after treatment (P < 0.01). **C.** HBV DNA significantly decreased in four groups after treatment (P < 0.01). Continuous variables are expressed as median (interquartile spacing). The Mann–Whitney U-test was used for statistical analysis.

doi: 10.53388/ghr2020-12-024

Table 1. Clinical and laboratory features of the patients

Feature		0 point (n=14)	1 point (n=45)	2 point (n=30)	3 point (n=118)	*P*-value
Age, year		30.5±5.0	28.0±7.0	26.0±7.8	29.0±9.0	0.1176
Sex	male	11	35	16	81	0.1284
	femal	3	10	14	37	
ALT, U/L		161.0(156.3)	197.0(185.5)	211.0(220.3)	178.5(174.5)	0.2825
AST, U/L		66.0(51.8)	124.0(89.0)	118.0(120.0)	92.5(83.3)	0.0128
HBV DNA		5.46(1.14)	7.10(1.02)	7.14(0.95)	7.44(0.98)	0.000

Continuous variables are expressed as mean ± SD or median (interquartile spacing).and categorical variables are described by count. Pearson chi-square test were used for statistical analysis. Abbreviations: HBcAg, hepatitis B core antigen; HBeAg, hepatitis B e antigen; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HBV, hepatitis B virus.

Table 2. Histologic necroinflammation grades of hepatitis and HBcAg expression intensity

Feature	0 point (n=14)	1 point (n=45)	2 point (n=30)	3 point (n=118)	Spearman rank correlation	coefficient of contingency
G1	1	1	0	2	0.1414	-0.1026
G2	6	25	17	82		
G3	6	15	13	29		
G4	1	3	0	5		

Categorical variables are described by count and proportions. Pearson chi-square test, Spearman rank correlation analysis and coefficient of contingency were used for statistical analysis.

Table 3. Histologic fibrosis stages of hepatitis and HBcAg expression intensity

Feature	0 point (n=14)	1 point (n=45)	2 point (n=30)	3 point (n=118)	Spearman rank correlation	coefficient of contingency
S1	7	26	11	75	0.0704	-0.1260
S2	4	14	15	38		
S3	3	3	2	5		
S4	0	2	2	0		

Categorical variables are described by count and proportions. Pearson chi-square test, Spearman rank correlation analysis and coefficient of contingency were used for statistical analysis.

Table 4. HBcAg expression intensity and viral response to Peg-IFNα-2a

Feature	0 point (n=14)	1 point (n=45)	2 point (n=30)	3 point (n=118)	Spearman rank correlation	coefficient of contingency
CR	8	16	7	19	0.0000	0.3029
PR	5	24	14	56		
NR	1	5	9	43		

Data are presented as number. Pearson chi-square test, Spearman rank correlation analysis and coefficient of contingency were used for statistical analysis.

Table 5. Multivariate analysis of clinical parameters independently associated with significant combination response.

clinical parameters	OR	combination response 95%(CI)	*P*-value
Age, year	0.082	0.037–0 .128	0.000
Sex(male)	0.982	0.376 –1.588	0.001
HBcAg expression intensity	-1.489	-2.287– -0.692	0.000

Data are presented as odds ratios (OR) and 95% confidence interval (CI).

HBcAg expression intensity and HBeAg seroconversion rate to 48 weeks of Peg-IFNα-2a treatment

After Peg-IFNα-2a treatment, the rate of HBeAg seroconversion was different in the four groups, and Fisher's exact test is 0.0017. HBeAg seroconversion in the 0 point group and 1 point group were higher than 3 point group (P = 0.0014). There was no significant difference between groups of 2 point and 3 point (P > 0.05) in Figure 3.

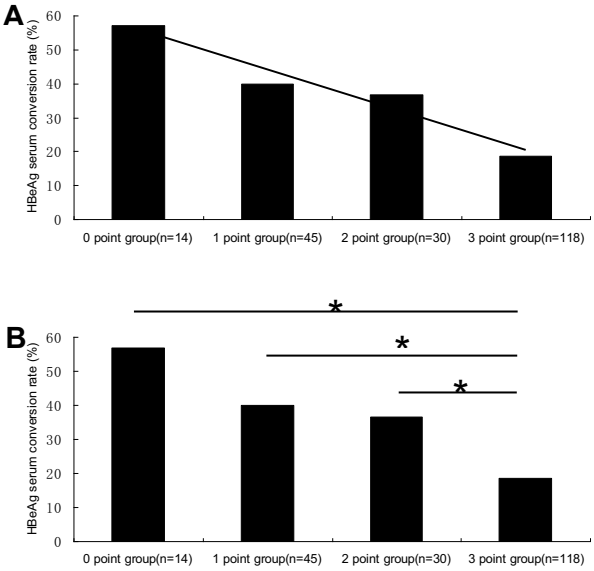

Figure 3. **HBcAg expression intensity and HBeAg seroconversion rate to 48 weeks of Peg-IFNα-2a treatment. A.** After Peg-IFNα-2a treatment, the rate of HBeAg seroconversion was difference in four groups. Data are presented as number (%) and Fisher's exact test was used for statistical analysis. **B.** Comparison of HBeAg seroconversion rate to 48 weeks of Peg-IFNα-2a treatment among four groups, 3 point group was the lowest compared to other groups (P < 0.05, P < 0.01). Pearson chi-square test were used for statistical analysis.

HBcAg expression intensity and viral response to 48 weeks of Peg-IFNα-2a treatment

After 48 weeks of Peg-IFNα-2a treatment, a significant difference in viral response was seen among the four groups (P < 0.01). We also found a sequential increase in combination response from 3 point group to 0 point group. The 0 point group shows the highest rate of combination response to Peg-IFNα-2a, reached 57.1% (Table 4).

Influence factor of combination response to 48 weeks of Peg-IFNα-2a treatment

Clinical parameters that were analyzed by the multivariate regression with combination response to 48 weeks of Peg-IFNα-2a treatment (Table 5). Age (P = 0.000),Sex(male) (P = 0.001) and HBcAg expression intensity (P = 0.000) were independently associated with combination response to Peg-IFNα-2a.

Discussion

The World Health Organization reported that about 350 million people are chronically infected with HBV. About one million people die from hepatic failure , cirrhosis and hepatocellular carcinoma caused by HBV infection each year [7].There had estimated 20-30 million patients with CHB in china [8].Antiviral therapy is the most fundamental treatment for CHB. Peg-IFNα was recommended as the first-line treatment of CHB for its higher combined response rate and higher HBsAg, HBeAg seroconversion rate [9]. The HBeAg seroconversion rate of Peg-IFNα fluctuates from 36% to 48%.Choosing a good timing of treatment such as baseline low HBV DNA level, baseline high ALT level, male can improve the efficacy of Peg-IFNα [10, 11]. Providing the most appropriate antiviral therapy to the most suitable patient can achieve the best response of Peg-IFNα in CHB patients. If we can find more response of predictors to guide the Peg-IFNα therapy, the better effects may acquire and the waste of financial resources may be reduced.

HBcAg is the main antigen causing the T cell immune reaction and has a stronger antigenicity than other viral proteins [12], which plays an important role in the immune injury of CHB. Serinoz et al. [5] suggested that expression of HBcAg correlates with the liver pathology and the three phases of chronic HBV infection: 1) the early immune tolerance phase is characterized by nuclear HBcAg, high expression intensity HBcAg, minimal liver injury, high HBVDNA load and low HBeAg seroconversion rate; 2) the virus elimination phase by cytoplasm HBcAg or negative HBcAg, low expression intensity HBcAg, frequent active liver injury, low HBV-DNA load and high HBeAg seroconversion rate; and 3) the inactive virus replication phase by frequent negative HBcAg. Thus, the rate of hepatocyte proliferation and expression of the HBcAg may be important in determining the prognosis and the viral response to treatment in CHB. Uzun et al. [6] investigated the role of HBcAg expression in response to antiviral treatment in patients with CHB, who were treated with lamivudine and interferon α combined or with lamivudine alone and supported the idea that the absence or a low level of HBcAg expression intensity may predict good viral response, especially in the HBeAg-negative group.

From the study, as the Spearman rank correlation analysis showed no correlation between histologic necroinflammation, fibrosis stages of hepatitis and the expression intensity of HBcAg (P > 0.05). But in subgroup analysis, the histologic necroinflammation in the 0 point group reached G3/G4 (50.00%), while 3 point group reached G3/G4(28.81%). 0 point group S3/S4 (21.43%), while 3 point group reached S3/S4

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doi: 10.53388/ghr2020-12-024

(4.24%). Histologic necroinflammation and fibrosis which expressed in 0 point group were significantly higher than 3 point group. 0 point group showed more piecemeal necrosis and focal necrosis by using microscope.

This study also showed that the 0 point group (HBcAg-negative group) had a stronger immune reaction and a better viral response to Peg-IFNα than with the HBcAg-positive group after 48 weeks of Peg-IFNα treatment, a significant difference in HBeAg seroconversion rate was seen among the four groups (P = 0.0014).

We also found a sequential increase in combined response from 3 points group to 0 point group. The 0 point group shows the highest CR rate and HBeAg seroconversion rate to Peg-IFNα-2a, reached 57.1%. 3 points group has the worst HBeAg seroconversion rate and CR rate, which seroconversion rate and CR rate was only 18.60%,16.10%. This study suggests that the expression intensity of HBcAg and Peg-IFNα-2a antiviral effect is closely related and whether to choose Peg-IFNα-2a according to HBcAg expression intensity before antiviral therapy. We can use Peg-IFNα-2a to increase the immune function and anti-HBV when the expression intensity of HBcAg was 0 point group or 1 point group, which can further promote viral clearance and increase treatment efficiency. We also analyzed the influence factor of CR rate to Peg-IFNα. Besides, we took CR rate as the dependent variable. Gender, age, baseline ALT level, baseline AST level, baseline HBV DNA level, baseline HBcAg expression intensity, histologic necroinflammation grades, histologic fibrosis stages as independent variables, and were analyzed by the multivariate regression. The results showed that age (P = 0.000), sex (male) (P = 0.001) and HBcAg expression intensity (P = 0.000) was associated independently with CR rate to Peg-IFNα.These were consistent with previous studies which suggested that young and female were predictive factors for the effects to Peg-IFNα [13,14]. Besides, this study revealed that HBcAg expression intensity is also a predictor of the effects to Peg-IFNα.

With this prospective study which has clear diagnostic criteria and unified treatment, the result was certainly value in clinical. However, the source of patients was only come from Xiamen Hospital of Traditional Chinese Medicine. and the target patientis we choose were with HBeAg-positive chronic hepatitis B, lacking inactive carriers and HBeAg-negative chronic hepatitis B. Moreover, HBcAg was not only one viral target for immune control [15]. In the future, a larger scale and the long-term study will be needed, in order to validate the value of the HBcAg expression intensity in hepatocytes to predict response to Peg-IFNα.

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