

Noninvasive Biomarkers in Assessment of Liver Fibrosis in Patients with HBeAg Negative Chronic Hepatitis B

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Abstract

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BACKGROUND: Liver biopsy for evaluation of liver fibrosis has several adverse effects, for which reason noninvasive tests have been developed.

AIM: To evaluate the usefulness of noninvasive biomarkers, qHBsAg and HBV DNA levels in predicting liver fibrosis in patients with hepatitis Be antigen (HBeAg) negative chronic hepatitis B (CHB).

MATERIAL AND METHODS: This prospective study included 50 patients with HBeAg negative CHB. All patients underwent laboratory and serology testing, quantification of HBV DNA and HBs antigen. The liver stiffness was measured with elastography. The patients were analysed for APRI and FIB-4, quantitative hepatitis Bs antigen and HBV DNA.

RESULTS: Logistic regression analysis showed that greatest significance in predicting liver fibrosis has FIB-4 (Wald = 3.24, P = 0.07), followed by HBV DNA $\geq 2\ 000$ IU/ml $\leq 20\ 000$ IU/ml (Wald = 2.86, P = 0.09), qHBsAg (Wald = 2.17, P = 0.14), HBV DNA $> 20\ 000$ IU/ml (Wald = 0.58, P = 0.45), APRI (Wald = 0.04, P = 0.84).

CONCLUSION: the FIB-4 index has the greatest value in predicting liver fibrosis while APRI has the lowest; the more advanced liver disease is associated with lower serum level of quantitative HBs antigen. Combination of noninvasive blood biomarkers and imaging tests can provide better diagnostic accuracy and exclude the need for liver biopsy.

Introduction

Chronic infection with hepatitis B virus (HBV) is a global health problem, with over 350 million people worldwide affected by it, remaining the predominant cause of chronic liver disease and liver-related morbidity worldwide. This clinical condition is considered to be the major risk factor for cirrhosis, end-stage liver disease and hepatocellular carcinoma (HCC) [1] [2] [3]. Hepatitis Be antigen (HBeAg) negative chronic hepatitis B (CHB) is characterised by fluctuating levels of hepatitis B virus deoxyribonucleic acid (HBV DNA) and aminotransferases, with temporary remissions during the disease [4]. HBeAg-negative CHB patients with active hepatic necrotic

inflammation and persistent viraemia have higher rates of complications in contrast to HBeAg-negative patients with CHB who are inactive carriers. Both forms of CHB have similar laboratory and serologic characteristics and are not always easy to distinguish [5]. Assessment of liver fibrosis and its timely detection is essential for evaluation of liver disease severity. This is of particular importance for decision making and starting antiviral therapy and consequently preventing the development of CHB caused complications [4] [5] [6] [7].

Liver biopsy is the standard gold method for assessing the stage of the liver diseases. It is an invasive procedure, associated with pain and complications, where accurate results rely not only on the tissue sample quality and size but also of the

pathologists' experience [8]. Recently noninvasive methods for predicting liver fibrosis as well as imaging techniques, including transient elastography (TE), ultrasonography (US), computed tomography (CT) and magnetic resonance imaging (MRI) have been developed [4][9]. Serum markers for assessing liver fibrosis and cirrhosis can be used individually, but most of the times are combined to achieve better diagnostic sensitivity and specificity. Direct markers are representative of liver fibro-genesis and include glycoproteins, collagens, collagenases and collagenase inhibitors. Indirect markers reflect liver damage and correlate with liver fibrosis. They include platelet (PLT) count, aspartate transaminase (AST) and alanine transaminase (ALT), globulin level, serum quantitative HBs antigen (qHBsAg), ceruloplasmin, TGF- α , red blood cell distribution width, and serum Golgi protein 73 (GP73) [6].

Fibrosis index (FIB-4) is based on the four factors: evaluation of age, AST, platelets and ALT. Moreover, the FIB-4 index has been used to evaluate significant fibrosis and liver cirrhosis in HBV-infected patients in numerous studies [10] [11]. Concerning aspartate transaminase-to-platelet ratio index (APRI), it was first developed in the study of patients with chronic HCV infection [12], but it has been concluded that has moderate sensitivity and accuracy when it comes to HBV related fibrosis [13] [14]. Evaluation of the level of quantitative hepatitis B surface antigen (qHBsAg) reflects the amount of transcriptional activity of cccDNA and the integrated DNA in the hepatocytes [15] [16] representing one of the main serologic markers in chronic HBV infection; accurately monitoring both disease progression and prognosis as well as response to antiviral therapy [17] [18]. In this context, several studies have observed the correlation between quantitative HBsAg and liver fibrosis indicating their mutual correlation [19] [20] [21] [22].

Moreover, serum HBV DNA levels directly reflect the degree of HBV replication and are considered a strong prognostic indicator for CHB infection. Increasing HBV DNA levels correlate with the higher rate of progression to cirrhosis, the incidence of hepatocellular carcinoma (HCC), and subsequent death from HCC or chronic liver disease [23]. However, high HBV DNA levels do not always predict significant hepatitis [23].

Lastly, transient elastography (TE) is performed to measure the speed of the shear wave which is directly associated with the liver stiffness. TE measures the liver stiffness (LS) which by itself is associated with the degree of fibrosis [24] [25].

In this study, we evaluated the usefulness of noninvasive biomarkers FIB-4, APRI, quantitative hepatitis B antigen and HBV DNA for prediction of liver fibrosis in patients with HBe antigen negative chronic hepatitis B.

Material and Methods

A prospective, non-randomized study was conducted at the University Clinic for infectious diseases and febrile conditions which included fifty patients with hepatitis Be negative antigen CHB. The inclusion criteria were: age over 18 years, patients with serologically confirmed chronic hepatitis B, patients who were hepatitis B e antigen negative. All patients have been examined at least twice, with a minimum follow-up period of at least 6 months. All patients signed informed consent. The exclusion criteria were co-infection with human immunodeficiency virus (HIV), hepatitis A (HAV) and hepatitis C (HCV) as well as other liver diseases with different aetiology. Patients who had received antiviral therapy, patients who are currently on antiviral therapy, patients with hepatocellular carcinoma (HCC) and patients with liver failure were not included in the study.

All patients underwent standard laboratory and serology testing. We evaluated APRI and FIB-4 score as well as the values of aspartate transaminase (AST), alanine transaminase (ALT), qHBsAg, HBV DNA and liver fibrosis.

Relevant clinical variables were age, platelet count, ALT, AST, HBsAg, hepatitis B e antigen, HBV DNA, and fibroscan. The value of ALT, AST, qHBsAg and HBV DNA were expressed in IU/ml.

Quantification of HBV DNA levels in the plasma was performed by real-time polymerase chain reaction (RT-PCR) on COBAS AmpliPrep COBAS TaqMan HBV test and Abbott m 2000 sp/m 2000 rt with a lower detection limit of 10 IU/mL.

The serum level of HBsAg (qHBsAg) was quantified with Architect HBsAg assay (Abbott Laboratories) according to the manufacturers' protocol. The detection level of HBsAg varies from 0.05 to 250 IU/ml. Sera with HBsAg level higher than 250 IU/ml were diluted 1:500.

Abdominal ultrasound, as well as transient elastography, was performed on all patients. The liver stiffness was measured with transient elastography (TE); fibroscan (EchoSens^R, Paris, France) and expressed in kilopascals (kPa). The mean value was obtained from 10 performed measures, with success rate more than 60% and interquartile range (IQR) < 0.25.

Aspartate transaminase-to-platelet ratio index (APRI) was calculated with the following formula: $((AST/ULN \text{ AST}) \times 100) / \text{Platelets} (10^9/L)$. APRI score greater than 1.0 has a sensitivity of 76% and specificity of 72% for predicting cirrhosis. APRI score greater than 0.7 has a sensitivity of 77% and specificity of 72% for predicting significant hepatic fibrosis. APRI > 1.5 is the cut-off value for significant fibrosis, whereas a score <0.5 can rule it out [26].

The fibrosis index (FIB-4) is based on the four factors and calculated by the following formula: Age (yr.) xAST (IU/ml)/PLT (x10⁹/L) x ALT (IU/ml) 1/2. A FIB-4 score <1.45 has a negative predictive value of 90% in patients with advanced fibrosis. FIB-4 score >3.25 has a 97% specificity and a positive predictive value of 65 % of patients [10] [11].

All data were processed using a statistical computer program Statistica 7.1 for Windows and SPSS Statistics 17.0. For a description of the numerical variables descriptive statistics ((Mean; Std. Deviation; ± 95, 00%CI; Minimum; Maximum) was used, where frequencies and percentages were used for the description of the categorical variables.

To identify the predictive values for FIB-4, APRI, qHBsAg and HBV DNA for fibrosis, logistic regression analysis (Wald, Exp (B), 95, 0% CI for Exp (B), and p) were used. For all analyses, the P-value < 0.05 was considered statistically significant.

Results

A total of 50 chronic hepatitis B treatment naïve HBeAg-negative patients were included in the study. There were 26% female and 74% male patients. The patients' age ranged from 19 to 67 years. The platelet count ranges between the intervals from 104 to 344 x 10⁹/L.

The mean values of ALT vary in the interval 44.42 ± 42.63 IU/ml, and AST value varies in the interval of 29.88 ± 18.62 IU/ml. Serum qHBsAg values vary in the interval of 6143.21 ± 9372.24 IU/ml; the level of HBV DNA vary in the interval of 492303.2 ± 1642234 IU/ml (Table 1).

Table 1: Statistical analysis of the patients from the cohort

	Valid N	Mean	Confidence -95.00%	Confidence +95.00%	Minimum	Maximum	Std.Dev.
Age	50	38.820	35.754	41.886	19.0000	67.00	10.787
Tr	50	208.240	194.391	222.089	104.0000	344.00	48.731
ALT	50	44.420	32.304	56.536	10.0000	210.00	42.631
AST	50	29.880	24.589	35.171	13.0000	103.00	18.616
qHBsAg	50	6143.205	3479.644	8806.767	0.0500	55513.63	9372.241
HVD DNA	50	492303.2	25585.48	959020.9	24	10451113	1642234

Abbreviations: Tr: thrombocytes, ALT: alanine aminotransferase, AST: aspartate aminotransferase, qHBsAg: quantitative hepatitis B antigen, HBV DNA: hepatitis B virus deoxyribonucleic acid

Stratification of the patients according to the level of HBV DNA and qHBsAg showed that 50% of the patients had HBV DNA level lower than 2 000 IU/ml, 22% had HBV DNA more or equal to 2 000 IU/ml and lower or equal to 20 000 IU/ml, and in 28% HBV DNA level was higher than 20 000 IU/ml, while 36% patients had qHBsAg level lower or equal to 1 000 IU/ml and in 64% the value of qHBsAg was higher than 1000 IU/ml (Table 2).

Table 2: Frequency table (%) for HBV DNA, qHBsAg, fibroscan, APRI, FIB-4

HBV DNA	Number	Cumulative Number	%	Cumulative %
HBV DNA <2000 IU/ml	25	25	50.00	50.00
HBV DNA ≥2000≤20 000IU/ml	11	36	22.00	72.00
HBV DNA >20 000 IU/ml	14	50	28.00	100.00
Missing	0	50	0.00	100.00
qHBsAg	Number	Cumulative Number	%	Cumulative %
qHBsAg ≤1000 IU/ml	18	18	36.00	36.00
qHBsAg>1000IU/ml	32	50	64.00	100.00
Missing	0	50	0.00	100.00
Fibroscan	Number	Cumulative Number	%	Cumulative %
f0/f1	42	42	84.00	84.00
f2/ f2/f3	5	47	10.00	94.00
f3,f3/f4, f4	3	50	6.00	100.00
Missing	0	50	0.0	100.00
APRI	Number	Cumulative Number	%	Cumulative %
APRI>0.7	4	4	8.00	8.00
APRI<0.7	46	50	92.00	100.00
Missing	0	50	0.00	100.00
FIB-4	Number	Cumulative Number	%	Cumulative %
FIB 4<1.45	44	44	88	88
FIB4>1.45<3.25	5	49	10	90
FIB 4>3.25	1	1	2	100.0
Missing	0	50	0.00	100.00

Abbreviations: HBV DNA: hepatitis B virus deoxyribonucleic acid, qHBsAg: quantitative hepatitis B antigen, APRI: Aspartate transaminase-to-platelet ratio index, FIB-4: Fibrosis index based on the four factors

Liver stiffness measured with transient elastography showed that 84% patients had no fibrosis (f0/f1), 10% had intermediate fibrosis (f2 and f2/f3), while significant fibrosis (f3, f3/f4 and f4) was detected in 6% of the patients (Table 2).

Aspartate transaminase-to-platelet ratio index (APRI score) greater than 0.7 was observed in 8% of the patients, while in 92% of them was lower than 0.7. For detecting significant fibrosis, APRI scores greater than 1.5 was not observed in any patient, while APRI scores lower than 0.5 was found in 42 (84%) of the patients (Table 2).

Fibrosis index (FIB-4) based on the four factors showed that 88% patients included in our study had a FIB-4 score < 1.45, and 2% had a FIB-4 score > 3.25 (Table 2).

The predictive values of APRI, FIB-4, qHBsAg and HBV DNA were evaluated for fibrosis, using the model of discrimination. The global accuracy of this model in predicting fibrosis is 90.00% with a sensitivity of 55.60% and specificity of 97.60% (Table 3).

Table 3: Model of discrimination -Prediction of fibrosis with APRI, FIB-4, HBsQ, HBsQ, HBV DNA

Observed	Fibrosis	Predicted		Percentage Correct
		Absence	Presence	
Step 1 Fibrosis	Presence	40	1	97.6
	Absence	4	5	55.6
Overall Percentage				90.0

a. The cut value is 500; The global accuracy of this model for predicting liver fibrosis is 86, 00%. Sensitivity is 44.40%, and specificity is 95.10%

Table 4 reports the data obtained through logistic regression analysis which showed that FIB-4 has the greatest significance in this model (Wald = 3.24, P = 0.07), followed by intermediate high level of HBV DNA ≥ 2 000 IU/ml ≤ 20 000 IU/ml (1) (Wald =

2.86, $P = 0.09$), qHBsAg (Wald = 2.17, $P = 0.14$), HBV DNA $>20\ 000$ IU/ml (1) (Wald = 0.58, $P = 0.45$), while APRI has the lowest prediction for liver fibrosis, (Wald = 0.04, $P = 0.84$).

Logistic regression analysis showed that the increase of FIB-4 for one single cut-off value enhances the probability for fibrosis for 9.34 (Exp (B) = 9.34)/(834%) insignificant in 95% CI for EXP (B): 0.82-106.54, $P > 0.05$. Evaluation of the level of HBV DNA showed that patients with intermediate values of HBV DNA level between 2 000 IU/ml and 20 000 IU/ml compared to patients with low values of HBV DNA ($< 2\ 000$ IU/ml) have 10 times more probability for liver fibrosis, (Exp(B) = 10.38) 95% CI for EXP (B): 0.69-156.17, $P > 0.05$. Patients with qHBsAg level more than 1 000 IU/ml compared to patients with qHBsAg level of lower or equal than 1000 IU/ml have 0.15 (Exp(B) = 0.15) times lesser probability for liver fibrosis insignificant in 95% CI for EXP(B): 0.01-1.87, $P > 0.05$. Patients with high HBV DNA level ($>20\ 000$ IU/ml) compared to patients with low HBV DNA level ($<2\ 000$ IU/ml) have 3.50 times more probability for liver fibrosis, insignificant for 95% CI for EXP (B): 0.14-88.72, $P > 0.05$ (Table 4).

Table 4: Assessment of the logistic regression model

	B	S.E.	Wald	df	Sig.	Exp(B)	95% CI for EXP(B)	
							Lower	Upper
Step 1 ^a APRI	-0.46	2.30	0.04	1	0.84	0.63	0.01	57.75
FIB-4	2.23	1.24	3.24	1	0.07	9.34	0.82	106.54
qHBsAg	-1.88	1.28	2.17	1	0.14	0.15	0.01	1.87
HBV DNA $\geq 20\ 000$ IU/ml $\leq 20\ 000$ IU/ml	2.34	1.38	2.86	1	0.09	10.38	0.69	156.17
HBV DNA $>20\ 000$ IU/ml	1.25	1.65	0.58	1	0.45	3.50	0.14	88.72
Constant	-3.85	1.23	9.85	1	0.002	0.02		

a. Variable(s) entered on step 1: APRI, FIB-4, qHBs, HBV DNA $\geq 20\ 000$ IU/ml $\leq 20\ 000$ IU/ml, HBV DNA $>20\ 000$ IU/ml; Abbreviations: APRI : Aspartate transaminase-to-platelet ratio index, FIB-4: Fibrosis index based on the four factors, qHBsAg: quantitative hepatitis B antigen, HBV DNA: hepatitis B virus deoxyribonucleic acid.

Analysis of APRI index showed that the increase of APRI score for one single cut-off value decreases the probability for liver fibrosis for 0.63 (Exp (B) = 0.63)/(37%) insignificant for 95% CI for EXP (B): 0.01-57.75, $P > 0.05$ (Table 4).

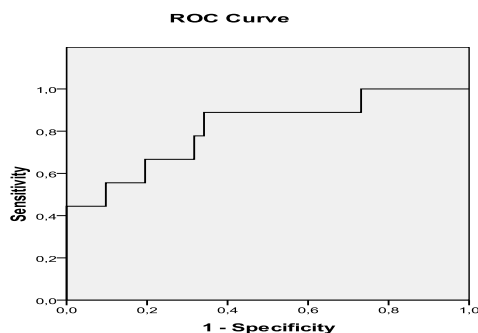


Figure 1: Area in the ROC curve model for fibrosis is 0.840 (95% CI 0.697-0.984), predicting the greater probability for fibrosis in 84% of all possible pairs of patients in which one has fibrosis, and the other does not

In the analysis of the area of the receiver operating curves (ROC) evaluating all four noninvasive biomarkers, the value of 0.840 means that in 84% of all possible pairs of patients where one has fibrosis, and the other pair is without fibrosis, this model will have higher predictive probability for fibrosis (Figure 1).

Discussion

The data from our study indicate that the greatest probability for distinguishing fibrosis in patients with HBeAg-negative CHB has a FIB-4 index. The clinical significance and applicability of this index is based on the following observations: the progression of the liver disease is age-related, and the disease duration is proportional with severe fibrosis; advanced fibrosis leads to mitochondrial injury of the liver cells and greater elevation of AST; more advanced fibrosis is associated with thrombocytopenia due to secondary hypersplenism and decreased production of thrombopoietin by liver cells [12].

Our study revealed that FIB-4 has the greatest significance in predicting liver fibrosis and that the increase of FIB-4 index for one single cut-off value increases the probability for fibrosis for 9 folds. The studies performed by Kim [10], as well as the study of Ma [14] showed that FIB-4 can be suitable for distinguishing significant and extensive fibrosis in patients with chronic hepatitis B. The Kim's study showed that AUROC's area of FIB-4 for predicting significant fibrosis, severe fibrosis and cirrhosis were 0.865, 0.910 and 0.923, respectively. The study conducted by Ma [14] also found that FIB-4 and Lock's model was the most effective models for distinguishing significant fibrosis in patients with chronic hepatitis B. A meta-analysis performed by Yin showed that FIB-4 has relatively high diagnostic value for detecting liver fibrosis in patients with hepatitis B when the diagnostic threshold value was more than 2.0. Similar to these studies, our report shows that patients, who had significant fibrosis, have about 9.3 fold greater chance of being FIB-4 positive (above 1.45) compared to patients without significant fibrosis.

Analysis of the impact of serum level of HBV DNA showed that intermediate high serum level of HBV DNA compared to high HBV DNA viraemia has higher significance in predicting liver fibrosis. Patients with HBV DNA levels ranging between 2 000 IU/ml and 20 000 IU/ml have 10 times more probability for liver fibrosis compared to patients with HBV DNA levels less than 2000 IU/ml, while patients with HBV DNA levels above 20 000 IU/ml have 3.50 times more probability for liver fibrosis compared to patients with HBV DNA level lower than 2000 IU/ml. Comparatively,

the study by Croagh [27] found that HBV DNA level was a predictor of significant fibrosis in HBeAg-negative CHB patients with varying ALT with an OR of 1.3 for every 1 log increment. HBV DNA levels also correlated with advanced fibrosis in HBeAg-negative CHB patients with normal ALT and varying ages as reported in the study of Xiao et al., [28]. In contrast, Shao reported that HBV DNA levels had no significant statistical association with liver histology regardless of HBeAg status [29]. It is known that HBV itself is not directly cytopathic and host immune response plays a pivotal role in HBV-related liver diseases [2] [30]. The role of HBV DNA in correlation with liver histology in HBeAg negative patients remains controversial because different methods and assays have been used in different studies [31]. Zacharakis et al., [32] reported that it is beneficial to follow HBV DNA levels in CHB patients who are HBeAg negative, as the HBV DNA levels correlate with the progression of hepatic damage. According to the data obtained from the patients included in our study, the level of HBV DNA is associated with the progression of fibrosis. In our study, patients with low HBV DNA viraemia have 3.5 and 10 times lower chance for liver fibrosis compared to the patients with intermediate and high HBV DNA level, respectively.

Quantitative Hepatitis B surface antigen represents a marker of CHB related liver damage, and qHBsAg levels are linked with progression of liver disease in HBeAg-negative patients [33]. Our study shows that patients with a qHBsAg level higher than 1 000 IU/ml compared to patients with a qHBsAg level lower or equal to 1000 IU/ml have 0.15 times lower probability for liver fibrosis. Patients who have lower qHBsAg level are associated with a higher probability of liver fibrosis. Our finding is discordant with the findings from several studies which reported that lower HBsAg levels are found in “inactive carrier” patients rather than in HBeAg-negative patients with “active” chronic hepatitis B [34] [35]. It is found that HBsAg production is reflective not only of cccDNA transcriptional activity but also originates from the integrated DNA in hepatocytes [16] [17] [34].

In contrast, it has been shown that the presence of mutations within the pre-S/S region reduces HBsAg production [36]. Patients with more advanced liver disease and liver cirrhosis had more frequent changes in the pre-S/S regions. This could, therefore, explain the lower total levels of qHBsAg in patients with advanced liver disease [34]. The study of Martinot-Peignoux [37] showed that there is a strong correlation between the stage of fibrosis and HBsAg level, but in HBeAg positive patients, while in patients with HBeAg negative CHB, qHBsAg was not found to be associated with any significant liver histologic changes. Unlike Martinot study, our study did not include patients with HBeAg positive CHB. Results from our study show that patients with high level of quantitative HBsAg (> 1000 IU/ml) have insignificant, but still, the lesser probability for liver fibrosis. This

can be a result of the pre-S/S region mutation found in patients with CHB genotype D or as a result of decreased liver cell mass associated with more extensive fibrosis. Unfortunately, in our institution, there is no possibility to perform HBV genotyping and detecting mutations.

In patients included in our cohort, the observed APRI score showed that it has the lowest prediction for liver fibrosis. The increase of APRI score for one single cut-off value decreases the probability for liver fibrosis for 0, 63 folds. Our finding is consistent with findings from the other studies which showed that APRI test designed as the “perfect noninvasive model” to evaluate liver fibrosis, has only moderate sensitivity and accuracy for assessing HBV related fibrosis [38].

At present, accurate diagnosis of liver fibrosis is essential for the prevention of disease progression and treatment of chronic liver disease. In our study, we evaluated the association of noninvasive biomarkers FIB-4, APRI, quantitative hepatitis B antigen and serum level of HBV DNA in correlation with transient elastography. The appraised clinical parameters age, platelet count, aspartate transaminase alanine transaminase, ultrasound and fibroscan in detecting liver fibrosis, correlate with noninvasive biomarkers in predicting liver fibrosis. Overall, our study shows that FIB-4 has the greatest predictive value for liver fibrosis in our patients with hepatitis be negative antigen CHB.

In conclusion, a whole plead of noninvasive markers is available for the determination of fibrosis and monitoring the progression and regression of fibrosis in chronic HBV patients. It appears that a combination of blood and imaging tests can provide the highest diagnostic accuracy and exclude the need for liver biopsy. Our study shows that FIB-4 index has the greatest impact in predicting liver fibrosis and that more advanced liver disease is associated with lower serum level of quantitative HBs antigen. It will be a challenge to define the best clinical strategy on how to apply validated noninvasive tests in the management of patients with chronic HBV infection. The drawback of our study was that the sample size may have been too small, our incapacity for HBV genotyping and determining pre-S/S region mutations. Further studies involving a greater number of patients and combination of more noninvasive biomarkers are needed for better evaluation of the applicability of these markers in distinguishing liver fibrosis.

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