Prevalence and Immune Response with interferons to BK Polyomavirus in Chronic Kidney Disease Patients

Ibrahim MS. Hussein [1*], Hajir A. Shareef [2]

Abstract

Background: BK polyomavirus (BKPyV) is recognized as a significant cause of renal impairment and graft failure in transplant recipients, with its reactivation posing severe complications, especially in immunocompromised individuals. Although BKPyV infection is well-documented in various populations, there is limited research on its prevalence and impact in Iraq, particularly among patients with chronic kidney disease (CKD). This study aimed to evaluate the prevalence of BKPyV in CKD patients and assess its relationship with interferon alpha and beta levels. Methods: The study included 130 CKD patients (76 males and 54 females) divided into hemodialysis (n=110) and conservative (non-dialysis) groups (n=20), along with a control group of 50 healthy blood donors. Blood samples were analyzed for BKPyV-specific antibodies (IgM and IgG) using ELISA. Interferon alpha and beta levels were also measured. Statistical analysis was conducted to assess the association between seropositivity, interferon levels, and demographic variables. Results: Among the non-dialysis CKD patients, 10% tested positive for anti-IgG, while all samples were negative for anti-IgM. In contrast, 19% of hemodialysis patients were anti-IgG positive, 14.5% were anti-IgM positive, and 8.2% were positive for both antibodies. Elevated levels of interferon alpha and beta were observed in seropositive patients compared to controls, with significant differences at $P < 0.05$. Male patients aged 20-29 and female patients aged 40-49 exhibited higher seropositivity rates. Conclusion: The study highlights a notable prevalence of BKPyV among CKD patients in Iraq, particularly those undergoing hemodialysis. Elevated interferon alpha and beta levels in seropositive patients suggest a significant role of these cytokines in the immune response against BKPyV infection. The findings also indicate demographic variations in seropositivity, emphasizing the need for targeted monitoring and management of BKPyV in CKD patients. Further research is needed to develop effective interventions for BKPyV-related complications in this high-risk population.

Keywords: BK Polyomavirus, Chronic Kidney Disease, Hemodialysis, Interferon Levels, Seropositivity

Significance | This study determined BKPyV prevalence in CKD patients, emphasizing the role of interferons in immune response and the need for vigilant monitoring.

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Introduction

BK polyomavirus (BKPyV) is a double-stranded DNA virus belonging to the Polyomaviridae family, which primarily infects humans and various other species such as birds, rodents, and primates (Bennett et al., 2012; Knowles, 2006). Since its discovery in 1971 from a kidney transplant patient with ureteric stenosis, BKPyV has become recognized for its potential to cause severe complications, especially in immunocompromised individuals like kidney transplant recipients (KTRs) and patients with human

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immunodeficiency virus (HIV) infection (Ambalathingal et al., 2017; Saade et al., 2020). While initial childhood infection with BKPyV is usually asymptomatic and leads to lifelong seropositivity in more than 80% of adults, the virus can reactivate in states of immunosuppression, leading to significant clinical complications, particularly in transplant recipients (Popik et al., 2019; Favi et al., 2019).

In kidney transplant patients, BKPyV reactivation can lead to BK virus-associated nephropathy (BKVAN), which is now acknowledged as the primary cause of renal impairment and graft failure (Sharma & Zachariah, 2020). Studies suggest that the median post-transplant BK viremia rate is approximately 19.5%, with a substantial proportion of affected patients progressing to BKVAN, significantly increasing the risk of allograft loss (Randhawa et al., 2008; Ali et al., 2020). In addition, BKPyV is associated with hemorrhagic cystitis (HC) in hematopoietic stem cell transplant (HSCT) recipients, highlighting its clinical relevance across different transplant populations (Najafabadi et al., 2020).

Upon viral infection, host cells generally mount a defense by releasing interferons, a group of cytokines that activate immune responses to eliminate the virus (Genin et al., 2009; Theofilopoulos et al., 2005). Interferons, particularly type I interferons such as interferon alpha (IFN-α) and interferon beta (IFN-β), are critical in inhibiting viral replication and facilitating immune communication to bolster antiviral defenses (Schmidt et al., 2014). However, viruses like BKPyV can encode genetic components that subvert the interferon response, contributing to their persistence and pathogenesis (Allam et al., 2009; Chen et al., 2019).

Given the limited studies on BKPyV in Iraq, the current study aimed to evaluate the prevalence of BKPyV among patients with chronic kidney disease (CKD) and to examine its relationship with the levels of interferons alpha and beta (Hussein et al., 2022). This study involved both hemodialysis and non-dialysis CKD patients. The findings revealed distinct patterns of seropositivity for BKPyV antibodies (anti-IgM and anti-IgG) between these groups. In non-dialysis CKD patients, all samples were negative for anti-IgM, while 10% tested positive for anti-IgG. In contrast, among hemodialysis patients, 19% were anti-IgG positive, 14.5% were anti-IgM positive, and 8.2% tested positive for both antibodies (Mohammad & Dawood, 2016; Marriott & Huet-Hudson, 2006).

Furthermore, the study observed a significant relationship between interferon levels and BKPyV seropositivity (Sharif et al., 2015; Voskuhl, 2011). Seropositive samples exhibited elevated levels of both interferon alpha and beta compared to controls, with statistically significant differences ($P < 0.05$). This correlation suggests that the host immune response, mediated by interferons, may play a role in controlling or modulating BKPyV infection in CKD patients (Egli et al., 2009; Schmidt et al., 2014). Additionally, the data highlighted age and sex-related differences in seropositivity rates, which may indicate varying susceptibility and immune response to BKPyV across different demographic groups (Klein et al., 2012; Hewagama et al., 2009).

By investigating the prevalence of BKPyV in CKD patients and its relationship with interferon levels, this study provides essential insights into the immunological landscape of BKPyV infection in Iraq. It underscores the need for vigilant monitoring and targeted interventions to manage BKPyV-related complications in immunosuppressed populations (Sharif et al., 2015; Sabri & Ibraheem, 2023).

2. Materials and methods

2.1 Subjects

2.1.1 Patient Group

This study included 130 patients (76 males and 54 females) with chronic kidney disease (CKD) aged between 15-64 years. The patients were divided into two groups:

Hemodialysis Group: Consisted of 110 patients with CKD in the dialysis end stage.

Conservative Group: Comprised 20 patients with CKD in a non-dialysis stage.

The patients were enrolled from the main dialysis units of Kirkuk General Hospital and Azadi Teaching Hospital, Kirkuk, Iraq, between October 2022 and May 2023. All participants provided informed consent prior to inclusion. A structured questionnaire was administered to collect epidemiological data, including age, gender, duration of dialysis, and dialysis interval. This study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki and was approved by the Ethics Committee of Kirkuk General Hospital and Azadi Teaching Hospital, Kirkuk, Iraq. All participants provided informed consent prior to inclusion in the study. The study adhered to all relevant guidelines and regulations to ensure the ethical treatment of participants and the validity of the research findings.

2.1.2 Control Group

The control group consisted of 50 healthy blood donors (40 males and 10 females), aged 21-49 years, without clinical signs of CKD and free from any systemic diseases. The controls were recruited from the blood donation center in Kirkuk city.

2.2 Specimen Collection

Venous blood samples (5 mL) were collected from each participant using standard phlebotomy techniques. The blood was placed in EDTA tubes to prevent coagulation. Plasma specimens were obtained by centrifuging the blood at 3000 rpm for 5 minutes. The plasma was then aliquoted into Eppendorf tubes and stored at -20°C until further analysis. The detection of IgG and IgM antibodies and the determination of interferon-alpha levels were performed using enzyme-linked immunosorbent assay (ELISA) kits, according to the

manufacturer's instructions (Ambalathingal et al., 2017; Sharma & Zachariah, 2020; Popik et al., 2019).

2.3 Statistical Analysis

Data were analyzed using IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp., Armonk, NY, USA). The Chi-square test was utilized to assess the association between categorical variables, such as seropositivity rates and patient characteristics. Hardy-Weinberg equilibrium calculations were performed to determine genotype and allele frequencies (Bennett et al., 2012; Saade et al., 2020).

3. Results and discussion

3.1 Detection of BKPyV IgM and IgG by ELISA

The results from the ELISA analysis indicated that all specimens from the control group were negative for both BK polyomavirus (BKPyV) anti-IgM and anti-IgG antibodies. Similarly, all patients with chronic kidney disease (non-kidney failure) tested negative for anti-IgM, with only two specimens testing positive for anti-IgG, representing a prevalence rate of 10%. For patients undergoing dialysis for kidney failure, a total of 110 specimens were analyzed, comprising both sexes with ages ranging from 15 to 64 years. Among these, 82 specimens (74.5%) were negative for both anti-IgG and anti-IgM antibodies. In contrast, 12 specimens (10.9%) were anti-IgG positive and anti-IgM negative, while 7 specimens (6.4%) were anti-IgG negative and anti-IgM positive. Additionally, 9 specimens (8.2%) were positive for both IgM and IgG simultaneously (see Figure 1).

The elevated prevalence of IgG seropositivity may be attributed to the cumulative effect of previous infections, reactivation, or new infections, as hemodialysis patients are considered immunosuppressed. These findings align with a study where 25 out of 72 cases (25.6%) had positive IgM results, and 21 out of 72 patients (29.2%) were positive for IgG, while the control group remained negative (Mohammad & Dawood, 2016). Similarly, Alireza et al. (2015) reported an infection rate of 3.03% for BK polyomavirus among hemodialysis patients. According to Randhawa et al. (2008), 71.4% of serum samples were positive for BKV IgG antibodies only, 14.3% were negative for both IgG and IgM, and 14.3% were positive for both IgG and IgM, with no samples positive only for IgM.

3.2 Seroprevalence of BKPyV Antibodies by Sex

The analysis revealed that 48 male specimens (75%) and 34 female specimens (74%) were negative for both anti-IgG and anti-IgM antibodies. For anti-IgG positive and anti-IgM negative results, 6 male specimens (9.4%) and 6 female specimens (9.4%) were observed. Furthermore, 4 male specimens (6.3%) and 3 female specimens (6.5%) tested positive for anti-IgM and negative for anti-IgG. Meanwhile, 6 male specimens (9%) and 3 female specimens (6.5%) were positive for both anti-IgG and anti-IgM (see Figure 2). Sexual dimorphism plays a significant role in influencing the regulation and responsiveness of immune systems to stimuli. Females generally exhibit more robust innate, cell-mediated, and humoral immune responses than males (Marriott & Huet-Hudson, 2006; Klein, 2012; Hewagama et al., 2009; Klein et al., 2012). This results in females having a heightened response to antigenic challenges, leading to reduced pathogen burden and quicker pathogen elimination but also a higher prevalence of immune-related diseases, such as autoimmune or inflammatory conditions (Voskuhl, 2011).

3.3 Seroprevalence of BKPyV Antibodies by Duration of Dialysis

The highest percentage of IgG-positive specimens was observed among patients who had been on dialysis for more than one year but less than two years (1 < 2) at 22.6%, followed by those on dialysis for two to three years (2 ≤ 3) at 20%. In contrast, the highest percentage of IgM-positive specimens was found in patients who had been on dialysis for more than three years (3 >) at 19.5%, followed by those on dialysis for one to two years (1 < 2) at 16.2%. These results were not statistically significant, as shown in Table 1.

3.4 Seroprevalence of BKPyV Antibodies by Dialysis Interval

The highest percentage of both IgG-positive and IgM-positive specimens was recorded in patients undergoing dialysis three times per week, at 20.6% each. This was followed by those receiving dialysis twice a week, at 19.4% for IgG and 16.6% for IgM, and finally by patients dialyzed once a week, at 17.5% for IgG and 7.5% for IgM. These results were not statistically significant, as shown in Table 2.

3.5 Seroprevalence of BKPyV Antibodies by Sex and Age Groups

3.5.1 Male Age Groups

Table 3 shows that the highest percentage of male samples positive for IgM and IgG antibodies were in the 20-29 age group, at 62.5% and 50%, respectively. This result was statistically significant.

3.5.2 Female Age Groups

The highest percentage of female specimens positive for IgM was found in the 40-49 age group at 27.3%, while the highest percentage of IgG-positive specimens was in the ≥60 age group at 25%. These results were not statistically significant, as shown in Table 4.

Table 4 highlights the relationship of seropositive antibodies to BKPyV across female age groups. In a study conducted by Schmidt et al. (2014), it was found that children under 10 years of age have very few CD4 T cells specific to BKV. Furthermore, BKV-specific IgG antibody levels increase with age, peaking in the 20-30 age range. Their research indicated that the immune response to BK virus is influenced by age, at both the cellular and humoral levels. Our analysis aligns with this study, demonstrating that the 20-30 age range exhibited the highest proportion of IgG and IgM antibodies. These results suggest that reactivation and a long-term IgM response to infection occur because the initial infection typically takes place during childhood, and the presence of IgM in

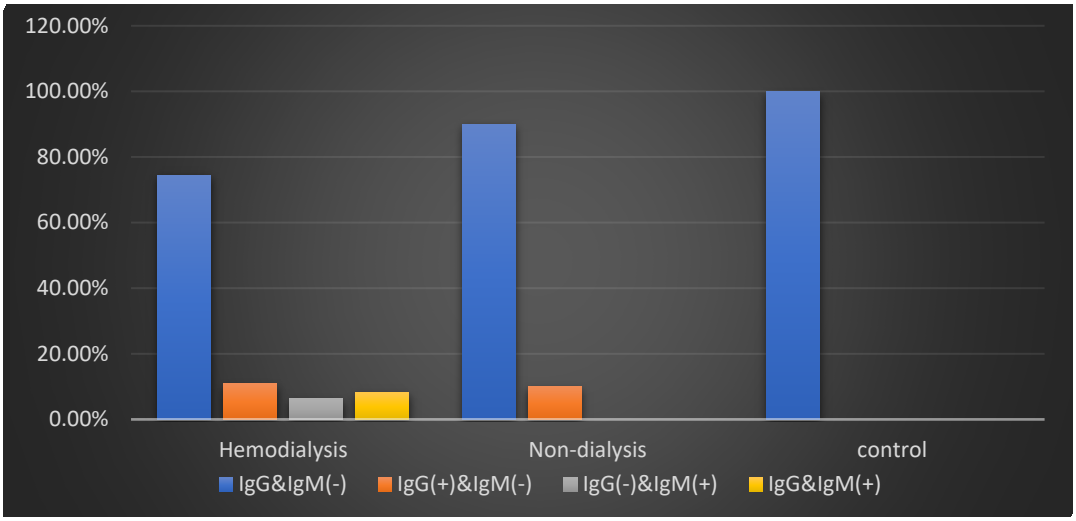

Figure 1. prevalence of BKPyV antibodies in study groups

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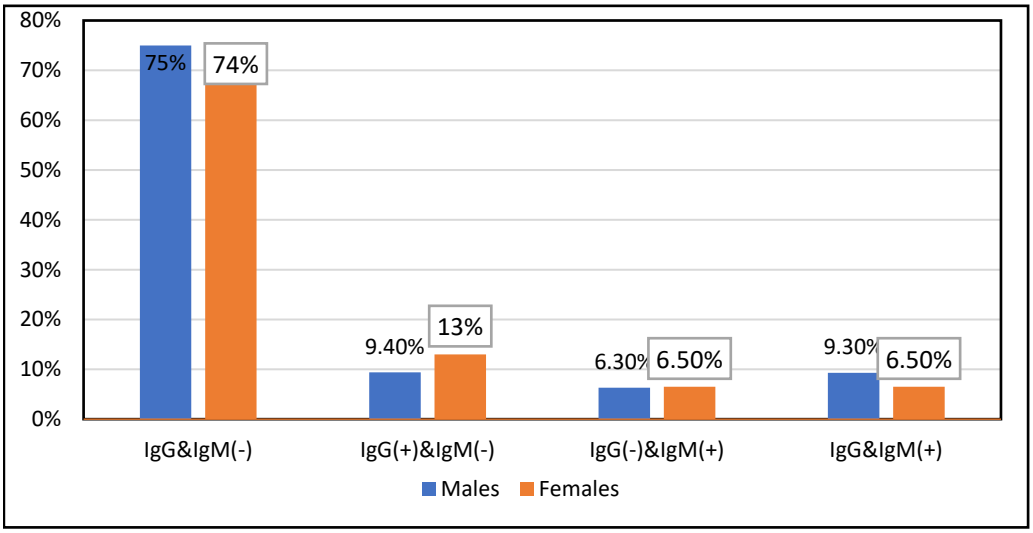

Figure 2. Prevalence of BKPyV antibodies in dialysis patients of both sexes.

Table 1. Seroprevalence of BKPyV antibodies by duration of dialysis.

Duration of dialysis (year)	IgG &IgM (-)	IgG(+) &IgM(-)	IgG(-) &IgM(+)	IgG &IgM (+)	Total
	No (%)	No (%)	No (%)	No (%)	
≤ 1	20(86.9%)	2(8.7%)	0(0%)	1(4.4%)	23(100%)
1 < 2	22(70.9%)	4(12.9%)	2(6.5%)	3(9.7%)	31(100%)
2 ≤ 3	15(75%)	2(10%)	1(5%)	2(10%)	20(100%)
3 >	25(69.4%)	4(11.1%)	4(11.1%)	3(8.4%)	36(100%)
Total	82(74.5%)	12(10.9%)	7(6.4%)	9(8.2%)	110(100%)
Chi-Square = 4.265 df = 9 P value = 0.893 P > 0.05 Non-significant (Ns)					

Table 2. Seroprevalence of BKPyV antibodies by dialysis interval.

Dialysis interval	IgG &IgM (-)	IgG(+) &IgM(-)	IgG(-) &IgM(+)	IgG &IgM(+)	Total
	No (%)	No (%)	No (%)	No (%)	
once a week	32(80%)	5(12.5%)	1(2.5%)	2(5%)	40(100%)
twice a week	26(72.3%)	4(11.1%)	3(8.3%)	3(8.3%)	36(100%)
three times a week	24(70.6%)	3(8.8%)	3(8.8%)	4(11.8%)	34(100%)
Total	82(74.5%)	12(10.9%)	7(6.4%)	9(8.2%)	110(100%)
Chi-Square = 2.997 df = 6 P value = 0.809 P > 0.05 Non-significant (Ns)					

Table 3. Relationship of seropositive antibodies to BKPyV to male age groups

Male age groups (years)	IgG &IgM (-)	IgG(+) &IgM(-)	IgG(-) &IgM(+)	IgG &IgM(+)	Total
	No (%)	No (%)	No (%)	No (%)	
< 20	2(100%)	0(0%)	0(0%)	0(0%)	2(100%)
20 – 29	1(12.5%)	3(37.5%)	2(25%)	2(25%)	8(100%)
30 – 39	9(69.2%)	1(7.7%)	1(7.7%)	2(15.4%)	13(100%)
40 – 49	17(89.4%)	1(5.3%)	0(20%)	1(5.3%)	19(100%)
50 – 59	11(78.7%)	1(7.1%)	1(7.1%)	1(7.1%)	14(19.7%)
≥ 60	8(100%)	0(0%)	0(0%)	0(0%)	8(100%)
Total	48(75%)	6(9.4%)	4(6.2%)	6(9.4%)	64(100%)
Chi-Square = 30.022 df = 15 P value = 0.012 P < 0.05 Significant (S)					

Female age groups (years)	IgG &IgM (-)	IgG(+) &IgM(-)	IgG(-) &IgM(+)	IgG &IgM(+)	Total
	No (%)	No (%)	No (%)	No (%)	
20 – 29	5(83.3%)	1(16.7%)	0(0%)	0(0%)	6(100%)
30 – 39	5(83.3%)	1(16.7%)	0(0%)	0(0%)	6(100%)
40 – 49	7(63.6%)	1(9.1%)	2(18.2%)	1(9.1%)	11(100%)
50 – 59	12(80%)	2(13.3%)	0(0%)	1(6.7%)	15(100%)
≥ 60	5(62.5%)	1(12.5%)	1(12.5%)	1(12.5%)	8(100%)
Total	34(73.9%)	6(13.1%)	3(6.5%)	3(6.5%)	46(100%)
Chi-Square = 6.101 df = 12 P value = 0.911 P > 0.05 Non-significant (Ns)					

Table 5. IFN α concentration rate of Seropositive antibodies in hemodialysis patients compared to the control group

groups	specimens No.	Interferon alpha pg/ml
		Mean ± Std. Deviation
Seropositive antibodies of BKPyV	28	611.643 ± 344.583
Control	28	407.495 ± 223.666
P. value = 0.025 T. value = 2.320 P < 0.05 Significant (S)		

Table 6. IFN β concentration rate of Seropositive antibodies in hemodialysis patients compared to the control group

groups	specimens No.	Interferon beta pg/ml
		Mean ± Std. Deviation
Seropositive antibodies of BKPyV	28	84.718 ± 75.493
Control	28	46.118 ± 27.755
P. value = 0.035 T. value = 2.178 P < 0.05 Significant (S)		

the serum of patients, both male and female, indicates reactivation of the virus (Schmidt et al., 2014).

A study conducted in Switzerland with 400 participants (200 females and 200 males) found that the incidence of the BK and JC viruses varied according to sex, although the presence of the BK virus remained relatively consistent in both male and female blood samples (83% vs. 82%) (Egli et al., 2009).

3.6 Relationship of Seropositive Antibodies of BKPyV to Serum Interferons

3.6.1 Interferon Alpha

Table 5 shows an increase in the average concentration of interferon alpha levels in the plasma of hemodialysis patients with seropositive anti-BKPyV antibodies (611.643 ± 344.583) compared to the control group (407.495 ± 223.666). The study results also indicated that 8 specimens exhibited significantly higher concentrations of interferon alpha levels compared to other specimens, with significant differences between the two groups at a probability level of $P < 0.05$. Although the kidney is typically a sterile organ, it can identify and react to pathogen-associated molecular patterns due to the presence of Toll-like receptors (TLRs) and other recognition molecules on both intrinsic renal parenchymal cells and intrarenal dendritic cells (DCs). Type I interferon (IFN), particularly sensitive to nucleic acids, is recognized by TLR3, which is expressed on both intrinsic renal cells and intrarenal conventional dendritic cells (Anders, 2007).

3.6.2 Interferon Beta

An increase in the mean level of interferon beta concentration was observed in the plasma of hemodialysis patients with seropositive anti-BKPyV antibodies (84.718 ± 75.493) compared to the control group (46.118 ± 27.755). The results also showed that 6 specimens had very high concentrations of interferon beta compared to other specimens, with significant differences between the two groups at a probability level of $P < 0.05$, as shown in Table 6.

The presence of polyomavirus in renal allograft tubular cells may stimulate the production of type I interferons; however, the onset or functional importance of type I IFN remains unclear. Unpublished research from our group suggests that cytosolic DNA sensors can identify polyomavirus DNA and induce cultured tubular epithelial cells to produce IFN-alpha and IFN-beta when DNA is translocated into the cytosol, similar to glomerular endothelial or mesangial cells. Transcription plays a significant role in regulating IFN induction caused by viral infections. The transcription factors IRF3 and IRF7, following phosphorylation by TBK1 and IKKe kinases, migrate to the nucleus and bind to IRF-binding sites in the promoters of IFN-alpha and IFN-beta (Allam et al., 2017; Marriott & Huet-Hudson, 2006).

4. Conclusion

This study demonstrated a significant prevalence of BK polyomavirus (BKPyV) among chronic kidney disease (CKD) patients in Iraq, particularly those undergoing hemodialysis. The findings indicate that hemodialysis patients exhibited a higher rate of seropositivity for both anti-IgG and anti-IgM antibodies compared to non-dialysis CKD patients and healthy controls. Additionally, the study demonstrates a significant correlation between increased levels of interferons alpha and beta and BKPyV seropositivity, suggesting that these interferons may play a crucial role in the immune response against BKPyV infection. Age and sex-related variations in seropositivity rates were also observed, indicating different susceptibilities to the virus across demographic groups. Overall, these results underscore the need for vigilant monitoring of BKPyV in CKD patients, particularly those receiving hemodialysis, to mitigate the risk of BKPyV-related complications. Further studies are warranted to explore targeted interventions that can effectively manage and control BKPyV infections in this vulnerable population.

Author contributions

I.M.S.H. is the principal author, responsible for setting objectives, data analysis, and the final revision of the paper. H.A.S. contributed by writing the abstract, methods, and materials sections. All authors reviewed and approved the final manuscript.

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Competing financial interests

The authors have no conflict of interest.

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