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ORIGINAL RESEARCH

Altered CD73-Adenosine Signaling Linked to Infection in Patients undergoing hemodialysis

Fangfang Xiang[1–4,*], Zhen Zhang[1,*], Yuxin Nie[1], Xuesen Cao[1], Yang Li[1], Xiaohong Chen[1], Shaomin Gong[1], Jianzhou Zou[1], Jie Teng[1], Xiaoqiang Ding[1–4], Bo Shen[1–4]

[1]Department of Nephrology, Zhongshan Hospital, Fudan University, Shanghai, People's Republic of China; [2]Shanghai Key Laboratory of Renal Disease and Blood Purification, Shanghai, People's Republic of China; [3]Shanghai Medical Center of Kidney, Shanghai, People's Republic of China; [4]Shanghai Institute of Kidney and Dialysis, Shanghai, People's Republic of China

*These authors contributed equally to this work

Correspondence: Bo Shen, Department of Nephrology, Zhongshan Hospital, Fudan University, No. 180 Fenglin Road, Xuhui District, Shanghai, People's Republic of China, Email shen.bo@zs-hosptial.sh.cn

Purpose: Infection is the most common cause of hospitalization and the second most common cause of mortality among patients undergoing hemodialysis (HD). The enzyme CD73, a cell surface 5′-nucleotidase, regulates the balance between pro-inflammatory nucleotides and anti-inflammatory adenosine. Diminished CD73-adenosine signaling contributes to severe infections.

Methods: In this prospective cohort study, 393 patients who underwent HD for over six months were evaluated for $CD73^+$Tcell ratios and were followed up for three years to track infection events. Kaplan–Meier curves and Cox regression analyses were used to evaluate the relationship between $CD73^+$T cells and infections; meanwhile, multiple logistic regression analysis was used to analyze differences among infection groups. In addition, a 5/6 nephrectomy (5/6 Nx) rat model and cecal ligation and puncture (CLP) were used to verify the effect of chronic kidney disease (CKD) and sepsis on CD73-adenosine signaling.

Results: Decreased $CD73^+$ T cells were independently associated with increased infection risk over one and three years. The hazard ratios for one- and three-year infection incidences were 3.173 (95% CI 1.782–5.650, $p < 0.001$) and 1.429 (95% CI 1.052–1.992, $p = 0.035$), respectively. Furthermore, they were associated with recurrent and fatal severe infections. Animal models demonstrated reduced CD73 mRNA transcript and adenosine receptor levels, along with decreased serum adenosine levels in CKD. Impairment of CD73-adenosine signaling was more pronounced after CLP in CKD rats.

Conclusion: Lower $CD73^+$T cell levels are strongly associated with infection complications in patients undergoing HD. Altered CD73-adenosine signaling likely plays a substantial role in immune dysfunction in CKD.

Keywords: adenosine, CD73, hemodialysis, infection, T cell dysfunction

Introduction

Chronic kidney disease (CKD), particularly end-stage renal disease (ESRD), is a substantial global public health concern, reducing human life expectancy, markedly increasing disability and mortality rates.[1] Despite advancements in technology, hemodialysis (HD), the most prevalent dialysis modality, still has a high mortality rate.[2] Although infection risk among patients with ESRD is well recognized,[3] this issue has received little attention. About 15.3% of patients undergoing HD hospitalized for infections required an intensive care unit stay, with 28.6% requiring prolonged hospitalization.[4] Furthermore, these patients exhibit 100–300 times higher rates of sepsis death than the general population.[5] During the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, patients undergoing HD had an infection rate of 105/10,000 person-weeks—considerably higher than the global infection rate of 5/10,000 person-week.[6] Moreover, the advanced age and high comorbidity burden of these patients render them more susceptible to infection. A seven-fold increase in mortality rate has been reported among dialysis patients infected with SARS-CoV-2, with approximately 20–25% of dialysis patients dying within one month of diagnosis.[7,8] Infection also plays a significant role in cardiovascular disease (CVD) development, increasing the risk by 25% in the first 30 d and

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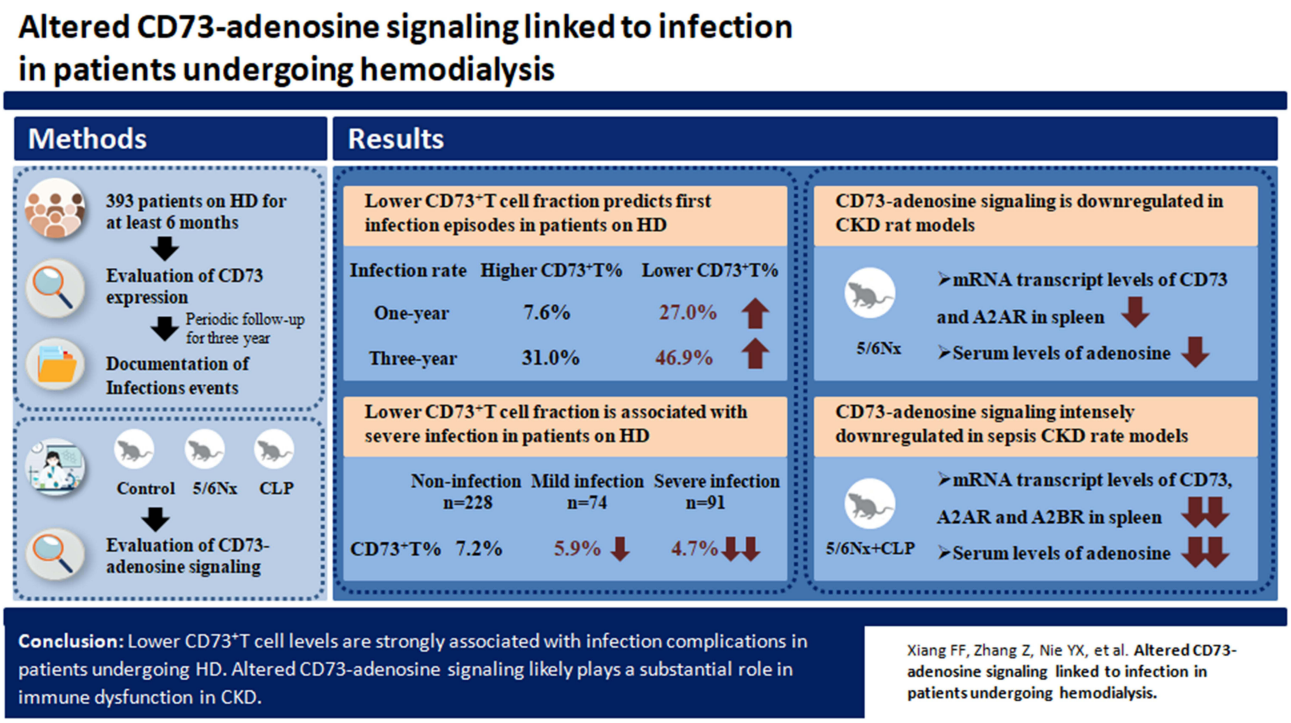
Graphical Abstract



18% in 90 d.[9] In addition, heart failure increased over fivefold in the six months following septicemia.[10] Therefore, infection and its prevention require greater attention in these patients, as can reduce at least one source of inflammatory stimulation, thus reducing CVD and mortality. Immune dysfunction is known to contribute to the high incidence of infection among these patients. However, studies have rarely focused on the mechanisms by which immune dysfunction causes infections in these patients and assessed the corresponding intervention measures.

Purinergic signaling is a fundamental mechanism used by all cells to regulate their internal activities and interactions with the surrounding environment. As an integral component of the purinergic system, CD73 functions as a rate-limiting enzyme that catalyzes extracellular ATP metabolism to form adenosine.[11] When exposed to inflammatory, hypoxic, metabolic, and other types of stress, increased ATP release from stressed or dying cells represents a pro-inflammatory signal. This signal is terminated when ATP is metabolized to the anti-inflammatory mediator adenosine, thus preventing excessive activation of tissue defense mechanisms.[12,13] Therefore, CD73-adenosine signaling is crucial to maintaining equilibrium within the immune system, exerting potent anti-inflammatory effects to protect against cellular damage and facilitate tissue repair.[14] CD73 also exerts non-enzymatic regulatory effects on T cell activation, homing, and differentiation.[15]

Several studies have indicated that diminished CD73 function may be a contributing factor to persistent or severe infections.[16–18] We previously demonstrated that reduced expression of CD73 on T cells is closely associated with systemic inflammation and can predict infection events in patients undergoing HD.[19] Here, we investigated the correlation between CD73-adenosine signaling and infection complications in patients with ESRD and explored the immunological mechanisms underlying infection susceptibility and the tendency for severe complications in these patients.

Materials and Methods

Study Population

Patients who had been on HD for at least six months at the Blood Purification Center of the Department of Nephrology at Zhongshan Hospital (Fudan University, Shanghai, China) were enrolled in November 2020.

We applied exclusion criteria to rule out diseases that have an impact on immune function per se or acute illness situations that could create an unstable immune status. First, individuals who had a cardiovascular event or infection within three months before enrollment were excluded. Next, patients with blood disorders, rheumatic conditions, active malignancies, use of immunosuppressants, or human immunodeficiency virus (HIV) infection were excluded from the study. All the patients who met the inclusion criteria provided written informed consent. This study complied with the Declaration of Helsinki and was approved by the Ethics Committee of Zhongshan Hospital (B2020-381R). This study was registered at ClinicalTrials.gov (NCT04658069) in December 2020.

Peripheral blood samples were collected from the arterial site of the vascular access point before the HD session, which typically occurred at midweek. All procedures were conducted at the Department of Clinical Chemistry of Zhongshan Hospital in accordance with standard protocols. Details of cell preparation and flow cytometric analysis are provided in the Figure S1.

Infection Determination and Outcomes

The patients were observed weekly for 36 months, concluding in December 2023. Infection episodes were defined as clinical manifestations of infection necessitating the administration of regular intravenous antibiotics in hospital or emergency department settings. Mild infections, such as those affecting the upper respiratory tract or urinary tract and requiring only oral antibiotic treatment, were excluded from the analysis. The primary outcome of this study was the initial episode of infection.

Animals and Models of CKD and Sepsis

CKD was induced by 5/6 nephrectomy (Nx) as previously described.[20] After isoflurane anesthesia, the lower and upper thirds of the left kidneys were resected in week 0, and the right kidney was removed in week 12. The second surgery was postponed due to the lockdown policy during the SARS-CoV-2 pandemic. CKD can be further classified into short-term CKD (sCKD) and long-term CKD (lCKD). The rats in the sCKD group were sacrificed or sepsis was induced on the 24th postoperative week. Rats from the lCKD group were sacrificed or sepsis was induced on the 30th postoperative week. Sepsis was induced via cecal ligation and puncture (CLP).[21] Twenty-four hours after the CLP procedure, rats were euthanized and blood and tissue samples were collected. The experimental design is illustrated in Figure 1. All the protocols were approved by the Animal Care and Use Committee of Zhongshan Hospital and were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Details about

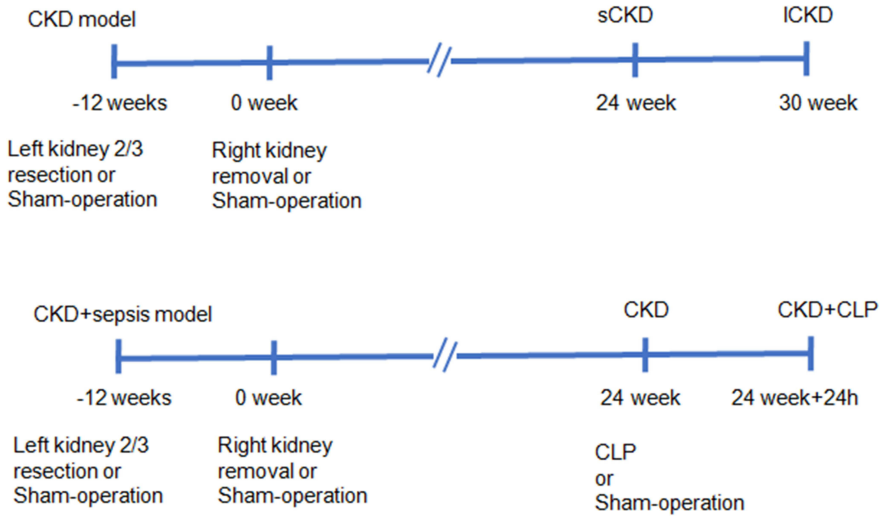


Figure 1 Experimental design. Blood and spleens were taken on the 24th week after the second 5/6 nephrectomy (5/6 Nx) in the control and short-term chronic kidney disease (sCKD) groups. Meanwhile, blood and spleen harvests were performed on the 30th week after the second 5/6 Nx in the long-term chronic kidney disease (lCKD) group. In the CKD+sepsis model, blood and spleen harvests were performed 24 h after cecal ligation and puncture (CLP) or sham operations.

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animal model, blood tests, and quantitative reverse transcription polymerase chain reaction are provided in the supplementary materials. Primer sequences are listed in Table S1.

Uremic Toxin and Nucleotide Measurements

The serum concentrations of indoxyl sulfate (IS) and p-cresyl sulfate (PCS) were determined using a high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) method, modified according to the methodology proposed by Wang and Korfmacher.[22] The detailed methodology has been published.[23,24] The levels of adenosine and ATP were quantified in plasma samples using ultra-HPLC-MS/MS, as previously described.[25] Details are provided in the supplementary materials.

Statistical Analysis

All data are presented as mean ± standard deviation or median (interquartile range). The time until the first infectious episode was estimated using the Kaplan–Meier curve, and differences between the groups were examined using the Log rank test. Univariate Cox regression analysis was used to ascertain the predictors of episodes of infection. Subsequently, significant predictors were incorporated into the multivariate model and backward stepwise Cox regression was employed to identify the most parsimonious model. The probability value used for stepwise regression was set at 0.05 for the entry of variables and 0.1 for their removal. One-way analysis of variance (ANOVA) or Mann–Whitney *U*-test was used to identify variables that differed between infection groups, and multinomial logistic regression was used to compare the percentages of $CD73^+$ T cells between groups. In animal experiments, a comparative analysis of the blood test results between multiple groups was conducted using one-way ANOVA with a post hoc least significant difference (LSD) test. PCR results were evaluated using the Kruskal–Wallis and Mann–Whitney *U*-tests. Statistical significance was set at $p < 0.05$. All statistical analyses were conducted using SPSS software, version 20.0.

Results

Clinical Characteristics and Infection Events of Patients on HD

We enrolled 393 patients (236 men, 157 women; average age, 59.8 ± 13.7 years). The median HD duration was 57 months (range, 22–106 months). Of patients, 103 (26.2%) had diabetes mellitus, whereas 154 (39.2%) had a history of CVD, including coronary artery disease, congestive heart failure, stroke, and peripheral arterial occlusive disease. Baseline patient information is presented in Table S2.

During the three-year follow-up period, a total of 119 patients died of their illness and 52 patients (43.7%) died of infection, including 18 SARS-CoV-2 deaths. We documented 239 cases of infection. A total of 165 patients (42.0%) experienced at least one infection; 113 patients had one infection, while 36 patients had two. Sixteen patients experienced over two infection episodes. The most prevalent infection was pneumonia (n = 159, 66.5%), including 43 instances of SARS-CoV-2 infection during the pandemic period (December 2023 to January 2024). Septic shock was the most severe complication, with 85.7% of patients dying after diagnosis. Details of infection episodes are presented in Figure 2.

Lower $CD73^+$T Cell Fraction Predicts First Infection Episodes in Patients on HD

In the initial year of observation, 68 patients had an episode of infection. Patients with lower $CD73^+$T cell fractions had tremendously higher infection rates (27.0% vs 7.6%). During the three-year follow-up period, 165 patients experienced an infection episode and patients with lower $CD73^+$T cell fractions also had higher infection rates (46.9% vs 31.0%). After taking age into consideration, patients with a lower $CD73^+$T cell proportion exhibited a significantly elevated incidence of infection across both age groups (Figure 3). Decreased $CD73^+$T cells more effectively predicted one-year infection events than age alone. However, the predictive value of a decreased $CD73^+$T cell proportion diminished with the prolongation of the follow-up period, particularly during the SARS-CoV-2 pandemic. The incidence and mortality rates of SARS-CoV-2 infection were significantly higher in the older group.

In the univariate Cox proportional hazards model, lower percentages of $CD73^+$ T cells were identified as predicting one-year and three-year infection (Tables 1 and 2). In the multivariate Cox proportional hazards model, a lower percentage of

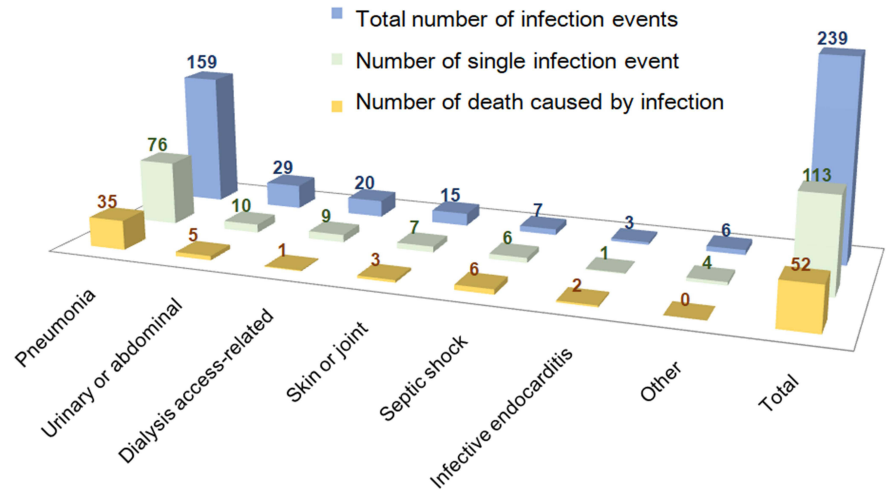

Figure 2 Characteristics of infectious events in patients undergoing hemodialysis (HD). During the three-years follow-up period, the most common infection was pneumonia, accounting for 66.5% of all infection events. Other infections included dialysis access-related infections (n = 20, 8.4%), skin or joint infections predominantly caused by peripheral arterial occlusion (n = 15, 6.3%), urinary or abdominal infections (n =29, 12.1%), infective endocarditis (n =3, 1.3%), septic shock (n =7, 2.9%), and infections at other or undocumented sites (n = 6, 2.5%). Septic shock was the most severe complication, with 85.7% of patients dying after diagnosis.

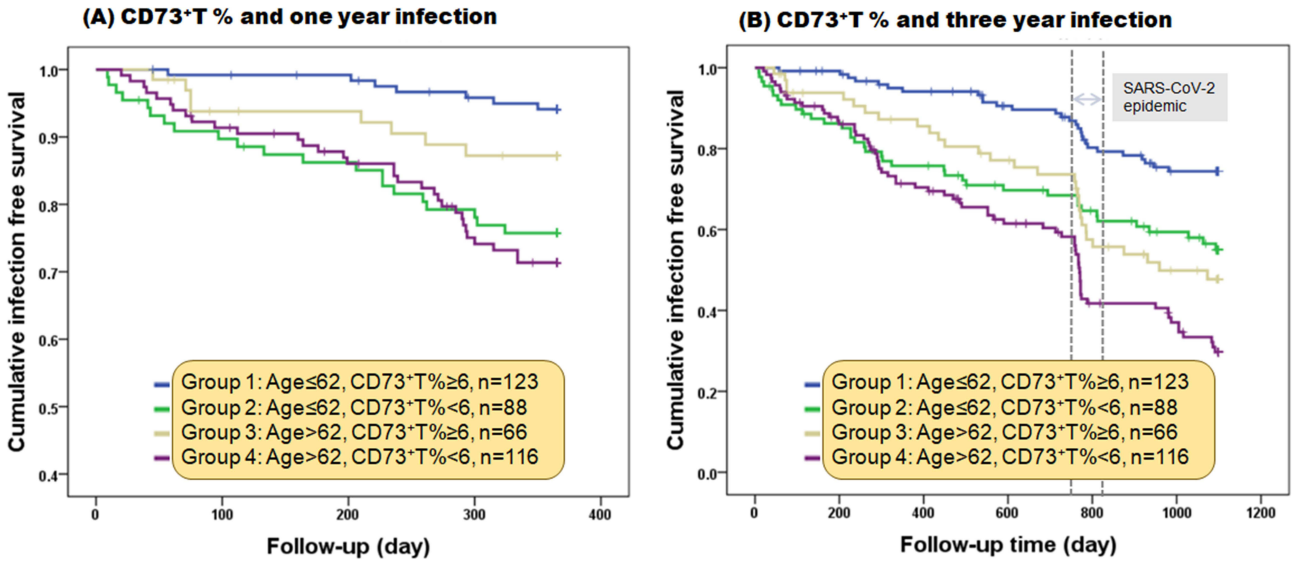

Figure 3 Infection-free survival curves according to the age-CD73$^+$T cell groups. The patients were divided into four groups based on their age and the proportion of CD73$^+$T cells. (**A**) One-year infection-free survival curves according to age and CD73$^+$T cell group. Kaplan–Meier analysis demonstrated a statistically significant difference in survival rates across the four age-CD73$^+$T cell groups ($p< 0.001$). Patients with a lower CD73$^+$T cell proportion exhibited a significantly higher infection incidence in both the younger (Group 1 vs Group 2, $p< 0.001$) and older groups (Group 3 vs Group 4, p =0.023). The data indicated that younger patients with a lower CD73$^+$T proportion exhibited a higher infection rate than older patients with a higher CD73$^+$T proportion, although the difference did not reach statistically significance (Group 2 vs Group 3, p =0.079). (**B**) Three-year infection-free survival curves in the age-CD73$^+$T cell group. Kaplan–Meier analysis demonstrated a statistically significant difference in survival rates across the four age-CD73$^+$T cell groups ($p< 0.001$). Patients with a lower CD73$^+$T cell proportion exhibited a significantly higher infection incidence in both the younger (Group 1 vs Group 2, p= 0.001) and older groups (Group 3 vs Group 4, p =0.023). No statistically significant difference was observed between younger patients with a lower CD73$^+$T cell proportion and older patients with a higher CD73$^+$T cell proportion.

CD73$^+$ T cells was an independent predictor of one-year and three-year infection incidence. The hazard ratio for one-year infection incidence was 3.173 (95% CI 1.782–5.650, $p < 0.001$), and the hazard ratio for three-year infection incidence was 1.429 (95% CI 1.052–1.992, $p = 0.035$).

Lower CD73$^+$T Cell Fraction is Associated with Severe Infection in Patients on HD

The patients were divided into three groups: non-infection (no infection event during follow-up), mild infection (one non-fatal infection event during follow-up), and severe infection (more than one non-fatal infection event or death caused by

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Table 1 Cox Hazard Model for One-year Infection Incidence in Patients on HD

Variable	Univariate Cox Hazard Model		Multivariate Cox Hazard Model[a]	
	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Age (year)	1.023 (1.004, 1.042)	0.016		
Diabetes mellitus (yes=1)	1.771 (1.082, 2.900)	0.023		
CVD (yes=1)	2.243 (1.387, 3.628)	0.001	1.534 (0.922, 2.552)	0.100
Central venous catheter (yes=1)	1.845 (1.134, 3.000)	0.014	1.550 (0.948, 2.537)	0.081
BMI (kg/m^2)	1.037 (0.973, 1.106)	0.260		
Kt/V_{urea}	0.343 (0.088, 1.332)	1.122		
Time on HD (months)	0.706 (0.997, 1.004)	0.706		
Hemoglobin (g/L)	0.979 (0.964, 0.993)	0.004		
White blood cells ($\times 10^9$/L)	1.199 (1.083, 1.327)	<0.001		
Albumin (g/L)	0.908 (0.848, 0.971)	0.005		
Prealbumin (g/L)	0.992 (0.989, 0.995)	<0.001		
Log-hsCRP (mg/L)	2.780 (1.897, 4.074)	<0.001	2.385 (1.611, 3.531)	<0.001
Log-NT-proBNP (pg/mL)	2.539 (1.567, 4.112)	<0.001	1.751 (1.018, 3.014)	0.043
Log-ferritin (pg/mL)	2.578 (1.407, 4.723)	0.002		
$CD73^+$T %<6% (yes=1)	3.979 (2.243, 7.060)	<0.001	3.173 (1.782, 5.650)	<0.001

Notes: [a]Backward conditional method. The model included the T cell parameter and was adjusted for age, history of CVD, diabetes mellitus, type of vascular access, hemoglobin, white blood cell count, albumin, prealbumin, log-ferritin, log-NT-proBNP, and log-hsCRP levels.

Abbreviations: BMI, body mass index; CVD, cardiovascular disease; HD, hemodialysis; Log-hsCRP, log-transformed high-sensitivity C-reactive protein; Log-NT-proBNP, log-transformed N-terminal pro-brain natriuretic peptide.

Table 2 Cox Hazard Model for Three-year Infection Incidence in Patients on HD

Variable	Univariate Cox Hazard Model		Multivariate Cox Hazard Model[a]	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age (year)	1.043 (1.030, 1.056)	<0.001	1.031 (1.017,1.045)	<0.001
Diabetes mellitus (yes=1)	1.605 (1.152, 2.235)	0.005		
CVD (yes=1)	2.048 (1.508, 2.782)	<0.001	1.823 (1.337, 2.487)	<0.001
Central venous catheter (yes=1)	1.854 (1.349, 2.548)	<0.001	1.442 (1.035, 2.010)	0.031
BMI (kg/m^2)	0.985 (0.941, 1.030)	0.511		
Kt/V_{urea}	0.827 (0.371, 1.841)	0.641		
Time on HD (months)	1.000 (0.998, 1.002)	0.972		
Hemoglobin (g/L)	0.992 (0.982, 1.002)	0.123		
White blood cells ($\times 10^9$/L)	1.048 (0.961, 1.143)	0.286		
Albumin (g/L)	0.895 (0.856, 0.936)	<0.001		
Prealbumin (g/L)	0.992 (0.989, 0.995)	<0.001	0.998 (0.995, 1.000)	0.056
Log-hsCRP (mg/L)	1.988 (1.547, 2.556)	<0.001	2.440 (1.666, 3.573)	<0.001
Log-NT-proBNP (pg/mL)	1.825 (1.339,2.487)	<0.001	1.734 (1.309, 2.298)	<0.001
Log-ferritin (pg/mL)	1.766 (1.215, 2.568)	0.003		
$CD73^+$T %<6% (yes=1)	2.199 (1.602, 3.019)	<0.001	1.429 (1.052, 1.992)	0.035

Notes: [a]Backward conditional method. The model included T cell parameter and was adjusted for age, history of CVD, diabetes mellitus, type of vascular access, albumin, prealbumin, log-ferritin, log-NT-proBNP, and log-hsCRP levels.

Abbreviations: BMI, body mass index; CVD, cardiovascular disease; HD, hemodialysis; Log-hsCRP, log-transformed high-sensitivity C-reactive protein; Log-NT-proBNP, log-transformed N-terminal pro-brain natriuretic peptide.

infection). The percentage of $CD73^+$ T cells was significantly lower in the severe infection group than in the others (Table 3). In the multinomial logistic regression analysis, the sole differentiating factor between each group, including mild and severe infection groups, was the decreased $CD73^+$ T cell proportion. Other parameters, including age, history of CVD, and serum levels of β2-MG and hsCRP, showed significant differences only between the non-infection and severe infection groups (Table 4).

Table 3 Patient Characteristics According to Various Infection Severity Groups

	Non-Infection N=228	Mild Infection N=74	Severe Infection N=91	*P*value[a]
Age, years	56.1±13.1	62.1±13.8*	66.8±11.8*#	<0.001
Time on HD, months	59(44,65)	50(22,87)	60(32,121)	NS
Male, %	139(61.0%)	44(59.5%)	53(58.2%)	NS
Hypertension, %	160(70.2%)	57(77.0%)	74(81.3%)	NS
Diabetes mellitus, %	52(22.8%)	22(29.73%)	29(31.9%)	NS
CVD history, %	69(30.3%)	36(48.6%)*	49(53.8%)*	<0.001
Central venous catheter, %	53(23.2%)	26(35.1%)*	34(37.4%)*	0.017
Hemoglobin, g/L	115.8±14.8	115.7±15.0	113.9±16.9	NS
White blood cells, $\times10^9$/L	6.31±1.68	6.59±2.01	6.36±2.38	NS
Lymphocytes, $\times10^9$/L	1.13±0.40	1.13±0.45	1.00±0.45*	0.46
Albumin, g/L	40.4±3.1	39.5±3.3*	38.5±3.5*	<0.001
Creatinine, μmol/L	1037.8±288.5	944.5±245.7*	878.7±228.0*	<0.001
β2-MG, mg/L	35.9(30.9, 41.0)	38.2(30.8, 43.0)	40.2(32.9, 44.6)*	0.005
Calcium, mmol/L	2.27±0.22	2.26±0.25	2.29±0.24	NS
Phosphorus, mmol/L	2.21±0.68	2.21±0.72	2.15±0.64	NS
iPTH, pg/mL	288.0 (190.0, 447.0)	268.5 (155.3, 394.3)	268.0 (188.0, 454.0)	NS
Ferritin, pg/mL	156.0 (66.0, 284.0)	162.5 (82.5, 367.0)	213.0 (75.9, 420.0)	0.054
NT-proBNP, pg/mL	3667.5 (1776.3, 8470.0)	3816.5 (2141.8, 12,179.5)	6400.0 (2802.0, 16,625.0)*	0.009
hsCRP, mg/L	3.1 (1.1,9.3)	4.6 (1.2,11.5)	7.2 (2.1,21.3)*	<0.001
$CD73^+$T cells, %	7.2(4.2,11.7)	5.9(4.0,8.7)	4.7(3.1,6.3) *#	<0.001

Notes: [a]One-way ANOVA or Mann–Whitney *U*-tests were used to investigate differences between the groups. *p<0.05, compared to the non-infection group. # p<0.05, compared to the mild infection group. NS: non-significant, p> 0.1.

Abbreviations: HD, hemodialysis; CVD, cardiovascular disease; β2-MG, beta-2-microglobulin; iPTH, intact parathyroid hormone; NT-proBNP, N-terminal pro-brain natriuretic peptide, hsCRP, high-sensitivity C-reactive protein.

Table 4 Multinomial Logistic Regression of Factors Associated with Infection Severity

	OR	95% CI	*P* value[a]
Non-infection			
Age (year)	0.955	0.930–0.980	<0.001
CVD (yes=1)	0.508	0.279–0.925	0.027
Central venous catheter (yes=1)	0.776	0.414–1.456	0.430
Lymphocytes ($\times10^9$/L)	1.676	0.841–3.340	0.142
Albumin (g/L)	1.041	0.946–1.145	0.408
Creatinine (μmol/L)	1.001	1.000–1.003	0.052
β2-MG (mg/L)	0.961	0.929–0.995	0.023
Log-hsCRP (mg/L)	0.515	0.316–0.839	0.008
Log-NT-proBNP (pg/mL)	0.993	0.514–1.918	0.984
Log-ferritin (pg/mL)	0.775	0.392–1.534	0.465
$CD73^+$T %<6% (yes=1)	0.503	0.274–0.922	0.026
Mild infection			
Age (year)	0.981	0.953–1.010	0.191
CVD (yes=1)	1.061	0.539–2.087	0.985
Central venous catheter (yes=1)	1.140	0.571–2.276	0.711
Lymphocytes ($\times10^9$/L)	1.977	0.937–4.174	0.074
Albumin (g/L)	1.013	0.910–1.126	0.817

(*Continued*)

Table 4 (Continued).

	OR	95% CI	*P* value[a]
Creatinine (μmol/L)	1.000	0.999–1.002	0.599
β2-MG (mg/L)	1.000	0.964–1.038	0.996
Log-hsCRP (mg/L)	0.613	0.353–1.064	0.082
Log-NT-proBNP (pg/mL)	0.751	0.358–1.574	0.448
Log-ferritin (pg/mL)	0.959	0.439–2.092	0.916
$CD73^+T$ %<6% (yes=1)	0.452	0.226–0.901	0.024

Notes: [a]Severe infection groups were listed as reference lines, and $CD73^+T$%, along with age, history of CVD, types of vascular access, lymphocytes, albumin, creatinine, β2-MG, log-ferritin, log-NT-proBNP, and log-hsCRP were included and analyzed in this model.

Abbreviations: CVD, cardiovascular disease; β2-MG, beta-2-microglobulin; Log-hsCRP, log-transformed high-sensitivity C-reactive protein; Log-NT-proBNP, log-transformed N-terminal pro-brain natriuretic peptide.

Blood Test Confirmed the Development of CKD Rat Models and Enhanced Chronic Inflammation

A comparison of the clinical parameters between the Control and CKD groups is shown in Table 5. The serum and urea nitrogen levels were significantly elevated in the sCKD and lCKD groups. Serum levels of β2-MG, IS, and PCS, which represent middle- and protein-bound uremic toxins, were elevated exclusively in the lCKD group. The inflammatory markers that were elevated only in the lCKD group included CRP, IL-1β, IL-2, IL-6, TNF-α, and INF-γ.

CD73-Adenosine Signaling is Downregulated in CKD Rat Models

The mRNA transcript levels of CD73 and the adenosine receptor A2AR notably declined in lCKD spleens. Serum levels of adenosine decreased in both sCKD and lCKD groups. The lCKD group exhibited elevated expression of CD39, although this difference was not statistically significant (Figure 4).

Table 5 Comparison of Clinical Parameters Between the Control and CKD Groups

	Control	sCKD	lCKD
Creatinine (μmol/L)	15.3±1.8	63.2±2.0*	68.9±1.7*
Urea nitrogen (mmol/L)	6.7±1.2	19.2±0.9*	22.2±1.1*
β2-MG (ng/mL)	123.5±7.7	128.4±4.0	197.0±8.0*
IS (μg/mL)	0.44±0.37	1.56±0.24	4.45±2.30*
PCS (μg/mL)	0.08±0.10	0.46±0.40	1.82±0.95*
CRP (mg/L)	291.7±24.8	316.8±15.3	418.0±14.4*
IL-1β (pg/mL)	8.15±3.10	8.36±1.90	10.08±2.34*
IL-2 (pg/mL)	14.85±2.38	15.61±1.30	22.36±1.76*
IL-6 (pg/mL)	20.28±1.16	21.13±1.92	29.81±1.38*
TNF-α (pg/mL)	4.92±0.60	5.19±0.46	7.81±0.37*
INF-γ (pg/mL)	7.37±1.76	8.16±2.78	12.08±2.78*

Notes: Values are presented as mean ± standard deviation (SD); Control, sham-operated.

Abbreviations: sCKD, short-term CKD; lCKD, long-term CKD; CKD, chronic kidney disease model (5/6 nephrectomized); β2-MG, β2-microglobulin; IS, indoxyl sulfate; PCS, p-cresyl sulfate; CRP, C-reactive protein; IL-1β, interleukin-1β; IL-2, interleukin-2; IL-6, interleukin 6; TNF-α, tumor necrosis factor-α; INF-γ, interferon γ; * $p < 0.05$, vs control.

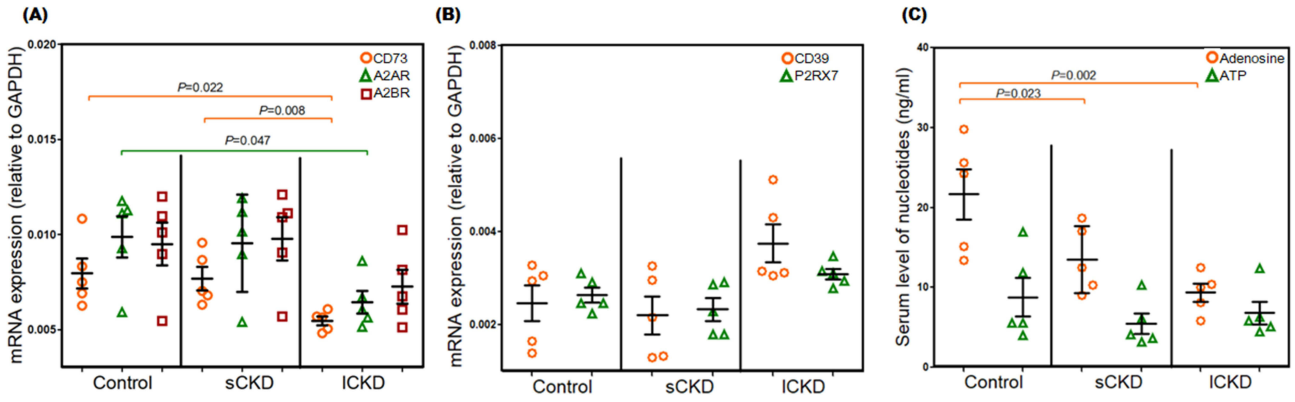

Figure 4 CD73-adenosine signaling is downregulated in CKD rat models (**A**) The mRNA expression of CD73 and adenosine receptors (A2AR) in the spleen were significantly downregulated in the ICKD group. (**B**) A trend of increased CD39 mRNA expression was observed in the spleens of the ICKD group, although the difference was not statistically significant. (**C**) The serum adenosine level was decreased in both the sCKD and ICKD groups (n = 5/group).

CD73-Adenosine Signaling Intensely Downregulated in Sepsis CKD Rate Models

The purinergic signaling pathway is activated in rats with sepsis. The mRNA transcript levels of CD39 and P2RX7 were upregulated at 24 h after CLP in both the control and CKD groups. CD73, A2AR, and A2BR mRNA levels were downregulated following CLP in both groups, with significantly lower levels in the CKD+CLP than in the Control+CLP group. Serum ATP levels only increased following CLP in the control groups. ATP and adenosine declined notably in the CKD+CLP group relative to the Control+CLP group. Furthermore, adenosine reduced substantially following CLP in the CKD group. Cytokine levels were elevated following CLP, exhibiting a significantly higher concentration in the CKD+CLP group than in the Control+CLP group (Figure 5).

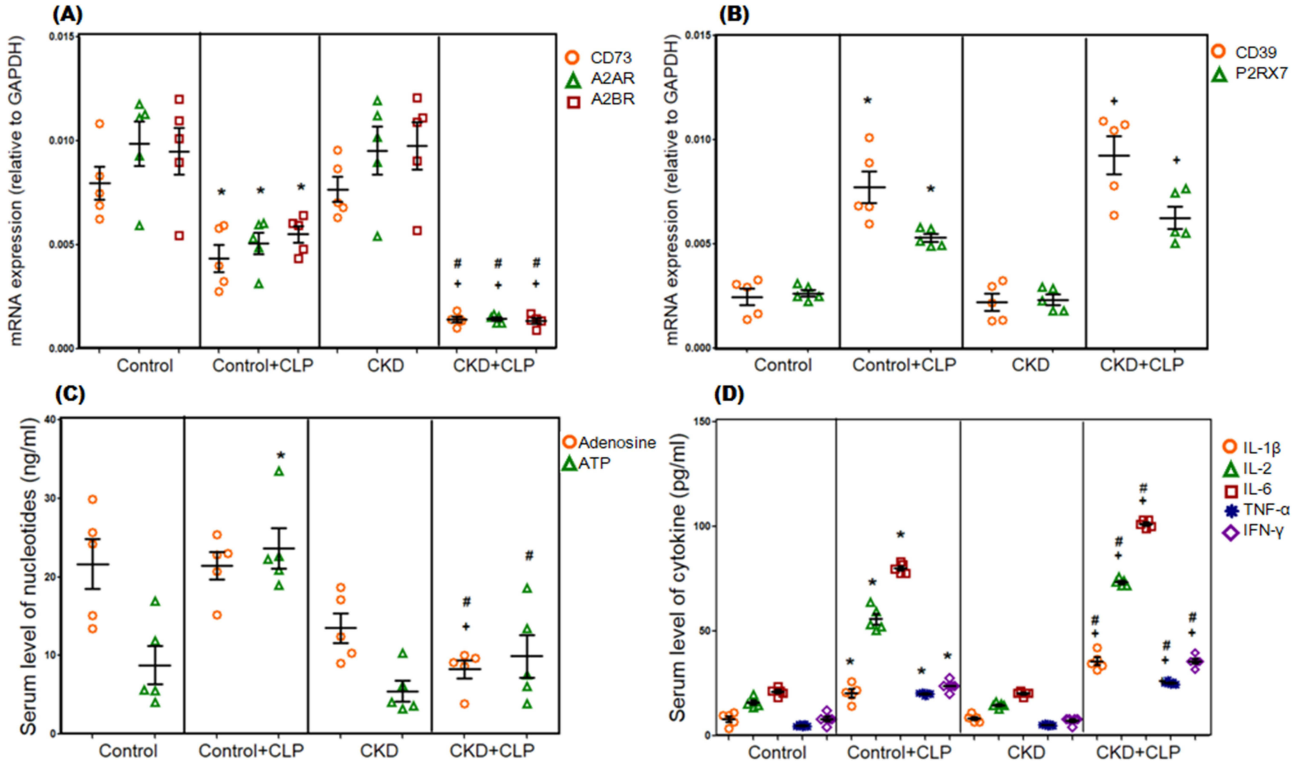

Figure 5 CD73-adenosine signaling was substantially downregulated in sepsis CKD rate models (**A**) mRNA transcript levels of CD73, A2AR, and A2BR were downregulated following CLP in both groups, with significantly lower levels in the CKD+CLP than in the Control+CLP group. (**B**) mRNA transcript levels of CD39 and P2RX7 were significantly upregulated at 24 h after CLP induction in Control and CKD groups; however, there were no significant differences between the Control+CLP and CKD+CLP groups. (**C**) ATP serum levels were elevated only after CLP in the control group. ATP and adenosine declined notably in the CKD+CLP group relative to the Control+CLP group. Furthermore, adenosine was substantially reduced following CLP in the CKD group. (**D**) Cytokine levels were elevated after CLP and were much higher in the CKD+CLP group than in the Control+CLP group (n=5/group). **Note**: **p*<0.05, compared to the Control group; + *p*<0.05, compared to the CKD group; # p<0.05, compared to the Control+CLP group.

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Discussion

We believe this is the first comprehensive study to demonstrate the relationship between CD73 signaling and infection in patients undergoing HD. Our study findings indicate that the reduced proportion of $CD73^+$ T cells is a robust and independent predictor of infection events and is associated with recurrent and fatal severe infections in these patients. CD73-adenosine signaling was significantly diminished in rats with CKD following sepsis, a finding that diverge markedly from the observed profile in rats with a non-CKD background. Our data suggest the mechanistic involvement of impaired CD73-adenosine signaling in infection with ESRD.

We previously demonstrated a significant reduction in $CD73^+$T cells in patients undergoing HD.[19] Here, we confirmed a decline in CD73 mRNA transcription levels in CKD rat model spleens. This decline may contribute to the observed reduction in extracellular adenosine levels and the subsequent downregulation of downstream signals. A trend of upregulated mRNA transcription levels of CD39 was observed, indicating that the purinergic signal of the CD39/CD73/adenosine pathway may be skewed, contributing to anabolic inflammation and disease progression in CKD.

The underlying cause of the pathological downregulation of CD73 signaling remains unclear. CD73 is a highly prevalent marker of age-related alterations in T cells, with a notable decline in the number of $CD73^+$T cells observed with advancing age.[26] Additionally, CKD induces premature senescence of T cells, particularly in individuals undergoing HD treatment.[27–29] Thus, downregulation of CD73 signal in CKD may share a common mechanism with immune senescence. Another potential explanation is the prolonged stimulation by uremic toxins. Our previous studies demonstrated that the uremic toxin IS stimulated T cells in vitro, significantly altering the expression of numerous immune regulatory genes, including an increase in CD39 and decrease in CD73 transcription.[30] This acute effect of uremic toxins is analogous to a damage-related signal that activates purinergic signaling. The chronic effects of uremic toxins on purinergic signals may vary, which can resemble the effects of chronic infection. Chronic pathogen infection can down regulate CD73 expression.[31–33] For instance, patients with HIV exhibit a decreased percentage of $CD73^+$T cells in peripheral blood, and this global downregulation of CD73 expression is associated with immune activation and functional impairment of T cells.[16] In patients with common variable immunodeficiency, decreased expression of CD73 in cytomegalovirus (CMV)-specific T cells was observed in patients with inflammatory disease compared to patients without inflammation or healthy controls.[34] Of note, latent infection with CMV is highly prevalent in patients undergoing HD.[35] We conducted an analysis between peripheral $CD73^+$T cells and CMV-IgG titer, showing these two factors are significantly associated regardless of age (data not shown). Therefore, it is necessary to determine whether CMV infection affects purinergic signaling in ESRD.

Our findings also indicated that patients with a lower proportion of $CD73^+$T cells experienced significantly higher rates of infection at both one and three years, with the predictive ability for one-year infection events exceeding that of age. After adjusting for traditional risk factors, a reduced proportion of $CD73^+$T cells was identified as the sole differentiating factor between mild and severe infection groups. CLP-induced sepsis resulted in upregulated CD39 and ATP receptor transcription in the spleen tissue, whereas the mRNA transcription levels of CD73 and adenosine receptors were downregulated, along with elevated extracellular ATP levels in peripheral blood, which describes a common mechanism involving purinergic signaling during early-stage infections, consistent with previous studies.[36] In contrast to the rats without CKD, those with CKD exhibited globally declining extracellular nucleotide levels after CLP, with adenosine dropping dramatically. CD73 mRNA downregulation was markedly more pronounced in rats with CKD than in controls after sepsis induction. Therefore, it can be concluded that impaired CD73-adenosine signaling is more pronounced after the induction of sepsis in CKD. The levels of inflammatory cytokines were markedly elevated in rats with sepsis-induced CKD compared to their counterparts without CKD. This observation provides compelling evidence that impairment of the CD73-adenosine signaling pathway may explain accelerated cytokine storm syndrome, exacerbated organ damage, and heightened mortality in CKD after infection.

Given the pivotal role of ectonucleotidases in immune regulation, researchers have studied their involvement in infectious disease progression. In a study by Hasko et al, CD73-deficient mice that underwent sepsis exhibited more severe inflammation and higher mortality rates than wild-type mice.[37] In humans, decreased levels of soluble CD73 in the peripheral blood have been associated with severe septic shock.[18] Pietrobon et al observed reduced CD73 expression in the whole blood of patients with SARS-CoV-2 and a further reduction in $CD73^+$T cells in patients with severe illness.[17] Elsaghir et al observed a significant reduction in serum adenosine levels in patients with severe illness due to SARS-CoV-2 infection, which was paralleled with

a notable decline in CD73 expression.[36] In addition, T cells lacking CD73 exhibited enhanced cytotoxic effector functionality compared to their CD73-positive counterparts.[38] Administration of adenosine may mitigate the inflammatory storm observed in patients with severe infection.[39] Besides its role in T cell regulation, CD73-adenosine is crucial to the recruitment and bactericidal function of polymorphonuclear leukocytes (PMNs).[40,41] PMNs from aged mice expressed significantly less CD73 and failed to efficiently kill pneumococci ex vivo. Supplementation with adenosine or transfer of PMNs expressing CD73 from young mice rescued this defect.[42] Therefore, an understanding of the disturbances to purinergic signaling in different immune cell subpopulations provides valuable insights into the pathogenesis of the disease and may facilitate the development of new treatments that target specific immune mechanisms.

This study has some limitations. First, this study was conducted at a single center, which may limit its statistical power and external validity issues during clinical trials. Second, the mechanism underlying CD73 downregulation caused by uremia remains unclear. Several factors, including uremic toxins, persistent inflammation, malnutrition, and dialysis, may contribute to this mechanism, which necessitates further confirmation. Third, although reduced adenosine concentrations have been observed in animal studies, CD73 enzymatic activity was not measured; therefore, its impact on infection incidence remains unclear whether the impact of CD73 on infection incidence depends on its enzymatic function. The absence of intervention in CD73 signaling makes it difficult to establish a causal relationship between CD73 signaling and infection. Further comprehensive and rigorous scientific experimentation must validate the role of CD73 signaling in infection immunity, particularly in uremic conditions.

In conclusion, patients on HD exhibited altered CD73 expression in T cells, which was positively associated with episodes of infection. Altered CD73-adenosine signaling may account for the high incidence and severity of infection in ESRD. Assessing T cell CD73 expression may benefit evaluations of immune function and prompt identification of patients at an elevated risk of significant complications. Further research must elucidate the underlying mechanisms and identify effective methods for preventing or reversing abnormal purinergic signaling in these patients. Finally, since animal experiments have previously shown that adenosine supplementation can improve the antibacterial ability of immune cells, this may also be worthy of further exploration.

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Disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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