Heliyon 9 (2023) e18907

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Application of immunomodulatory therapy in a human brucellosis patient with pancytopenia: A case report

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ARTICLE INFO

Keywords:
Brucellosis
Pancytopenia
Bone marrow suppression
Immunomodulatory therapy

ABSTRACT

Brucellosis is a common zoonotic infectious disease with diverse and non-specific clinical manifestations caused by *Brucella*. Although *Brucella* can cause damage to multiple systems in the human body, hematological complications are relatively rare. We present a case of a 47-year-old male brucellosis patient with pancytopenia. In May 2018, the patient was diagnosed with brucellosis and recovered after receiving antibiotic treatment (rifampicin 600 mg/day and doxycycline 200 mg/day) for six weeks. However, after three years, the patient experienced a recurring high fever. Brucellosis relapse was confirmed based on the patient's clinical history, Rose Bengal plate agglutination test and standard tube agglutination test results. Routine blood examination revealed a decrease in the whole blood cell count, suggesting bone marrow suppression. Bone marrow aspiration and bacterial culture confirmed the diagnosis of brucellosis with pancytopenia. Antibiotic treatment failed to effectively improve the patient's condition. Therefore, a combination of immunomodulatory and antibiotic treatments was used. The antibiotic regimen included oral rifampicin 600 mg/day, intravenous doxycycline hydrochloride 200 mg/day, and subcutaneous injection of human granulocyte-stimulating factor (0.2 mg/day). Immunomodulatory therapy consisted of 20,000 mg/day intravenous human immunoglobulin (pH 4) for five days and 800 mg/day oral pidotimod liquid for 20 days. As the treatment progressed, the count gradually recovered to normal levels, and the symptoms of bone marrow suppression were alleviated. PCR testing revealed the absence of *Brucella* DNA in both monocyte and serum samples. Furthermore, negative standard tube agglutination test results were obtained. These findings indicate that the immunomodulatory therapy resulted in a complete clearance of *Brucella*. Therefore, immunomodulatory therapy could be an effective option in cases of brucellosis with pancytopenia that are unresponsive to conventional antibiotic treatment. Further research and clinical evidence are required to confirm and optimize the use of immunomodulatory therapies in patients with brucellosis.

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https://doi.org/10.1016/j.heliyon.2023.e18907
Received 20 October 2022; Received in revised form 21 July 2023; Accepted 2 August 2023

1. Introduction

Brucellosis is a widespread zoonotic infectious disease caused by *Brucella* [1]. Endemic areas are mainly distributed in the Middle East, Central Asia, North Africa, China, South America, and other developing countries, with at least 500,000 new cases reported per year [2]. In China, the Inner Mongolia Autonomous Region is among the areas with a high incidence of brucellosis [3]. Brucellosis is characterized by nonspecific and diverse symptoms, making its clinical diagnosis challenging and often resulting in misdiagnosis or delayed treatment [4]. *Brucella* can cause damage to multiple systems in the human body. However, hematological complications such as pancytopenia and thrombocytopenia are relatively rare [5].

Pancytopenia is a hematological condition defined as a decrease in three peripheral blood cell lines: leukopenia, thrombocytopenia, and anemia [6]. Based on previous case reports, the mechanism underlying the association between brucellosis and pancytopenia remains unclear. Currently, it is believed to be potentially associated with splenomegaly, impaired phagocytic function, compromised immune function, and bone marrow suppression [7,8].

Herein, we report a case of brucellosis presenting with pancytopenia. After the failure of antibiotic treatment alone, a combination of immunomodulatory therapies was administered. The patient's condition significantly improved, demonstrating the potential effectiveness of this approach. Research on the use of immunomodulatory therapies to treat brucellosis is limited. By sharing our experience and the positive outcomes observed in this patient, we aim to contribute to the existing knowledge and encourage further exploration of immunomodulatory therapies as a complementary strategy for improving treatment outcomes in similar cases.

2. Case report

We report a case of a 47-year-old man with a history of livestock exposure who was previously in good health and engaged in the breeding industry. The timeline in Fig. 1 shows the patient's clinical history. In May 2018, the patient was diagnosed with brucellosis using Rose Bengal plate agglutination test (RBT) and standard tube agglutination test (SAT). The patient was administered rifampicin 600 mg once daily and doxycycline 200 mg once daily. The treatment regimen lasted six weeks. After antibiotic treatment, the manifestations disappeared, and the whole blood cell count substantially recovered to normal levels, except for a low total white blood cell (WBC) count of 2.0–2.4 $\times$ 10^9/L.

In August 2021, the patient suffered from a high body temperature that reached 40 °C due to working overtime day and night. The patient's body temperature increased severely overnight but typically dropped to normal levels by the following morning. Consequently, the patient visited the Horqin Moli Temple Sumu Health Center, a rural local health center, seeking treatment. However, the

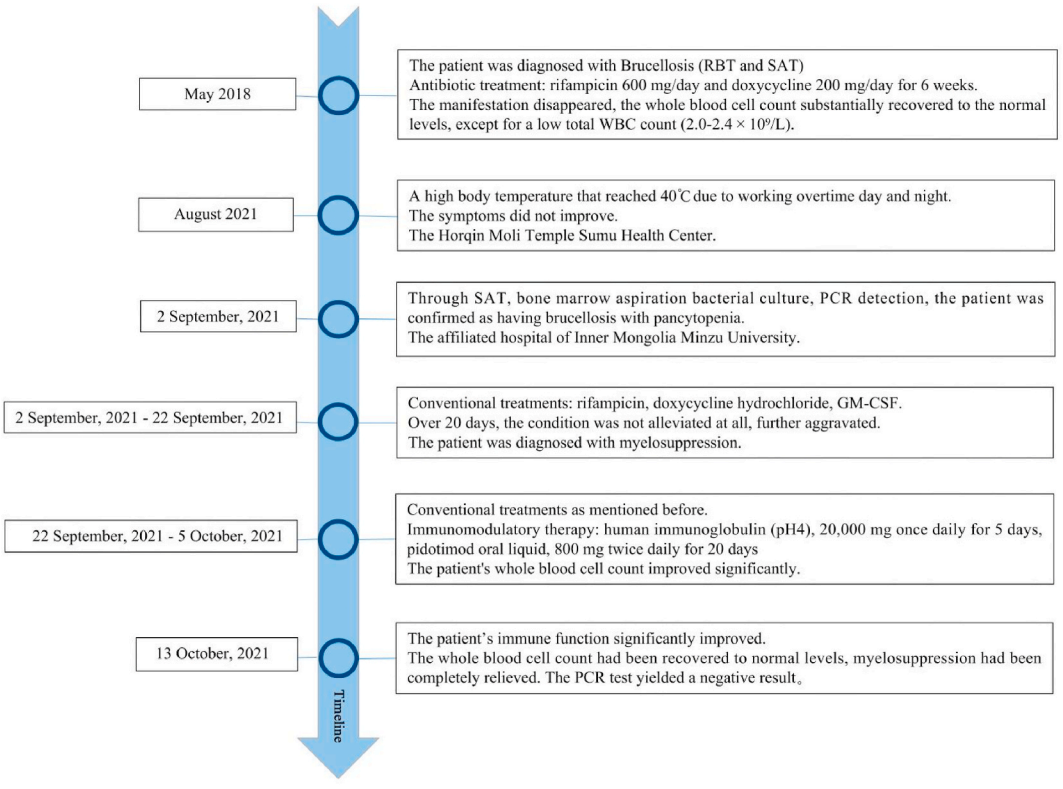

Fig. 1. Timeline of the patient's clinical history.

symptoms did not improve. He then visited the Affiliated Hospital of Inner Mongolia Minzu University, on September 2, 2021. Laboratory examination results were as follows: SAT >1:200; routine blood tests: WBC count, 0.89×10^9/L; red blood cell (RBC) count, 3.33×10^{12}/L; hemoglobin (HGB) level, 106 g/L, platelet (PLT) count, 97×10^9/L (Table 1). According to the results of the routine blood examination, there was a decrease in the whole blood cell count. Considering the possibility of bone marrow suppression, bone marrow aspirate was collected to perform bacterial cultures. The bone marrow biopsy sample measuring 2 × 8 mm was collected. The sample was fixed in a 4% formaldehyde solution (pH 7.0). To prepare for Gomori staining, routine dehydration and paraffin-embedded tissue sections were performed. The following steps were carried out: 2% oxalic acid solution for 1 minute, 2% oxalic acid solution for 1 minute, ammonium iron sulfate solution for 5 minutes, Gomori silver ammonia solution for 3 minutes. Before immersing the sections in each respective solution, they were rinsed with distilled water. Subsequently, the sections were reduced with a 4% neutral formaldehyde solution for 1 minute. After an 8-min water wash, followed 0.2% gold chloride solution for 1–2 minutes. Another rinse with distilled water was conducted, and the sections were fixed with a 5% sodium thiosulfate solution for 2 minutes. Routine dehydration was performed, followed by sealing with neutral gum. The microscopic examination revealed an MF-0 result (Fig. 2A and B). The scattered distribution of bacteria was observed on the blood agar plates, albeit not significant (Fig. 3A). Subsequently, the Gram-stained bacterial smears were observed on a 1000× lens using oil immersion. Interestingly, beach-like Gram-negative coccobacilli were observed, confirming their presence (Fig. 3B). Transmission electron microscopy (TEM) procedure: On the well-cultured bone marrow aspirate sample on blood agar medium, a single bacterial colony was selected and mixed with an appropriate amount of sterile water to prepare a bacterial suspension. Microscopy forceps were used to pick up a carbon film, and the forceps place it onto a sample holder. Using a micropipette (10 μL), an appropriate amount of the bacterial suspension was added onto the carbon film. An adequate amount of 2% phosphotungstic acid solution (pH 7.0) was added onto the membrane. After staining for approximately 1 minute, any excess dye was absorbed using filter paper. The specimen was left to air dry naturally and was subsequently subjected to observation and analysis using a HITACHI H-7650 TEM (Japan) operating at 8 kV with a magnification of 8000×. The TEM image also confirmed these characteristics (Fig. 3C).

Subsequently, to assess the presence of *Brucella*, *Brucella* DNA was extracted from monocytes (intracellular) and serum (extracellular) simultaneously [9]. Samples were analyzed using conventional PCR and abortus-melitensis-ovis-suis PCR (AMOS-PCR). bcsp31 was selected as the target gene for both detection methods**.** Conventional PCR showed a negative serum result and a positive result in monocytes, suggesting that the patient had not completely cleared the *Brucella* infection despite receiving conventional antibiotic treatment. Furthermore, AMOS-PCR identified the strain as *B. melitensis biovar* 3, which is the predominant epidemic strain in the Tongliao area of Inner Mongolia, China. Given the above test results, the patient was concurrently diagnosed with brucellosis and pancytopenia, with the bone marrow suppression attributed to *Brucella* infection. The patient was administered rifampicin tablets (600 mg orally once daily), doxycycline hydrochloride injections (200 mg once daily via an intravenous drip), and granulocyte-macrophage colony-stimulating factor (GM-CSF) subcutaneous injections (0.2 mg once daily). However, the patient showed no signs of improvement and, instead, developed acute fever, fatigue, and drowsiness without lymphadenopathy or hepatosplenomegaly.

Given the ineffectiveness of antibiotic treatment alone, we administered a combination therapy comprising conventional antibiotics and immunomodulatory agents during the period from September 22 to October 13. The same aforementioned dosages of conventional antibiotics were used. Immunomodulatory therapy included an intravenous drip of human immunoglobulin (pH 4) at a dose of 20,000 mg once daily for 5 days and pidotimod oral liquid at a dose of 800 mg twice daily for 20 days. As the treatment progressed (from September 22 to October 5), significant improvements were observed in the patient's WBC count and immune function. The WBC count was 4.61×10^9/L, the RBC count was 2.13×10^{12}/L, the HGB level was 65 g/L, and the PLT count was 114×10^9/L (Table 1). As shown in Table 1, during the 20 days of treatment (from September 22 to October 13), the patient's immune function significantly improved.

Unfortunately, the patient presented herein resisted undergoing bone marrow aspiration after the immunomodulatory treatment. Considering the invasive nature of the procedure, we decided not to perform the bone marrow aspiration. Nonetheless, it is noteworthy that the patient's blood parameters normalized, immune indicators approached normal levels, and clinical symptoms resolved on October 13, 2022. Additionally, the PCR test yielded a negative result, providing evidence of complete eradication of *Brucella* infection and resolution of bone marrow suppression following the immunomodulatory treatment. These findings prove the effectiveness of

Table 1
Blood routine.

Clinical indicators	Laboratory data						Unit	Reference range
	9.2	9.9	9.22	9.29	10.5	10.13		
White blood cell	0.89	0.29	0.23	0.57	4.61	10.24	10^9/L	3.50–9.50
Neutrophil	0.02	0.02	0.04	0.25	3.52	8.42	10^9/L	1.80–6.30
Neutrophil rate	2.3	6.9	17.4	43.9	76.4	82.3	%	40.0–75.0
Lymphocyte	0.85	0.27	0.18	0.28	0.84	1.09	10^9/L	1.10–3.20
Lymphocyte rate	95.5	93.1	78.3	49.1	18.2	10.6	%	20.0–50.0
Monocyte	0.01	0.0	0.01	0.04	0.24	0.70	10^9/L	0.10–0.60
Monocyte rate	1.1	0.0	4.3	7.0	5.2	6.8	%	3.0–10.0
Red blood cell	3.33	2.64	1.89	1.65	2.13	2.41	10^{12}/L	4.30–5.80
Hemoglobin	106	85	59	53	65	75	g/L	130–175
Platelet	97	65	72	73	114	229	10^9/L	125–350

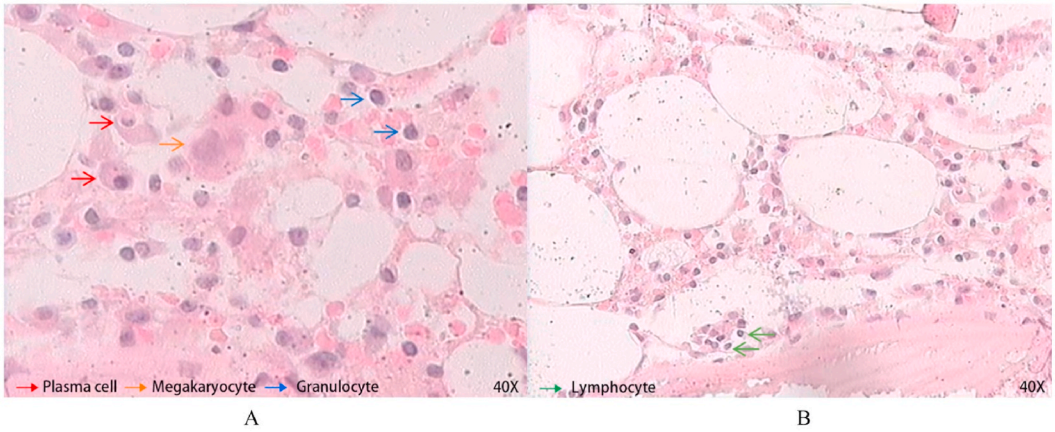

Fig. 2. Bone marrow biopsy under the microscope with Gomori stain (MF-0).
A: Clearly visible plasma cells (red arrow), granulocytes (blue arrow) and megakaryocytes (orange arrow), decreased myeloid and erythroid proliferation, and increased adipose tissue. Magnification, 400×. B: Clearly visible lymphocyte (green arrow), Magnification, 400×.

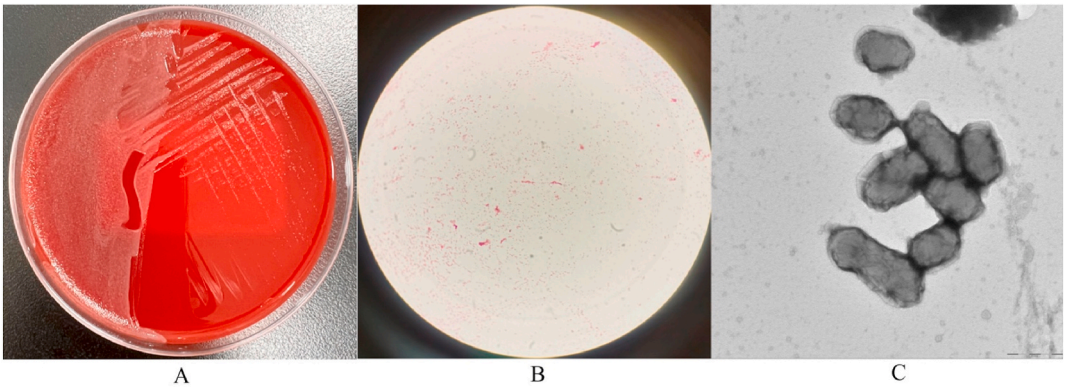

Fig. 3. Bone marrow culture and microscopy.
A: The bone marrow aspirate sample was placed in a 5% CO_2 incubator after being inoculated in blood agar. After 24 h, we observed the blood plate, but it was not clear enough to identify the colony morphology. The culture was maintained until 48 h later when small, protruding, neat-edged, gray-white, and moist colonies were observed. B: On the Gram-stained bacterial smear, Gram-negative coccobacilli with beach-like structures were observed on a 1000× lens using oil immersion. C: TEM (Japan, HITACHI H-7650) showed no flagella, capsules, or spores. Magnification, 8000×.

immunomodulatory therapy in treating *Brucella*-related conditions.

3. Discussion

Brucella is a facultative anaerobic Gram-negative intracellular parasitic bacterium, that can evade the host immune response via different strategies and by expressing different virulence factors [10–12]. *Brucella* can survive persistently in human macrophages as *Brucella*-containing vacuoles, and the human immune system cannot effectively clear this form of intracellular parasitic bacteria [13]. This further explains the possible reasons for the chronicity or relapse of brucellosis, including irregular antibiotic treatment, poor antibiotic efficacy, and misdiagnosis or underdiagnosis due to the complex and diverse clinical manifestations of brucellosis. As the body's immune system weakens, *Brucella*, which hides within phagocytic cells, proliferates. This leads to the lysis of the parasitized macrophages and the release of *Brucella*, which can then infect more phagocytic cells. Consequently, bacteria can spread throughout the body via the lymphatic and blood circulation, leading to more severe manifestations [14]. In addition to the common clinical manifestations, such as fever, fatigue, excessive sweating, and backache, the most remarkable manifestations in this patient were decreased whole blood cell count (Table 1) and significant weight loss. The bone marrow aspirate report (Fig. 2) suggested significantly reduced myeloproliferation and pancytopenia due to suppression of bone marrow hematopoietic function (Fig. 2A). The main cause of pancytopenia in this patient was bone marrow suppression.

Currently, the antibiotic treatment recommended by the World Health Organization remains the first-line treatment for clinicians in the management of patients with brucellosis [15]. Most patients have favorable outcomes after treatment. However, a small

proportion of patients may encounter treatment limitations due to individual factors such as immune system deficiencies or dialysis [16,17]. In previous reports, patients with brucellosis and pancytopenia or other hematological complications generally achieved complete recovery with antibiotic treatment, including regimens comprising rifampicin and doxycycline or streptomycin [5,7,8,18,19]. However, the case described in this report experienced worsening of symptoms and did not sufficiently respond to conventional antibiotic treatment. As shown in Table 1 and Fig. 3, the whole blood count and bone marrow culture revealed pancytopenia, suggesting bone marrow suppression caused by *Brucella* infection. To investigate this further, we simultaneously extracted peripheral blood monocyte and serum samples for *Brucella* nucleic acid detection. The serum test result was negative, indicating the absence of *Brucella*, whereas the monocytes showed positive results. This method has high clinical value in aiding the diagnosis of brucellosis and in assessing treatment efficacy. Additionally, immune function indicators provided further evidence that conventional antibiotic treatment alone was insufficient to alleviate the symptoms of this patient (Table 2). Based on these findings, we implemented a combination of immunomodulatory therapies and conventional antibiotic treatment. Throughout the treatment process, the patient showed no adverse reactions or symptoms of discomfort.

The immunomodulatory therapies included intravenous human immunoglobulin (pH 4) and oral pidotimod liquid. The intravenous human immunoglobulin (pH 4) liquid formulation contains immunoglobulins derived from normal human plasma [20]. It exhibits a broad range of activity against viruses, bacteria, and other pathogens and can rapidly increase IgG levels in the bloodstream while also regulating immune mediators and enhancing cellular immune capabilities [21]. Consequently, the intravenous immunoglobulin formulation has been increasingly used as an “immunomodulator” in various immunodeficiency and inflammatory diseases. The oral pidotimod liquid is a commonly used immunomodulator that effectively controls respiratory tract infections in children [22]. Pidotimod can enhance the phagocytic activity of macrophages and neutrophils and strengthen innate and adaptive immune responses. It also promotes lymphocyte proliferation, modulates the secretion of interferon-gamma (IFN-γ) and interleukin-2 (IL-2), and stimulates the activation of cellular immunity [23]. Furthermore, it promotes B lymphocyte proliferation, enhances immune function, and prevent bacterial infections. Both medications can dynamically modulate the Th1/Th2 immune response. As shown in Tables 1 and 2, the patient achieved good clinical treatment results and had significantly improved immune function; the whole blood cell count also recovered to normal levels. The possible mechanism of immunomodulatory therapy is as follows: immunomodulatory drugs can activate $CD4^{+}$T cells and increase the production of IFN-γ, tumor necrosis factor-alpha (TNF-α), and IL-2, thereby enhancing their function of killing and clearing *Brucella*; these drugs also decrease the levels of interleukin-4 (IL-4), interleukin- (IL-10), and IgE, indicating that the activation of Th2-type responses and inhibitory T cells both decreased [21,23]. Therefore, Th1-type responses increased. In other words, immunomodulatory therapy improves the effectiveness of Th1-type responses. An increase in interleukin-6 (IL-6) levels can contribute to specific B cell activation as well as the synthesis and secretion of specific antibodies against *Brucella* [23].

Owing to the complexity of the clinical manifestations of brucellosis, the diagnosis should consider the patient’s clinical presentation, epidemiological history, and laboratory tests [24]. It is worth noting that in endemic regions, many patients may exhibit persistent high antibody titers even after successful treatment. Thus, antibody titers must be interpreted with caution [24,25]. Additionally, the simultaneous extraction of nucleic acids from monocytes and serum holds significant clinical value in assisting the diagnosis of brucellosis and in assessing the efficacy of treatment, particularly in cases where the serum tests negative but the monocytes test positive.

In this report, we have focused on describing clinical findings and potential mechanisms. While the patient’s condition improved with immunomodulatory therapy, it is important to consider that individual responses to therapeutic interventions may vary. Future studies should explore how immunomodulatory treatment improves the condition of brucellosis patients with pancytopenia at the molecular level and how it exerts its effects, as well as the potential for personalized therapeutic approaches.

4. Conclusions

This report describes the case of a 47-year-old man who presented with fever and fatigue and was diagnosed with brucellosis accompanied by pancytopenia. Conventional antibiotic therapy failed to improve the patient’s condition. However, after undergoing immunomodulatory therapy, the patient’s clinical manifestations disappeared, the whole blood cell count recovered to normal levels, and myelosuppression was completely relieved. These findings highlight the importance of considering immunomodulatory therapy as an adjunct treatment for cases of brucellosis with pancytopenia that are unresponsive to conventional antibiotics.

Ethics approval and consent to participate

In this report, the requirement for ethics approval was waived by the Research Ethics Committee of Inner Mongolia Minzu Univeristy. (No. NM-LL-2022-05-31-01).

Consent for publication

Written informed consent was obtained from the patient for publication of this case report.

Author contribution statement

All authors listed have significantly contributed to the investigation, development and writing of this article.

Table 2
Immunological examination parameters.

Clinical indicators	Laboratory data		Unit	Reference range
	9.22	10.13		
$CD3^+$	96.80	96.54	%	50.00–80.00
$CD3^+CD4^+$	45.90	49.41	%	27.00–51.00
$CD3^+CD8^+$	47.70	44.68	%	15.00–44.00
$CD3^-CD19^+$	1.40	3.34	%	5.00–18.00
$CD16^+CD56^+$	0.20	2.36	%	7.00–40.00
Interleukin 2	1.07	0.81	pg/mL	0–5.71
Tumor necrosis factor α	0.85	0.37	pg/mL	0–4.60
Interferon γ	1.02	3.92	pg/mL	0–7.42
Interleukin 4	0.96	0.21	pg/mL	0–3.00
Interleukin 6	74.99	209.23	pg/mL	0–5.30
Interleukin 10	11.45	5.10	pg/mL	0–4.91

Data availability statement

No data was used for the research described in the article.

Additional information

No additional information is available for this paper.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

We thank Prof. Changlong Lv for the assistance with enlightening discussions.

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