

**Correlation Between Monocyte / Lymphocyte Ratio and Risk of Death and
Cardiovascular Events in Patients Receiving Peritoneal Dialysis: a Multicenter
Prospective Cohort Study**

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Background

The epidemiological survey of chronic kidney disease in China shows that the current incidence of chronic kidney disease (CKD) in China is about 10.8%, with about 119 million patients. About 2% of patients will enter the stage of end-stage renal disease (ESRD) and require dialysis or kidney transplantation to support life. Currently the main renal replacement therapy is: hemodialysis (HD) or peritoneal dialysis (PD). Among them, PD patients are increasing year by year. As of 2016, more than 900,000 people in Asia are undergoing maintenance dialysis (218 cases per million population). Statistics from the CNRDS system show that as of the end of 2017, the number of PD patients in China has reached 86264. Compared with HD, PD is easy to operate, better preserves residual renal function, has early survival advantages, and is more cost-effective. In spite of this, the prognosis of PD patients is still unsatisfactory. At present, the main causes of death in PD patients are cardiovascular disease, peritoneal dialysis-related peritonitis, gastrointestinal bleeding, and tumors. Among them, cardiovascular disease (CVD) is the most important, accounting for about 50% of the deaths of PD patients. Among all CVD events, coronary heart disease, heart failure, and stroke are the most common.

CVD risk factors for dialysis patients can be divided into traditional and non-traditional categories. Traditional Framingham risk factors can only explain some of the CVD risks of PD patients. Non-traditional risk factors, especially persistent inflammatory states, are essential in the pathogenesis of CVD such as atherosclerosis and vascular calcification, and lead to protein energy expenditure and premature death outcomes in patients with CKD. The causes of chronic inflammation in patients with peritoneal dialysis are mainly divided into two aspects. Dialysis-related factors include: catheter-related infections, continuous exposure to biologically incompatible PD solutions, peritonitis, increased adipose tissue, and adipose factor balance disorders; associated with low GFR Factors include decreased clearance of pro-inflammatory factors, accumulation of uremic toxins, endotoxin exposure, oxidative stress, increased volume load, oral or other organ infections, and susceptibility to infections. The causes of inflammation in peritoneal dialysis are interconnected, leading to a persistent state of inflammation that ultimately increases the risk of cardiovascular events. Therefore, it is important to screen for markers that predict the risk of CVD in dialysis patients.

At present, various inflammatory mediators, such as c-reactive protein (CRP), interleukins (IL) and tumor necrosis factor (TNF), have been studied and proven to independently predict the risk of CVD in dialysis patients. CRP can induce the expression of adhesion molecules in endothelial cells, increase the adhesion of vascular endothelial cells and monocytes; promote the formation of foam cells and atherosclerosis; aggravate vascular endothelial dysfunction; TNF- α can increase the expression and activity of alkaline phosphatase, enhance Isolated

vascular wall calcification; aggravates vascular endothelial dysfunction; promotes left ventricular remodeling and aggravates left ventricular dysfunction. IL-6 can stimulate macrophages to secrete monocyte chemoattractant protein 1, induce endothelial cell adhesion molecule expression; stimulate vascular smooth muscle cell proliferation and migration; aggravate vascular endothelial dysfunction; and induce cardiac hypertrophy. Although the mechanisms are different, they all increase the risk of CVD death in dialysis patients by aggravating vascular endothelial dysfunction, promoting ventricular remodeling and inducing myocardial hypertrophy. However, such markers are expensive and their detection is not easy to limit their clinical application. This prompted researchers to devote themselves to mining new markers of inflammation.

In recent years, experts and scholars have become interested in blood cell parameters. Previous studies have shown that white blood cells and their subgroups (neutrophils, lymphocytes, monocytes, Neutrophil / lymphocyte ratio (NLR), monocyte /lymphocyte ratio (MLR), platelet / lymphocyte ratio (PLR) and other indicators have important predictive value for all causes and prognosis of cardiovascular disease in dialysis population. They have the advantages of low cost and easy detection. Among them, MLR has been proven to be an independent predictor of death in cardiovascular diseases such as coronary heart disease and heart failure. Previous research found. Monocytes play a key role in the occurrence and development of atherosclerosis. After the initial injury, endothelial cells are activated and promote monocytes to roll, attach and migrate under the endothelium. Monocytes that migrate to the subendothelial can differentiate into dendritic cells, which are key participants in activating adaptive immunity, or differentiate into macrophages, which secrete pro-inflammatory cytokines, thereby recruiting more immune cells and Promote inflammation. Macrophage phagocytosis of lipoprotein particles can lead to the formation of fatty streaks, the earliest ultrastructural changes in the formation of atherosclerosis. The migration of smooth muscle cells from the medial membrane to the intimal membrane further promotes the atherogenic process. Monocyte-derived cells transform this early lesion into advanced atherosclerotic plaques, which contain lipid-rich and macrophage-rich necrotic cores that eventually cause the plaque to rupture. In addition, previous studies have shown that physiological stress can lead to a significant increase in systemic cortisol production, which has led to a shift in leukocyte differentiation toward a decrease in lymphocytes and an increase in the percentage of granulocytes. Measuring lymphocyte counts can reflect stress levels. In patients with coronary heart disease, studies have shown that a decrease in lymphocyte count is an independent predictor of prognosis in patients with coronary heart disease; there is a phenomenon of lymphocyte apoptosis on the endothelium of atherosclerotic injured blood vessels. Therefore, low lymphocytes can reflect the occurrence and development of atherosclerotic diseases. In patients with end-stage renal disease, there will be changes in the number of immune cells such as increased

monocytes and decreased lymphocytes. First, the decline in renal function causes retention of uremic toxins and cytokines, which leads to increased proinflammatory cytokines and oxidative stress. It further stimulates the proliferation of monocytes, and at the same time down-regulates immunity, resulting in a decrease in the number and function of lymphocytes, which in turn promotes inflammation and oxidative stress, and continues a vicious cycle. MLR can integrate pro-inflammatory and anti-inflammatory effects, and simultaneously reflect inflammation and immune deficiency. It may be an important marker of inflammation in patients with end-stage renal disease. In 2017, Xiang F et al. For the first time found in a prospective cohort study of 355 hemodialysis patients that high MLR levels were independent predictors of all-cause and CVD mortality in hemodialysis patients, and exceeded the predictive value of NLR. However, there are no reports about MLR and prognosis in patients with peritoneal dialysis at home and abroad. Our previous retrospective analysis showed that the all-cause and cardiovascular disease survival rates of patients in the low MLR group were significantly higher than those in the high MLR group; high MLR levels were associated with all-cause and increased risk of cardiovascular death in patients with peritoneal dialysis. However, because single-center retrospective studies cannot determine causality, there may be problems such as selection bias. Therefore, a multicenter, prospective, large-sample study is needed to further explore the correlation between MLR and the prognosis of PD patients.

Summary

Title	Correlation Between Monocyte / Lymphocyte Ratio and Risk of Death and Cardiovascular Events in Patients Receiving Peritoneal Dialysis: a Multicenter Prospective Cohort Study.
Sponsor	Department of Nephrology of Zhujiang Hospital, Southern Medical University
Study official	Jun Zhang
Research purposes	Main research purpose: To explore the correlation between MLR and risk of death and cardiovascular events in patients receiving peritoneal dialysis. Secondary research purpose: To explore the correlation between MLR and peritoneal dialysis-associated peritonitis in patients with peritoneal dialysis.
Research hypothesis	The level of MLR is related to the prognosis of patients with peritoneal dialysis. High levels of MLR may increase the risk of death, cardiovascular events and dialysis-associated peritonitis in patients with peritoneal dialysis.
Study Model	prospective cohort study

Sample size	<p>This study is based on a balanced control design of two groups to test whether there is a significant difference in survival rates. All-cause mortality and cardiovascular mortality are Primary Outcome Measures. Divided into high MLR group and low MLR group according to the median of monocyte / lymphocyte ratio (MLR). The previous research results show that the one-year survival rate of patients in the high MLR group was 86.6%, and the survival rate of patients in the low MLR group was 97%. Taking 0.05 as the statistical significance level on both sides, setting the test efficiency to 80%, adopting a 1: 1 balanced design, calculated by software PASS.11, it requires 84 cases in the high MLR group and 84 cases in the low MLR group. The one-year cardiovascular survival rate of patients in the high MLR group is 90.7%, and the cardiovascular survival rate of patients in the low MLR group 98.6%, taking 0.05 as the statistical significance level on both sides, setting the test efficiency to 80%, adopting a 1: 1 balanced design, calculated by software PASS.11, 127 cases are required in the high MLR group and 127 cases are in the low MLR group. Further assuming that the maximum rate of lost follow-up in each group will not exceed 5%, the final sample size is determined as 133 cases in the high MLR group and 133 cases in the low MLR group, for a total of 266 cases.</p>
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Eligibility	<p>Study Population: Patients who underwent continuous ambulatory peritoneal dialysis in the Department of Nephrology of Zhujiang Hospital of Southern Medical University, Guangzhou First People's Hospital, and Jiangmen Central Hospital, and voluntarily signed informed consent to join the study.</p> <p>Inclusion Criteria:</p> <ul style="list-style-type: none">• Patients who underwent continuous ambulatory peritoneal dialysis for at least 3 months. <p>Exclusion Criteria:</p> <ul style="list-style-type: none">• Age <18 years, with acute or chronic infections such as lung infection, peritoneal dialysis-related peritonitis, urinary system infection, etc.• With history of malignant tumor or blood disease.• With rheumatic immune diseases, such as systemic lupus erythematosus, vasculitis, sjögren's syndrome, etc.• Used immunosuppressants, such as glucocorticoids, calcineurin inhibitors, etc.• Women during pregnancy.
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Follow up	<p>The follow-up period was from the beginning of the study to the first outcome event (death or cardiovascular event, peritoneal dialysis-associated peritonitis). For patients who did not have an outcome event or survived, the follow-up period is 1 year. The lymphocyte / monocyte ratio should be tested every 3 months after the start of the study, and the final value is the average.</p>
Exposure factor	Monocyte / lymphocyte ratio (MLR)
Outcome Measures	<p>Primary Outcome Measure:</p> <ul style="list-style-type: none"> • All-cause mortality • Cardiovascular mortality <p>Secondary Outcome Measure:</p> <ul style="list-style-type: none"> • The Incidence of cardiovascular events (including acute heart failure, angina, acute myocardial infarction, arrhythmias requiring treatment, transient ischemic attack (TIA), cerebral infarction or cerebral hemorrhage, peripheral vascular disease) • The Incidence of peritoneal dialysis-associated peritonitis

Data Sources	<p>Use a combination of electronic medical record system, test report system, national peritoneal dialysis information registration system, cardiovascular event registration form to collect patient clinical and demographic data, laboratory data, and survival outcomes. For out-of-hospital events, relevant information is obtained by telephone follow-up.</p> <p>Clinical and demographic data : age, gender, body mass index (BMI), dialysis time, smoking history, family history, medication history, medication status, primary kidney disease and complications</p> <p>Laboratory data: Peritoneal balance test (PET), dialysis adequacy assessment (KT/v), c-reactive protein (CRP), erythrocyte sedimentation rate (ESR), procalcitonin (PCT), interleukin 6 (IL-6), whole blood count and classification of leukocyte count, calcium, phosphorus, intact parathyroid hormone (IPTH), serum iron, ferritin, transferrin, transferrin saturation, urea nitrogen, serum creatinine, uric acid, urinary protein ,estimated glomerular filtration rate (eGFR), blood glucose, total cholesterol, triglyceride, high-density lipoprotein, low-density lipoprotein, albumin (ALB), N-terminal pro-brain natriuretic peptide, pulse pressure (PP), blood pressure,(BP) , left ventricular mass index (LVMI), ejection fraction (EF), carotid artery intima-media thickness (IMT).</p>
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<p>Statistical plan</p>	<p>Statistical hypothesis: test hypothesis H0: $\lambda 1 = \lambda 2$, that is, there is no difference in mortality and the Incidence of cardiovascular events between the high MLR group and the low MLR group.</p> <p>Alternative hypothesis H1: $\lambda 1 \neq \lambda 2$, there is a statistical difference in mortality and the Incidence of cardiovascular events between the high MLR group and the low MLR group.</p> <p>Main statistical analysis methods: Spss23.0 software was used for data statistical analysis. The continuity variables were tested for normality first. The continuous variables of normal distribution are represented by 'x \pm s'; the continuous variables of skew distribution are represented by median m; the data of classification or counting type are displayed by percentage. Two independent samples were used to compare the differences between groups in normal distribution measurement data, Mann Whitney U test was used to compare the differences between groups in non normal distribution measurement data, and chi square test was used to compare the differences between groups in classification or counting data. For missing values such as missing values and censored values, when the missing values are concentrated in a few variables and these variables are not the main variables for analysis, deletion can be considered, or the mean substitution method can be used to find alternative values for the missing values. The correlation between MLR and other indexes was analyzed by Spearman correlation and linear regression. Kaplan Meier curve was drawn to estimate the survival rate and cardiovascular disease incidence rate, and log rank test was used to check the difference. Single factor and multiple factor Cox regression models were used to explore the independent risk factors of death and cardiovascular disease in peritoneal dialysis patients, and to evaluate the correlation between MLR level and clinical all-cause and cardiovascular disease-related survival outcomes in peritoneal dialysis patients. Using HR risk ratio (95% CI confidence interval) to show the results, bilateral</p>
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	statistical standard p value < 0.05 has statistical significance.
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