Original Article

Relationship Between Serum DNA Replication, Clinicopathological Characteristics and Prognosis of Hepatitis B Virus-associated Glomerulonephritis with Severe Proteinuria by Lamivudine Plus Adefovir Dipivoxil Combination Therapy*

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Abstract

Objective To explore the relationship between HBV DNA and the clinical manifestations, pathological types, injury severity, and prognosis with HBV-GN.

Methods 102 patients with HBV-GN were divided into 3 groups, according to the serum titer of the HBV DNA. 24-h urine protein excretion, and other parameters were measured. Renal biopsy were performed. The association between HBV DNA and the pathological stage of membranous nephropathy was analyzed in 78 patients with HBV-MN. 24-h urine protein excretion was used for the evaluation of the prognosis, and the relationship between HBV DNA and prognosis were analyzed.

Results Several findings were demonstrated with the increase of serum HBV DNA: 24-h urine protein excretion, plasma cholesterol, and triglycerides increased significantly (P<0.05), while the plasma level of albumin decreased significantly (P<0.05); The changes of serum creatinine, C3 and C4 were found but no statistical significance. Glomerular deposition of HBVAg increased, and the pathological injury was more severe. The clinical remission rate was lower in the high replication group after treatment as compared with the low replication group (P<0.01).

Conclusion With the increase of serum HBV DNA, the urine protein excretion and the kidney injury were more severe, and the clinical remission rate was decreased.

Key words: Hepatitis B virus; Nephritis; Pathology; Proteinuria; Lamivudine; Adefovir dipivoxil

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INTRODUCTION

The incidence of HBV infection is very high in China. According to the Chinese national serosurvey in 2006, the weighted prevalence of HBsAg for the Chinese population aged 1-59 years was 7.18%[1]. Therefore, approximately 93 million people are carriers, and 30 million are chronically infected[2]. The incidence of Hepatitis B virus-associated glomerulonephritis (HBV-GN) is higher in China than in other countries. HBV-GN remains one of the most common secondary glomerular diseases in China[3]. Most HBV-GN patients present with nephritic syndrome or nephrotic syndrome, and some exhibit mild to moderate proteinuria with hematuria, even

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Biographical note of the first author: JIANG Wei, male, born in 1980, MD, majoring in mechanism of HBV-GN.

nephrotic-range proteinuria[4]. Quantitative detection of serum HBV DNA level plays an important role in the monitoring of virus replication and the evaluation of infectivity, as well as the choice of treatment methods. Although there are still debates on the relationship between the virus replication and the liver injury, several studies have already demonstrated some links between these two factors[5-6]. Few studies have focused on the relationship between the replication level of HBV DNA and the pathological injury of kidney, as well as the clinical manifestations. In the present study, the serum replication level of HBV DNA was measured, and its relationship with the clinical manifestation, pathological type, injury severity, and prognosis after medication in patients with HBV-GN were investigated.

MATERIALS AND METHODS

Criteria of the Patients

Patients with HBV-GN that had been treated in our hospital between January 2009 and October 2012 were selected. The inclusion criteria of the patients were as follows: (1) carrying hepatitis B virus and with HBV DNA replication; (2) were found with renal disease for the first time with the 24 h urine protein excretion level higher than 3.0 g, and the eGFR was not less than 60 mL/min/1.73 m^2 according to CKD-EPI formula[7]; (3) with normal ALT and AST level, and imaging examinations (ultrasound examination or CT) showed no abnormality; (4) without other primary or secondary renal diseases; (5) renal biopsy examination confirmed carrying hepatitis virus related antigens (including HBsAg, HBcAg, and HBeAg), and the patient could be diagnosed as HBV-GN according to pathology; and (6) had never been treated with antivirus drugs or immunosuppressor such as glucocorticoids. Finally there were 102 eligible patients (73 males and 29 females, male-to-female ratio of 2.5:1) included. The mean age of the patients was 36.3±10.1 years (ranging from 20 to 66 years). All the 102 patients were found with HBV DNA replication. As shown in Table 1, results of pathological examination of renal biopsy demonstrated that 78 (76.5%) of the patients were with membranous nephropathy (MN), 13 (12.7%) were with membranoproliferative glomerulonephritis (MPGN), and 11 (10.8%) were with mesangial proliferative glomerulonephritis (MsPGN). Parameters including 24-h urine protein excretion level and plasma levels of albumin, cholesterol, triglycerides, creatinine, C3, C4, ALT, and AST during the hospitalization and outpatient follow-up study period were collected for all the patients. Data before the treatment and at 6-, 12-, and 18-month after the treatment were collected and analyzed.

The present study was approved by the Ethics Committee of The Affiliated Hospital of Qingdao University and informed consent were obtained.

Standard of the Groups

Serum replication level of HBV DNA were determined by fluorescence quantitative polymerase chain reaction (with the detection range of 10^3-10^7 copies/mL) for all the 102 patients. Then the patients were divided into 3 groups according to the replication level[8], namely low- replication group (DNA<10^4 copies/mL), moderate- replication group (10^4 copies/mL≤DNA≤10^5 copies/mL), and high-replication group (DNA>10^5 copies/mL). No significant age or gender difference was found among these 3 groups.

Clinicopathological Characteristics

Lamivudine 100 mg/d and adefovir dipivoxil 10 mg/d were administered orally for all the patients. Parameters including 24-h urine protein excretion level and plasma levels of albumin, cholesterol, triglycerides, creatinine, C3, and C4 before the treatment and at 6-, 12-, and 18-month follow-up examinations were collected and compared. The mutation of the virus was also determined periodically during the follow-up period. The 24-h urine protein excretion levels, which were used to evaluate

Table 1. Baseline Demographic and Clinical Characteristics in Subjects with Patients

Items	*n*	Low Group	Mid Group	High Group
Gender				
Male	73	15	34	24
Female	29	6	16	7
Pathology				
MN Stage I	13	8	3	2
MN Stage II	37	6	24	7
MN Stage III	28	4	10	14
MsPGN	11	2	6	3
MPGN	13	1	7	5

the diagnosis of the patients, were compared among the 3 groups. Efficacy of the treatment was evaluated using the following parameters: (1) Complete remission of proteinuria was defined as a value for urinary protein excretion that was below 0.3 g/24 h; (2) Partial remission was defined as a decline in urinary protein excretion by 50% or more over baseline value but the absolute amount of proteinuria was over 0.3 g/24 h; (3) Treatment failure was defined as persistence of urinary protein excretion that exceeded 50% of the baseline value[9].

Immunofluorescence study of renal biopsy was used to detect the hepatitis virus related antigens (including HBsAg, HBcAg and HBeAg). In brief, each immunofluorescence slice was randomly collected five high power field (200-fold) by two pathologists to quantitative analysis (Image-Pro Plus version 6.0) total of positive glomerular area and integrated optical density (IOD) of positive staining area. The average IOD = IOD/area SUM. The five images of each slice calculated the average was IOD values of the slice[10]. The effects of serum HBV DNA replication level on the disposition of HBVAg was analyzed.

Seventy-eight of the 102 patients had been diagnosed with HBV-MN (including 18 patients in the low replication group, 37 in the moderate replication group, and 23 in the high replication group). The pathological stage of these 78 patients were determined and found that 13, 37, and 28 patients were with stage I, II, and III HBV-MN, respectively. The relationship between the pathological stage of these 78 patients and the virus replication level was analyzed. All HBV DNA replication values of the patients were converted into logarithmic values, correlation analysis was performed between HBV DNA replication level and urine protein excretion.

Statistical Analysis

SPSS 18.0 software was used for the statistical analysis. Quantitative data were expressed as mean±SD, and one-way ANOVA was used to compare the data among 3 or more groups, while *t*-test was used to compare the data between 2 groups. Chi-square test was used for the comparison of qualitative data. Spearman correlation coefficient was used for correlation analysis.

RESULTS

Comparison of 24-h Urine Protein Excretion and Other Parameters among the 3 Groups before the Treatments

Table 2 showed that as HBV DNA replication increased, a significant reduction in plasma albumin level was observed in the mid and high groups when compared with that in the low group, the 24-h urine protein excretion, cholesterol and triglycerides level in the mid or high group was significantly increased compared with that in the low group. While no significant differences were observed in serum creatinine, C3 or C4 in three groups.

Relationship between Serum HBV DNA Replication Level and Pathological Stage, Urine Protein Excretion of HBV-MN

Pathological injury was impaired progressively along with the increase of serum HBV DNA replication in HBV-MN patients. 44.5%, 33.3%, and 22.2% of the patients in the low- replication group were with stage I, II, and III disease, respectively; in contrast, 8.7%, 30.4%, and 60.9% of the patients in the high- replication group were with stage I, II, and III disease, respectively. The distribution of the pathological stages in different groups represented significant difference in Table 3. Figure 1 showed that there was significant correlation between urine protein excretion and HBV DNA replication level (log value) in HBV-MN.

Table 2. Comparison of Parameters Among Different Groups Before the Treatments (mean±SD)

Index	Low Group (*n*=20)	Mid Group (*n*=51)	High Group (*n*=31)
Urine protein (g/d)	3.6±0.6	4.7±0.8*	6.3±2.0$^{*,\#}$
Serum albumin (g/L)	37.9±4.7	30.3±6.4*	26.2±5.4$^{*,\#}$
Cholesterol (mmol/L)	5.7±1.0	7.7±1.5*	9.5±2.2$^{*,\&}$
Triglyceride (mmol/L)	0.9±0.4	1.5±0.4*	1.7±0.4*
Serum creatinine (μmol/L)	87.6±19.4	95.0±31.8	97±29.5
Serum C3 (mg/dL)	108.8±35.1	106.1±31.9	96.7±22.5
Serum C4 (mg/dL)	23.3±14.1	19.8±10.2	17.4±11.3

Note. $^{*}P<0.01$ as compared with low group; $^{\#}P<0.05$ and $^{\&}P<0.01$ as compared with mid group.

Effects of Serum HBV DNA Replication Level on the Deposition of HBVAg

Immunofluorescence examination in Figure 2A demonstrated that the average IOD value of the slice increased with serum HBV DNA replication, and the glomerular deposition of HBVAg (including HBsAg, HBcAg, HBeAg) and the extent of the lesion increased accordingly. Immunofluorescence examination of HBeAg demonstrated that the average IOD value was 0.22±0.13, 0.33±0.14, and 0.48±0.14 in the low-, moderate-, and high-replication groups, respectively, and the difference was statistical significant among these 3 groups. The average IOD value of HBcAg was 0.17±0.12, 0.22±0.13, and 0.31±0.16 in the low-, moderate-, and high-replication groups, respectively. The average IOD value of HBsAg was 0.16±0.14, 0.21±0.15, and 0.27±0.12 in the low-, moderate-, and high-replication groups, respectively. Figure 2B showed that significant differences in the average IOD value of HBsAg, HBcAg and HBeAg were found in high group when compared with that in the low group, while the average IOD value of HBeAg in mid group was significant higher than that in low group.

Table 3. Relationship between Serum HBV DNA Replication Level and HBV-MN

Groups	HBV-MN (*n*)		
	Stage I	Stage II	Stage III
Low group	8	6	4
Mid group	3	24	10
High group	2	7	14

Note. Spearman correlation coefficient between HBV DNA and HBV-MN was 0.3757, $P<0.01$.

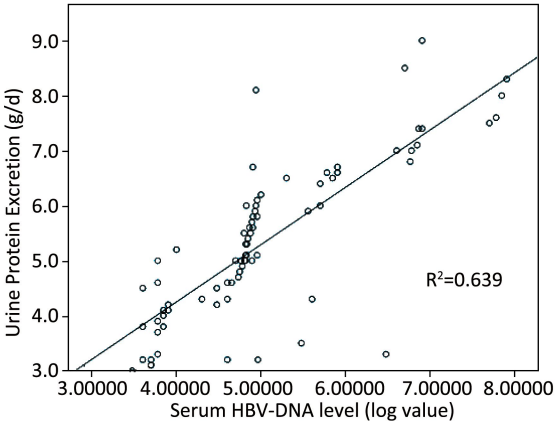

Figure 1. Spearman correlation coefficient between HBV DNA level and urine protein excretion was 0.823, $P<0.01$.

Comparison of the Efficacy of the Treatment among the 3 Groups

Adefovir dipivoxil in combination with lamivudine were administered orally for the patients in all three groups for more than 18 months. No liver function impairment, virus mutation, or renal function deterioration was found during the 18-month treatment. 24-h urine protein excretion level was used for the evaluation of the prognosis. The 24-h urine protein excretion level at 3 different time points after the treatment, namely 6-, 12-, and 18-month follow-up, was compared with the level before the treatment in all three groups. 24-h urine protein excretion level significantly decreased at 18-month treatment as compared with the level before the treatment in Figure 3 (A). The level of C3 increased after the treatment, however, no significant differences were observed in Figure 3 (C). In contrast, no significant change in the serum creatinine level was found after the treatment in Figure 3 (B). The HBV DNA replication level was significant lower than that in three groups after the treatment in Figure 3 (D). The overall remission rate was calculated at 18-month treatment, and Table 4 demonstrated difference among the patients in the 3 groups. The overall remission rate was 95.0%, 70.6%, and 54.8% in the low-, moderate-, and high-replication groups, respectively. There was significant correlation between HBV DNA lever in three groups and protein remission.

Table 4. Comparison of the Remission among the 3 Groups

Groups	*n*	CR	PR	NR	CR+PR (%)
Low group	20	13	6	1	19 (95.0)
Mid group	51	18	18	15	36 (70.6)
High group	31	9	8	14	17 (54.8)

***Note*.** Spearman correlation coefficient between HBV DNA level and protein remission was 0.2998, $P<0.01$.

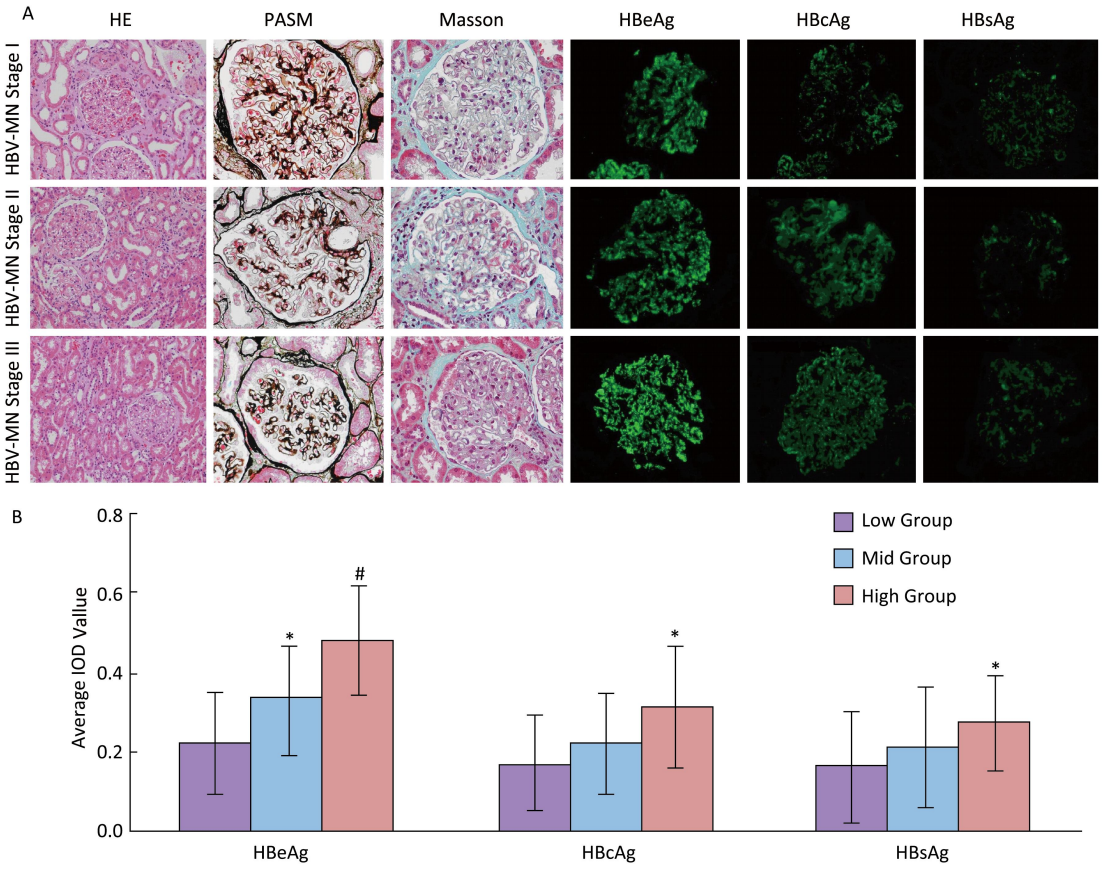

Figure 2. Effects of serum HBV DNA replication level on the deposition of HBVAg. (A) The pathology of light microscope and immunofluorescence in different stage of HBV-MN. (B) $^{*}P<0.05$ and $^{\#}P<0.01$ as compared with low group.

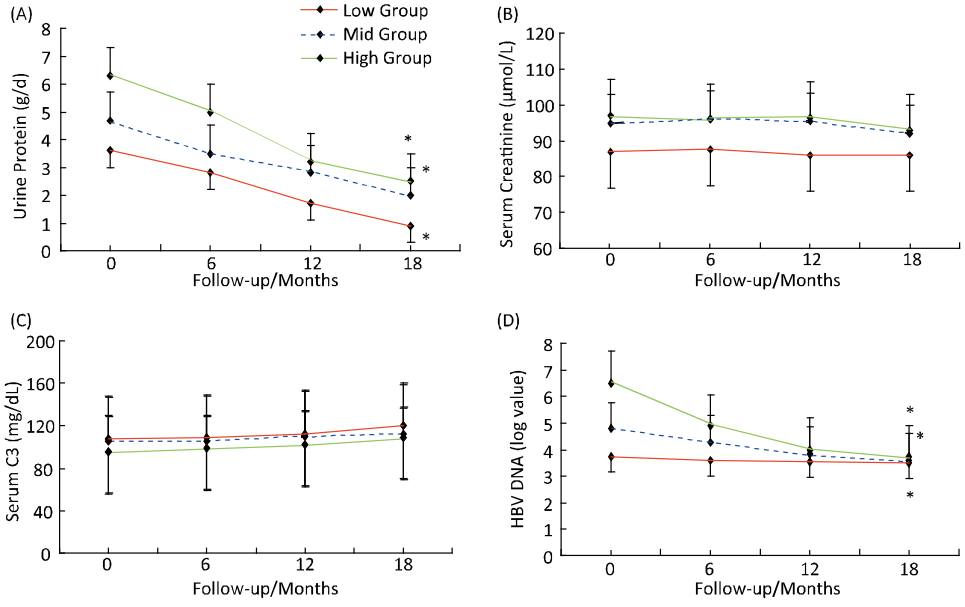

Figure 3. The index of patients were followed up in 0, 6, 12, and 18 months after treatment in three groups. (A) 24-h urine protein excretion. (B) serum creatinine. (C) serum C3. (D) serum HBV DNA replication level. *: $P<0.01$ *vs*. the level before treatment.

DISCUSSION

Generally, the serum HBV DNA replication level with less than 10^3 is of little significance for people's health. However, as the quantity of the virus increased, the replication of the HBV DNA is more active, and the infectivity is higher. HBV-GN is a very common secondary kidney disease, which is caused directly or indirectly by HBV. Combes. et al. have firstly identified the deposition of HBVAg on the glomerular basement membrane of a 53-year-old patient with membranous nephropathy[11]. The pathological mechanisms of HBV-GN are not yet known clearly. Four major pathogeneses have been suggested as the major routes of lesion to renal tissue by HBV in HBV-GN[12-13]: cytopathic collisions induced by cellular virus infection, deposition of immune complex particles attributed to viral antigens and host antibodies, virus-induced specific immunological mechanisms damaging the kidney, and the indirect adverse effects of virus-induced cytokines or mediators in the renal tissue. The immunopathogenetic mechanism is the most common cause for renal lesions[14]. Although several studies have not found the relationship between the serum level of HBV DNA and the liver injury[15-17], many clinical studies have confirmed that HBV replication can cause liver disease progression[18-19]. Such studies as the relationship between HBV replication and HCC, along with the increase of virus replication, the incidence of HCC was significantly increased with statistical difference[20-22]. Very few studies have investigated and found that the renal injury was associated with the replication level of HBV DNA[23]. In addition, many clinical studies have demonstrated that the renal injury relieved after anti-HBV therapies had been successfully performed, which also suggested the relationship between the serum replication level of HBV DNA and the pathological injury of kidney[3,24]. In the present study, we observed that the clinical manifestations and other biochemical parameters changed with the increase of serum level of HBV DNA. The level of 24-hour urine protein excretion, serum level of cholesterol and triglycerides increased with the increase of serum replication level of HBV DNA, while the serum complement level decreased accordingly. The immunofluorescence examination of the renal biopsies also demonstrated that the deposition of HBVAg increased and the pathological injury impaired progressively in the kidney with the increase of serum HBV DNA level. Pathological examinations of the 78 patients with HBV-MN showed that most of them were atypical membranous nephropathy. Patients with low replication level of HBV DNA were found with diffuse or irregular thickening of basement membrane, while no spike-like structure or substantial mesangial proliferation was found; increased glomerular capillary wall deposition of positive-staining substances was also found, but only limited glomeruli were involved. However, with the increase of the serum HBV DNA replication, spike-like structure and obvious mesangial proliferation, or even segmental glomerulosclerosis could be found, and increased deposition of positive-staining substances could be observed with immunofluorescence examinations, which could involve more glomeruli. These findings suggested that there were significant relationships between the serum replication level of HBV DNA and the deposition of immune complex, as well as the pathological injury of the kidney. Although the main pathological mechanisms involved in the kidney injury are inflammatory and immunologic injuries, We think that there are two mechanisms for the results. First, high level of HBV DNA replication can also lead to increased possibilities of renal deposition of related antigens and form immune complex, which in turn can lead to the activation of various inflammatory cells and cytokines. Decreased level of serum complements found in the present study also suggest that the increase of virus replication can activate the immune response and finally aggravate the pathological injuries of the kidney. Second, many studies have shown that gene mutation of HBV can result in virus replication and have more aggressive for tissues, which is specifical choice for chronic HBV infection[25-26]. The incidence of HBV-GN is markedly increased. However, there may be HBV mutations in patients with HBV-GN, gene sequencing and sequence alignment are needed for the patients.

Zhou Yi et al. showed that most patients with HBV-GN were successfully treated by anti-viral therapy. The clinical remissions of pediatric and adult patients in therapy groups were significantly higher than control groups[3]. Hossein et al.'s analysis of 119 patients from 10 studies also showed that lamivudine therapy was significantly associated with better outcome compared with IFN therapy, all patients receiving lamivudine had partial or complete remission, and none of them lost their kidneys[27]. Tang's study also showed lamivudine

treatment improved renal outcome in HBV carriers with MN and evidence of liver disease[9]. However, lamivudine had a high incidence of drug-resistance[28-29], adefovir dipivoxil was an antiviral nucleoside analog with a low drug resistance rate. Many studies had demonstrated lamivudine plus adefovir dipivoxil combination therapy resulted in effective in viral suppression and less risk in genotypic resistance[30]. In the present, lamivudine plus adefovir dipivoxil were administered orally for all the patients for more than 18 months. No liver function damage, drug resistant-HBV mutation, or renal function deterioration was found during the 18-month follow-up study. The serum replication level of HBV DNA was significantly associated with the urine protein level. The overall remission rate, which was evaluated with 24-h urine protein excretion level, was 95.0%, 70.6%, and 54.8% in the low-, moderate-, and high- replication groups, respectively, and statistical significant difference was found in three groups. These findings suggested that the replication level of HBV DNA played an role in the prognosis of the patients, and could be used as a serum parameter to evaluate the prognosis of the patients with HBV-GN.

In summary, the present study demonstrate that the serum replication level of HBV DNA can affect the degree of the renal injury in HBV-GN. The results of the clinical treatment also demonstrate different levels of the biochemical parameters and prognosis, suggesting that the replication level of HBV DNA could be used to help the treatment and evaluate the prognosis, and the pathological injury of the kidney. However, HBV-GN is a very complex disease which involves various pathological mechanisms and complex progression, and many factors including humoral immunity, cellular immunity, inflammatory cells, and cytokines are involved. What's more, genetic factors are also playing important roles in the pathogenesis of the disease as well as biopsychosocial factors[31]. The present study is limited by the relatively small sample size, more multi-central epidemiological studies with larger sample size are warranted to further investigate and validate these mechanisms.

CONFLICT OF INTEREST

No author has any financial/conflict interest to disclose.

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