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Case report

Gamma heavy chain disease (γ-HCD) as iatrogenic immunodeficiency- associated lymphoproliferative disorder: Possible emergent subtype of rheumatoid arthritis-associated γ-HCD

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Gamma heavy chain disease (γ-HCD) is a rare B-cell neoplasm that produces a truncated immunoglobulin γ-heavy chain lacking the light chain. The clinical features of γ-HCD are heterogeneous, resembling different types of B-cell lymphomas. Although rheumatoid arthritis (RA) is one of the common underlying diseases of γ-HCD, the therapeutic modality for RA has changed greatly in recent years; therefore, γ-HCD as iatrogenic immunodeficiency-associated lymphoproliferative disorder (LPD) should be taken into consideration. Here, we report such a γ-HCD case. A 69-year-old female was admitted because of fever, multiple lymph node swelling in the abdominal cavity, and peritoneal effusion. She had been treated using methotrexate for RA for 14 years, and using infliximab and adalimumab for Crohn's disease for one year. The serum concentration of IgG was 3,525 mg/dL, which was revealed to be monoclonal IgG lacking the light chain by rocket immunoselection assay. $CD19^{+}$/$CD20^{-}$/$sm\kappa^{-}$/$sm\lambda^{-}$ large abnormal lymphocytes were observed in the peritoneal fluid, which were demonstrated to be clonal B-cells by PCR examination. Discontinuation of methotrexate did not improve her condition and she died of pneumonia. Many abnormal lymphocytes positive for IgG and *EBER* but negative for the light chain were found on immunohistological examination of necropsy specimens from the spleen and bone marrow.

Keywords: Gamma heavy chain disease, methotrexate, infliximab, iatrogenic immunodeficiency-associated lymphoproliferative disorder, Epstein-Barr virus

INTRODUCTION

Gamma heavy chain disease (γ-HCD) is a B-cell neoplasm that produces a truncated immunoglobulin γ-heavy chain lacking the light chain-binding sites, subsequently forming an incomplete immunoglobulin molecule without a corresponding light chain.[1-5] γ-HCD is rare and to date, approximately 150 cases have been reported.[5] The clinical features of γ-HCD are heterogeneous, resembling marginal zone lymphoma, plasmacytoma, lymphoplasmacytic lymphoma (LPL), and chronic lymphocytic leukemia, and most patients with this disorder have generalized disease.[5,6] Some γ-HCD patients (25-30%) also have autoimmune diseases, commonly rheumatoid arthritis (RA).[3,6-8]

The therapeutic modality for RA, however, has greatly changed in recent years, including treatments with different biologicals and methotrexate (MTX), in addition to corticosteroid and non-steroidal anti-inflammatory drugs. As a consequence, iatrogenic immunodeficiency-associated lymphoproliferative disorder (LPD), most commonly MTX-associated LPD, has emerged as a complication in recent years.[9] Iatrogenic immunodeficiency-associated LPD exhibits a wide spectrum of clinical features, including diffuse large B-cell lymphoma, follicular lymphoma, MALT lymphoma, lymphoplasmacytic lymphoma (LPL), and peripheral T-cell lymphoma.[9] Along with the changes in the therapeutic modality for RA, γ-HCD as iatrogenic immunodeficiency-associated LPD should be taken into consideration. We encountered a γ-HCD patient who had been treated with MTX, and infliximab and adalimumab for RA and Crohn's disease, respectively.

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Here, we report this RA patient with γ-HCD as iatrogenic immunodeficiency-associated LPD following 2 such cases.[10,11]

CASE REPORT

A 69-year-old female was admitted because of fever, general fatigue, swelling of multiple lymph nodes in the thoracic and abdominal cavities, and pleural and peritoneal effusion in October 2015. She had been treated using MTX for RA since 2001, and using infliximab and adalimumab for Crohn's disease in 2011. She also underwent right hip and knee replacement surgeries in 2010.

Physically, she was febrile (38.4°C) and the saturation of percutaneous oxygen was as low as 94%. The abdomen was distended and edema of the lower body was noted. Neither superficial lymphadenopathy nor hepatosplenomegaly was observed. On auscultation, mild stridor was heard and vesicular sounds were diminished in the lower bilateral lungs. She did not complain of arthralgia, although mild deformity of extremity joints was observed. CT of the chest and abdomen demonstrated abundant ascites, and multiple small-sized lymphadenopathies at the mediastinum, right pulmonary hilum, abdominal paraaortic, right lilac, and bilateral inguinal regions, suggesting malignant lymphoma. Organized inflammatory lesions in the bilateral lower lobes of the lungs were also noted on chest CT. Therefore, the fever on admission may have been caused by tumoral fever of the suspected lymphoma or organized pneumonia. In addition, the serum concentration of soluble interleukin-2 receptor was markedly increased to 8,640 U/mL (normally 145 to 519 U/mL) (Table 1).

The results of laboratory examinations on admission are shown in Table 1. The hemoglobin concentration was 9.0 g/dL, white blood cell (WBC) count was 7.9×10^9/L with a low percentage of lymphocytes (14.0%), and platelet count was 100×10^9/L. The percentage of reticulocytes was high at 3.5% (normally 0.5 to 1.5%). Hemostat tests revealed PT-INR, APTT, and D-dimer to be 1.93 (normally 0.85 to 1.15), 77.9 seconds (normally 25.0 to 35.0 seconds), and 6.4 μg/mL (normally 0.0 to 1.0 μg/mL), respectively. Although the Coombs tests were negative, serum concentrations of total bilirubin and indirect bilirubin were elevated to 2.7 mg/dL (normally 0.2 to 1.3 mg/dL) and 1.5 mg/dL (normally 0.0 to 0.8 mg/dL), respectively, in addition to low levels of haptoglobin (below 10 mg/dL) and total cholesterol (61 mg/dL), suggesting Coombs-negative autoimmune hemolytic anemia (AIHA) or severe liver dysfunction of unknown cause. The hemoglobin concentration, however, remained around 9.0 g/dL thereafter regardless of suspected AIHA without any specific treatments. Serological tests for human hepatitis B and C viruses were negative, but those for Epstein-Barr virus (EBV) demonstrated a previous infection pattern with negative EBV-early antigen (EA)-IgG test (Table 1), suggesting chronic active EBV infection to be unlikely. On the other hand, the multiplex virus PCR assay[12] detected 2.2×10^2 copies of EBV genome/mL, which later increased to 7.8×10^5 copies/mL.

The serum concentration of IgG was increased to 3,525 mg/dL (normally 870 to 1,700 mg/dL) and an M peak was observed on serum electrophoresis (Figure 1A), revealed to be monoclonal IgG lacking the light chain by serum immunofixation (Figure 1B), indicating γ-HCD. γ-HCD was further confirmed by rocket immunoselection assay (Figure 2). On bone marrow aspiration, the nucleated cell count and granulocyte/erythrocyte ratio were 45×10^9/L and 1.23,

Table 1. Laboratory findings on admission

Hematology		Chemistry			
WBC	7.9×10^9 /L	AST	9 IU/L	T-Cho	61 mg/dL
RBC	$2,690\times10^9$ /L	ALT	8 IU/L	TG	36 mg/dL
Hb	9.0 g/dL	ALP	414 IU/L	Haptoglobin	<10 mg/dL
MCV	99.6 fL	T.Bil	2.7 mg/dL	Ferritin	874 ng/dL
MCH	33.5 pg	I.Bil	1.5 mg/dL	**Serology**	
Platelets	100×10^9 /L	LDH	141 U/L	CRP	5.77 mg/dL
seg	69.5 %	γGTP	54 U/L	IgG	3,525 mg/dL
bas	0.5 %	ChE	37 U/L	sIL-2R	8,630 U/mL
mon	15.5 %	BUN	10.4 mg/dL	RF	(-)
lym	14.0 %	CRE	0.45 mg/dL	EBV-EA-IgG	0.1
atyp. lym	0.5 %	UA	2.4 mg/dL	EBV-VCA-IgM	0.0
Retic	3.5 %	Na	130 mEq/L	EBV-VCA-IgG	1.3
Blood coagulation		K	3.9 mEq/L	EBV-EBNA 1-IgG	1.6
PT(INR)	1.93	TP	4.9 g/dL	EBV-PCR	2.2×10^2 copies/mL
APTT	81.7 Sec	Alb	1.8 g/dL	Direct Coombs	(-)
D-dimer	6.4 μg/mL			Indirect Coombs	(-)

Atyp. lym: atypical lymphocytes. Retic: reticulocytes (normally 0.5 to 1.5%). The normal range of PT-INR: 0.85 to 1.15; APTT: 25.0 to 35.0 seconds; D-dimer: 0 to 1.0 μg/mL; IgG: 870 to 1,700 mg/dL. sIL-2R: soluble interleukin-2 receptor (normally 145 to 519 U/mL). RF: rheumatoid factor. EBV-VCA: Epstein-Barr virus viral capsid antigen. EBV-EA: EBV early antigen. EBNA: EBV nuclear antigen. The results were evaluated as negative, faintly positive, and positive when the value was below 0.5, 0.5-0.9, and greater than 1.0, respectively.

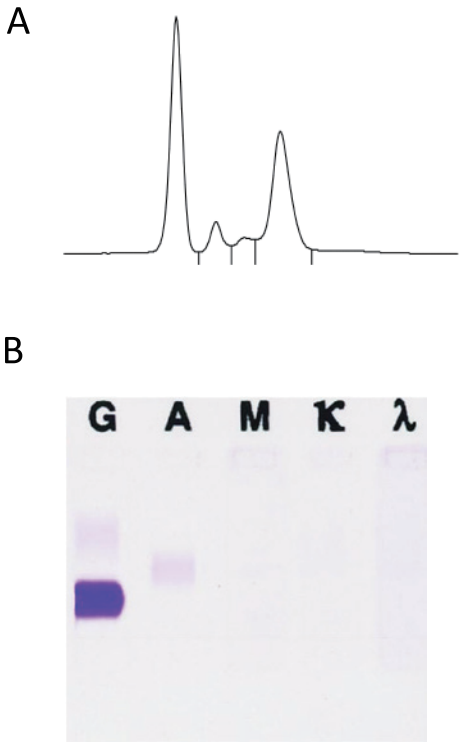

Fig. 1. An M-peak was noted on serum electrophoresis (***A***). This M-protein was revealed to be monoclonal IgG lacking the light chain by immunofixation (***B***).

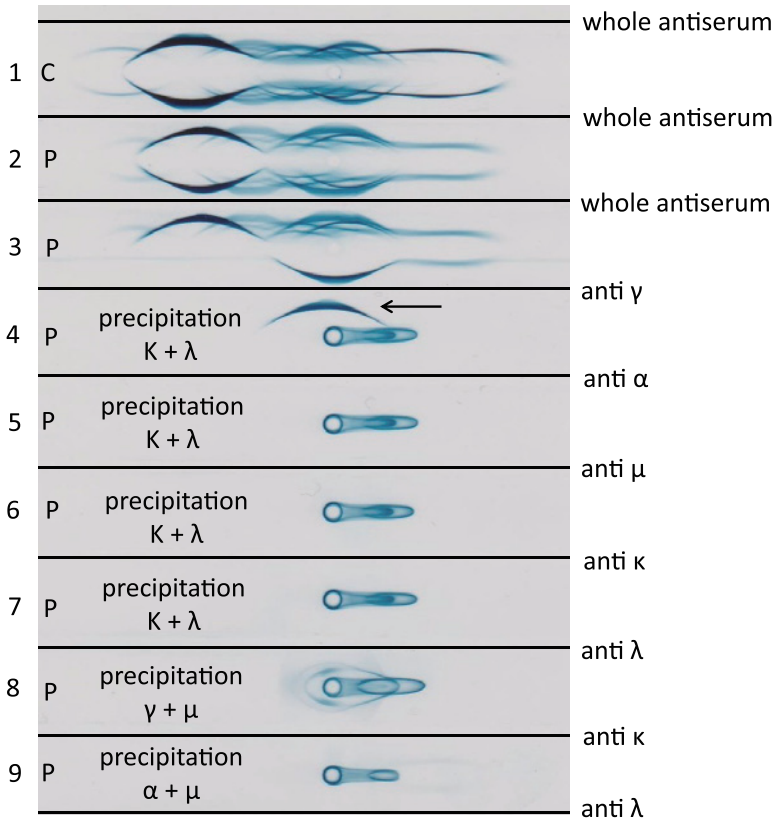

Fig. 2. Rocket immunoselection assay. A precipitation line representing a reaction with the anti-γ-antibody was observed (arrow) even after immuno-precipitation of whole immunoglobulins with anti-κ and -λ antibodies (lane 4).

respectively. A small number of large immature lymphoid cells were observed; however, analysis of flow cytometry (FCM) was difficult because of the small number of cells. These abnormal lymphocytes were also observed in the peripheral blood (0.8% of WBC), and on FCM, these cells were positive for CD19, but negative for CD20 (dim), CD5, CD10, CD23, sm/cyIg (α, μ, γ, δ), and sm/cyκ and λ light chains. Furthermore, many immature abnormal lymphocytes were observed in the ascites (Figure 3). FCM analysis of these cells yielded identical results to those of the peripheral blood, although cytoplasmic immunoglobulin heavy and light chains were not examined. PCR examination of these lymphocytes demonstrated monoclonal rearrangement of the immunoglobulin heavy chain (IgH) gene, but not the T-cell receptor-γ gene, suggesting neoplastic B cells. Based on these results, a diagnosis of iatrogenic immunodeficiency-associated LPD, which was substantially γ-HCD, was established.

We discontinued oral MTX and administered oral prednisolone (20 mg/day), but not chemotherapy, because of her poor general condition. This treatment reduced the size of generalized lymphadenopathy as evaluated by CT. However, her general condition did not improve and the multiplex virus PCR assay[12] detected multiple virus genomes in the serum (EBV: 7.8×10^5, cytomegalovirus: 1.7×10^2, BK virus: 7.5×10^2 copies/mL), suggesting increased tumor burden of the EBV-related γ-HCD and exacerbated immunodeficiency.

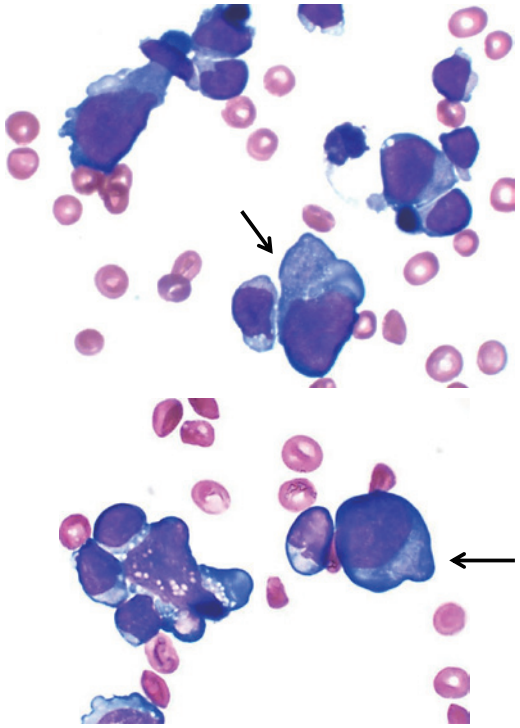

Fig. 3. Cytospin preparation of ascites. Many large abnormal lymphoid cells were observed. Their nuclear chromatin was fine and some of these abnormal cells were morphologically plasma cell-like (arrows).

She died of pneumonia 2 months after admission. Immunohistological examination of necropsy specimens from the spleen and bone marrow revealed many abnormal lymphocytes positive for IgG, but not for κ and λ light chains (Figure 4) These abnormal lymphocytes were positive for EBV-encoded small RNA (*EBER*) (Figure 4) but negative for CD20, CD79a, CD138, and Pax5 (data not shown). Only a few abnormal lymphocytes positive for IgG were observed in the necropsied liver.

DISCUSSION

Although γ-HCD is a rare B-cell neoplasm with a wide spectrum of clinical features,[1-5] RA-associated γ-HCD is relatively common. To the best of our knowledge, 15 γ-HCD patients with RA as an underlying disease have been reported.[3,6-8,10,11,13-20] Two γ-HCD patients were treated using MTX and corticosteroid[10] or MTX alone[11] for RA before the onset of LPD, and were later found to have γ-HCD; therefore, γ-HCD in these 2 patients is considered to be iatrogenic immunodeficiency-associated LPD, because this LPD subtype is tentatively defined as that arising in patients with autoimmune diseases with a history of treatment with immunosuppressive agents such as MTX,[9] although the causative mechanism of MTX for the development of LPD has not been elucidated. One more γ-HCD patient had been treated using MTX for seronegative RA; however, the patient did not have constitutive symptoms, lymph node swelling, hepatosplenomegaly, tumoral lesions, or bone marrow abnormality at the time of γ-HCD diagnosis.[20] Furthermore, the cells that produced the monoclonal γ-heavy chain were not found or identified in this patient. Therefore, γ-HCD in this patient lacked the clinical features of LPD and the diagnosis of iatrogenic immunodeficiency-associated LPD was unlikely. In the remaining 12 γ-HCD patients with RA, MTX or immunosuppressants were not used for RA treatment,[3,6-8,13-19] although the treatment agent was not described for one patient.[7] Therefore, the present case may be the third reported case of γ-HCD as iatrogenic immunodeficiency-associated LPD. Although γ-HCD is currently rare, it may be an emergent disorder because of the increased use of immunosuppressive agents including MTX for the treatment of autoimmune diseases, especially RA, in recent years. In addition, the EBV genome was detected in the serum, and γ-chain-producing cells were positive for *EBER* in the present patient. *EBER* was faintly positive in the biopsied lymph node from the similar γ-HCD patient.[11] In another γ-HCD patient, the EBV infection status was not described.[10] Thus, to our best knowledge, there has been no report of confirmed EBV-related γ-HCD. Immunodeficiency-associated LPD

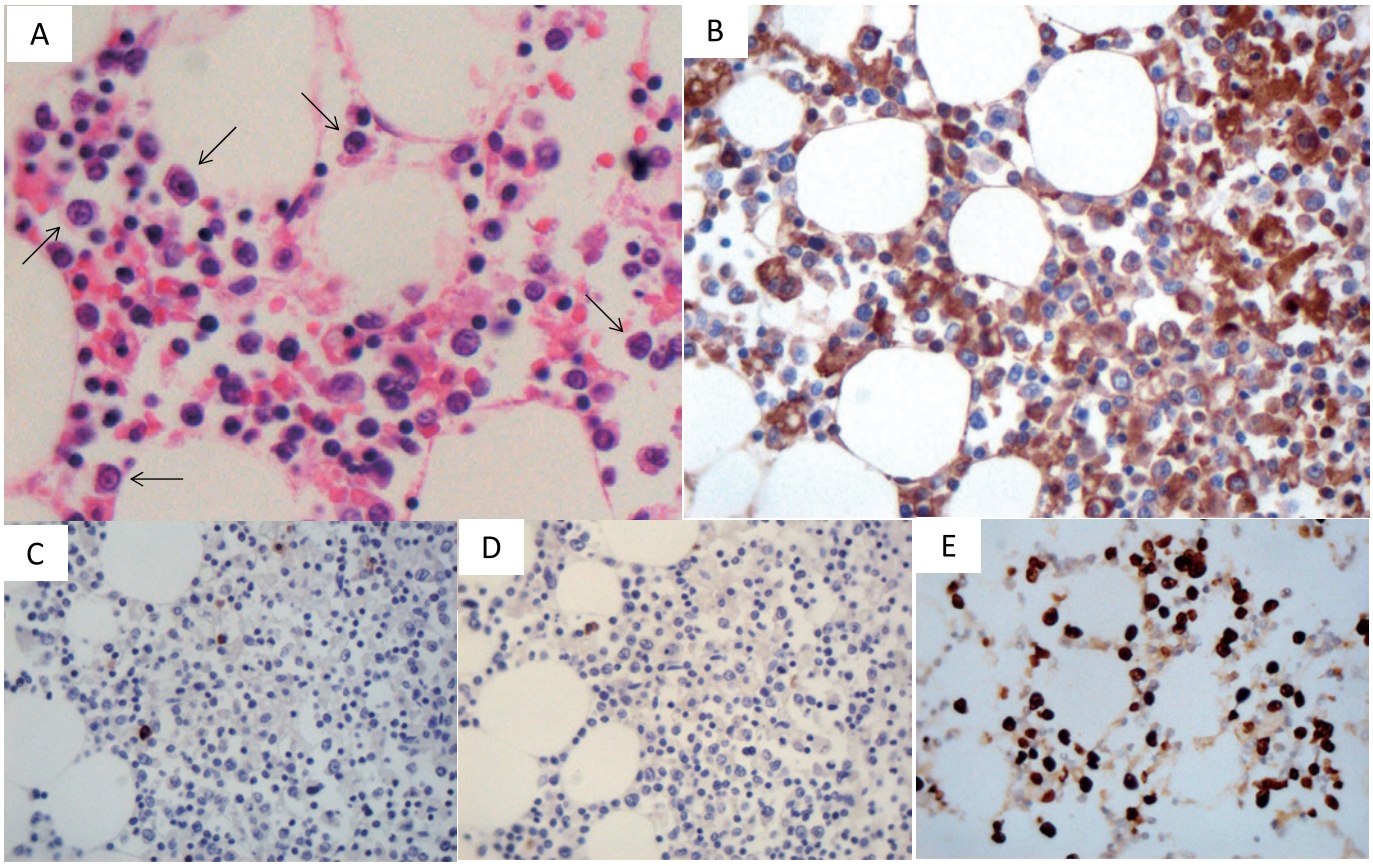

Fig. 4. Immunopathological examination of necropsied bone marrow. ***A***: many immature large lymphoid cells can be seen (arrows) (HE staining, ×400). ***B***: These abnormal cells were positive for IgG (immunostaining with polyclonal anti-IgG, ×400). ***C*** and ***D***: Few cells were positive for κ- and λ- light chains (immunostaining with anti-κ and -λ antibodies, respectively, ×200). ***E***: These abnormal cells were positive for EBV-encoded small RNA (*EBER*) (×200).

is usually associated with type III EBV latency, which is characterized by full EBV gene expression, including EBV nuclear antigen (EBNA) 2 to 5.[21] The tumor cells in the present patient may have exhibited type III latency because the tumor cells expressed *EBER* and EBNA 1 was positive on the serological test. However, we did not examine EBNA 2 or latent membrane proteins (LMP) 1 and 2; therefore, the exact type of EBV latency in the present patient was unclear. The relationship between EBV infection and γ-HCD development should be clarified in the future.

The clinical picture of iatrogenic immunodeficiency-associated LPD in the present patient was generalized disease involving the spleen, lymph nodes, bone marrow, peripheral blood, and peritoneum without a bulky mass. The B-cell neoplasm in the present patient, therefore, resembled LPL based on its pattern of tumor infiltration and monoclonal protein production, as described by Wahner-Roedler *et al*. for a cohort of γ-HCD patients.[6] However, the phenotype of the present case was different from that of typical LPL,[22,23] as well as iatrogenic immunodeficiency- or EBV-associated LPD,[9,24,25] in terms of being negative for CD20, CD138, CD79a, and Pax5. Although the reason for the negativity of these antigens is unclear, they may have been lost through accumulated genetic alteration in the process of tumor development. Alternatively, this phenotype may be characteristic of immunodeficiency- and EBV-associated γ-HCD. Thus, phenotypic and molecular investigations will be required in the future in a cohort of similar patients.

In FCM analysis of abnormal lymphocytes in the peripheral blood, cyIgG was undetectable regardless of the high possibility of γ-chain production by these cells. The negative result may have been due to the monoclonal anti-γ antibody used in FCM analysis. The γ-chain produced in γ-HCD is subsequently truncated, being an incomplete IgG molecule (one-half to three-quarters of the length of the normal γ-chain[4]); therefore, detection of incomplete IgG by the monoclonal antibody may not have been possible. Indeed, in another γ-HCD case, we were able to detect cyIgG using the polyvalent anti-IgG antibody but not the monoclonal antibody in FCM analysis.[26] On immunohistochemistry of a necropsy specimen from the present patient, we successfully detected IgG in abnormal lymphocytes because we used the polyvalent anti-IgG antibody. The presence of many IgG-positive tumor cells in the necropsied bone marrow may reflect cumulative tumor infiltration over 2 months because the marrow aspirate on admission only included a small number of abnormal cells on morphological and FCM evaluation.

In conclusion, we reported a rare case of iatrogenic immunodeficiency-associated γ-HCD, which may be an emergent subtype of γ-HCD. Further phenotypic and molecular investigations are required involving similar cases in the future.

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CONFLICT OF INTEREST

The authors declare no conflict of interest regarding this study.

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