

## Original Article



# Onset of Coronary Heart Disease is Associated with HCMV Infection and Increased CD14<sup>+</sup>CD16<sup>+</sup> Monocytes in a Population of Weifang, China\*

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## Abstract

**Objective** To investigate the relationship between human cytomegalovirus (HCMV) infection and peripheral blood CD14<sup>+</sup>CD16<sup>+</sup> monocytes in the pathogenesis of coronary heart disease (CHD), and to elucidate the mechanism of pathogenesis in CHD by analyzing the correlation between infection, inflammation, and CHD, to provide a basis for the prevention, evaluation, and treatment of the disease.

**Methods** In total, 192 patients with CHD were divided into three groups: latent CHD, angina pectoris, and myocardial infarction. HCMV-IgM and -IgG antibodies were assessed using ELISA; CD14<sup>+</sup>CD16<sup>+</sup> monocytes were counted using a five-type automated hematology analyzer; mononuclear cells were assessed using fluorescence-activated cell sorting; and an automatic biochemical analyzer was used to measure the levels of triglyceride, cholesterol, high- and low-density lipoprotein cholesterol, lipoprotein, hs-CRP and Hcy.

**Results** The positive rates of HCMV-IgM and -IgG were significantly higher in the CHD groups than in the control group. HCMV infection affects lipid metabolism to promote immune and inflammatory responses.

**Conclusion** HCMV infection has a specific correlation with the occurrence and development of CHD. The expression of CD14<sup>+</sup>CD16<sup>+</sup> mononuclear cells in the CHD group was increased accordingly and correlated with acute HCMV infection. Thus, HCMV antibody as well as peripheral blood CD14<sup>+</sup>CD16<sup>+</sup> mononuclear cells can be used to monitor the occurrence and development of CHD.

**Key words:** Human cytomegalovirus; Coronary heart disease; Antibody; CD14<sup>+</sup>CD16<sup>+</sup> monocytes; Weifang

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## INTRODUCTION

Coronary heart disease (CHD) affects cardiac function (hypoxia, ischemia, and necrosis) due to stenosis and the obstruction of arterial blood vessels<sup>[1-3]</sup>. The pathological changes are mainly caused by atherosclerosis (AS), which has a high mortality rate in European and American countries. In China, it currently affects about 11 million patients and this number is increasing every year<sup>[4,5]</sup>. Epidemiological studies have shown that classical risk factors, such as hypertension, hyperlipidemia, smoking, obesity, and diabetes cause AS, but the etiology of the disease still remains unclear<sup>[6-8]</sup>. In recent years, the prevalence of these risk factors has successfully reduced; however, there is still high incidence of cardiovascular diseases with AS<sup>[9]</sup>. Further research studies have revealed that chronic, repeated, and persistent inflammation might be an important cause of AS<sup>[10-12]</sup>. Moreover, infective factors and their inflammatory reactions are closely associated with the disease<sup>[13-15]</sup>. Some reports suggest that human cytomegalovirus (HCMV) may be involved in the initiation of AS<sup>[16-19]</sup>. HCMV infection and the number of peripheral blood mononuclear cells have been shown to be independent risk factors for AS; in addition, different subsets of mononuclear cells seem to play different roles in the AS inflammatory response<sup>[20-22]</sup>. HCMV infection can initiate the process of AS formation, as elucidated by the finding that regulatory T lymphocytes are reduced in HCMV-infected patients<sup>[23,24]</sup>, which disturbs the balance of immune suppression, thus inducing a highly inflammatory state, and further leading to the occurrence and development of coronary artery disease in patients with CHD. Inflammatory response is one of the important factors that cause AS<sup>[25]</sup>. Studies have shown that HCMV antigen and antibody form immune complexes in the AS lesion<sup>[21,26]</sup>. These are deposited on the vascular wall and induce vascular endothelial cells, macrophages, smooth muscle cells, foam cells, and infiltrated T lymphocytes to express the mononuclear cell chemical AS-1 (MCp-1). This subsequently stimulates macrophages to release many inflammatory cytokines such as interleukin (IL)-1, -6, -8, and -10, and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ). These factors activate monocytes in the blood and induce migration into the arterial intima<sup>[27]</sup>.

HCMV is an opportunistic pathogen that belongs to the beta herpes virus subfamily<sup>[28]</sup>. It is highly species-specific and spreads only from person to

person. HCMV infection is characterized by susceptibility, persistence, and repetition, and it can infect people of any age. Close contact with the body fluids of the infected persons causes it to spread in the human population; once infected, the host produces specific HCMV-IgM, HCMV-IgG, and other antibodies. The IgM and IgG antibodies produced are generally not affected by other factors, can be used as direct evidence of infection, and have predictive value for the condition<sup>[29]</sup>. The periodic activation of HCMV causes abnormal lipid metabolism and local chronic immune or inflammatory response. HCMV infection upregulates the endothelial adhesion molecules E-selectin and intercellular adhesion molecule-1 that favor the adhesion of monocytes<sup>[30]</sup>. HCMV infection also induces monocyte chemoattractant protein-1 (MCp-1), MCp-2, and macrophage colony-stimulating factor<sup>[31]</sup>, and stimulates macrophages to produce and release interleukins such as IL-1, IL-6, IL-8, and IL-10, as well as inflammatory factors such as TNF- $\alpha$ . These cytokines mainly act to activate monocytes and promote their entry into the endothelium<sup>[32]</sup>.

Mononuclear macrophages play an important role in the pathogenesis of AS; one-third of the proliferating cells in the artery endothelial injury are monocyte macrophages. Sinclair et al.<sup>[33]</sup> found that HCMV is cytosolic in mononuclear macrophages to avoid degradation and elimination. HCMV persists in monocyte precursors in the bone marrow, so monocytes become carriers and transporters of HCMV. Under the combined action of adhesion molecules, chemokines, and cytokines, monocytes transfer HCMV to the site of vascular damage, enter the endothelium to transform into macrophages, and after taking in a large amount of cholesterol, turn into foam cells. Therefore, HCMV may initiate or promote the development of AS *via* the movement of infected mononuclear macrophages into the coronary arteries<sup>[34]</sup>.

From the early lipid aggregation to plaque formation and instability until rupture, a series of ischemic symptoms and malignant events are triggered during this process, in which the migration, aggregation, and inflammation of monocytes play a key role<sup>[35]</sup>. Yang et al.<sup>[36]</sup> detected HCMV and virus antibody IgM in peripheral blood mononuclear cells using PCR and ELISA. The results showed that the positive rates of HCMV and IgM antibody are significantly higher in patients with coronary heart disease (CHD) than in the controls. Furthermore, the study found that the ratio of the peripheral blood classical monocyte subgroup (CD14<sup>++</sup>CD16<sup>-</sup>) in

patients with acute coronary syndrome does not differ significantly from those with stable angina pectoris and controls, while the ratio of the CD14<sup>+</sup>CD16<sup>+</sup> mononuclear cell subgroup is significantly higher. The group showed that CD14<sup>+</sup>CD16<sup>+</sup> monocytes are more strongly associated with inflammation. Compared to CD14<sup>++</sup>CD16<sup>-</sup> mononuclear cells, CD14<sup>+</sup>CD16<sup>+</sup> monocytes have a stronger ability to induce TNF and IL-6 in the process of inflammation, and upregulate the expression of inflammatory chemokine receptors more evidently<sup>[37,38]</sup>. Based on the expression of CD14 and CD16, we divided monocytes into four subgroups: CD14<sup>++</sup>CD16<sup>-</sup>, CD14<sup>++</sup>CD16<sup>+</sup>, CD14<sup>+</sup>CD16<sup>+</sup>, and CD14<sup>+</sup>CD16<sup>-</sup>. The role of HCMV infection and CD14<sup>+</sup>CD16<sup>+</sup> mononuclear cells in the development and pathogenesis of CHD and its interrelated mechanism were investigated based on HCMV infection and CD14<sup>+</sup>CD16<sup>+</sup> mononuclear cells in 192 patients with CHD, to provide a reference for its prevention, evaluation, and treatment.

## MATERIALS AND METHODS

### Human Materials

Between September 2017 and January 2018, we recruited 192 patients with cardiovascular disease from Weifang People's Hospital and Yidu Central Hospital. All participants provided written informed consent for the study protocol, which was approved by the Ethics Committee of Weifang Medical University (reference number, wfm2017012). Patients with heart disease caused by thyroid disease, rheumatic heart disease, non-rheumatic valvular disease, dilated or hypertrophic cardiomyopathy, hypertensive, congenital, pulmonary, and other heart diseases, were excluded. Patients were divided into three groups: latent CHD ( $n = 77$ ), angina pectoris ( $n = 64$ ), and acute myocardial infarction ( $n = 51$ ). Seventy-nine healthy individuals were recruited as controls.

### Criteria for Risk Factors

**Smoking:** Continuous smoking for > 12 months (not less than once per day) and still smoking, or long-term smoking for more than six months.

**Hypertension:** A history of hypertension or systolic blood pressure  $\geq 140$  mmHg or diastolic blood pressure  $\geq 90$  mmHg, or on antihypertensive drugs.

**Diabetes:** Fasting blood glucose  $\geq 7$  mmol/L or blood glucose  $\geq 11.1$  mmol/L 2 h after a meal.

**Family history:** First-degree relatives of women < 55 years of age (or males < 65 years of age) who have associated cardiovascular disease.

### Detection of HCMV IgG and IgM Antibodies

Peripheral venous blood was collected from patients and controls after fasting for at least 8 h and assessed as follows: The serum was obtained by collection in a standard coagulant tube, followed by separation from whole blood using centrifugation. HCMV-IgG and HCMV-IgM antibodies were detected by enzyme immunoassay (HCMV-IgM and HCMV-IgG detection kits were obtained from Beijing Baer Bioengineering Co. Ltd.). Four wells in the reaction plates were reserved for negative and positive controls (3 wells for negative control and 1 for positive control, according to the manufacturer's protocol), and the reaction was stopped within 10 min. The detection parameters of the microplate reader were set according to the number of samples to be tested and the wells of the reaction plate; the double wavelength was set at 450/630 nm, and the absorbance (A value) of each reaction well was detected and recorded. The A values were classified as positive or negative, based on thresholds. PCR assays were used for measuring HCMV DNA in peripheral blood leukocytes to reduce false positives and improve sensitivity.

### Detection of CD14<sup>+</sup>CD16<sup>+</sup> Mononuclear Cells and Measurement of Blood Lipid Indexes

Whole blood was collected into EDTA-K2 anticoagulant tubes. First, routine analysis was performed using the BC-6800 automatic blood analyzer (Mindray Shenzhen, China) to record the number of white blood cells, mononuclear cells, and their proportions in whole blood. Following that, mononuclear cell CD14 and CD16 were detected using FACS (FACSCalibur, BD Biosciences, CA, USA). The cells were divided into four subgroups: CD14<sup>++</sup>CD16<sup>-</sup>, CD14<sup>++</sup>CD16<sup>+</sup>, CD14<sup>+</sup>CD16<sup>-</sup>, and CD14<sup>+</sup>CD16<sup>+</sup> upon counting the percentages of 20,000 mononuclear cells expressing CD14 and CD16 (Figure 1).

The blood lipid indexes, such as triglycerides (TG), cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), lipoprotein A [Lp (a)], Hypersensitive C-reactive protein (hs-CRP) and Homocysteine (Hcy) were assessed after standard coagulant collection and serum separation by centrifugation at 3,500 rpm for 5 min using the BS-800 automatic biochemical analyzer (Mindray).

### Statistical Analysis

All data were analyzed using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). Data are shown as the mean  $\pm$  standard deviation; counts are expressed as positive and positive rates. All data were analyzed using the Chi-square and ANOVA tests. Multivariate logistic regression was used for correlation analysis. Differences between groups were considered to be statistically significant at a  $P$  value  $< 0.05$ .

### RESULTS

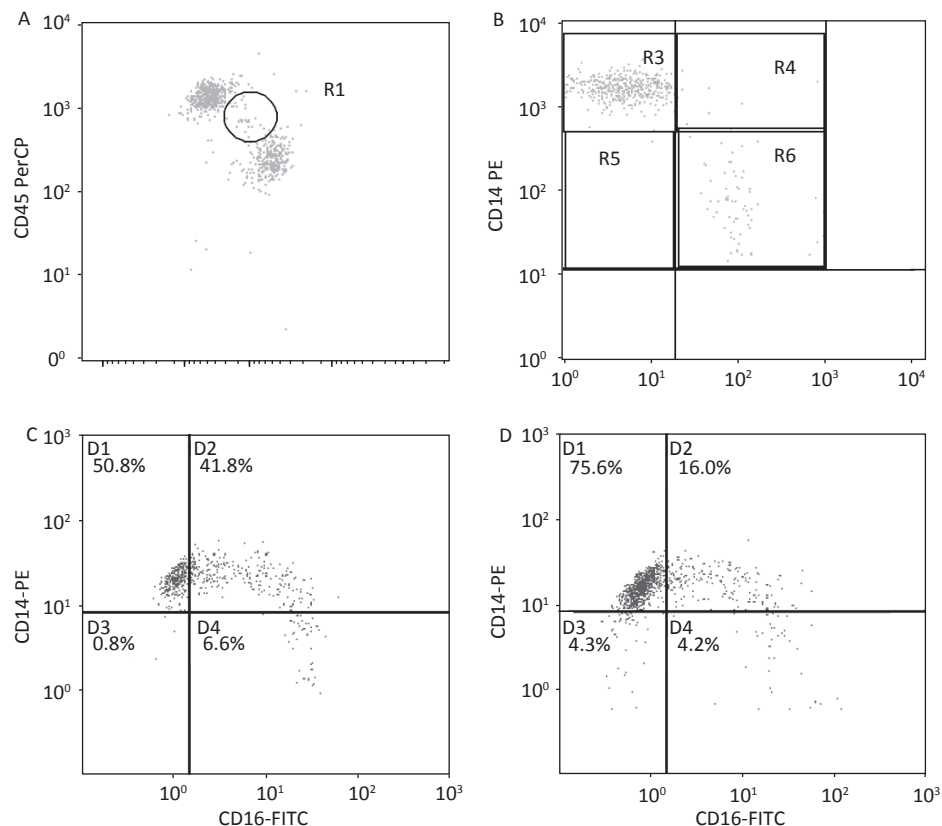
#### Gradual Increase in the Positive Rates of Viral Antibodies in the CHD Groups

To assess the relationship between the levels of HCMV antibodies and the incidence of various types of CHD, we first measured the IgM and IgG levels in the latent CHD, angina pectoris, myocardial infarction, and control groups. The positive rates of HCMV-IgM in the control, latent CHD, angina pectoris, and myocardial infarction groups (Table 1)

were 19.0%, 33.8%, 40.6%, and 52.9%, respectively. The positive rates in the latent CHD, angina pectoris, and myocardial infarction groups were higher than in the control group ( $\chi^2 = 4.396$ ,  $P = 0.036$ ;  $\chi^2 = 8.094$ ,  $P = 0.004$ ;  $\chi^2 = 16.338$ ,  $P < 0.001$ ). The positive rates of HCMV-IgG in the control, latent CHD, angina pectoris, and myocardial infarction groups (Table 1) were 44.3%, 62.3%, 67.2%, and 80.3%, respectively. The positive rates in the latent CHD, angina pectoris, and myocardial infarction groups were higher than in the control group ( $\chi^2 = 5.094$ ,  $P = 0.024$ ;  $\chi^2 = 7.468$ ,  $P = 0.006$ ;  $\chi^2 = 16.621$ ,  $P < 0.001$ ) with a gradually increasing trend.

#### Gradual Increase in CD14<sup>+</sup>CD16<sup>+</sup> Monocyte Expression in the CHD Groups and Differences in the Concentrations of TG, TC, HDL-C, LDL-C, Lp (a), hs-CRP, and Hcy in Various Groups

To compare the CD14<sup>+</sup>CD16<sup>+</sup> monocytes in CHD patients versus controls, we assayed the cells using FACS and found that there was an increase in the ratio of mononuclear cells and the expression of CD14<sup>+</sup>CD16<sup>+</sup> mononuclear cells in the latent CHD,



**Figure 1.** FACS analysis of CD14<sup>+</sup>CD16<sup>+</sup> monocytes in CHD patients and controls. (A) Area of R1 represents gated monocyte population. (B) Areas of R3-R6 represent CD14<sup>+</sup>CD16<sup>-</sup>, CD14<sup>+</sup>CD16<sup>+</sup>, CD14<sup>-</sup>CD16<sup>-</sup>, and CD14<sup>-</sup>CD16<sup>+</sup> monocyte subsets, respectively. (C–D) CD14<sup>+</sup>CD16<sup>+</sup> monocytes rates in CHD patients.

angina pectoris, and myocardial infarction groups (Figure 2A). Compared to the control group, the expression of CD14<sup>+</sup>CD16<sup>+</sup> mononuclear cells in the latent CHD, angina pectoris, and myocardial infarction groups were significantly different ( $t = 4.1776$ ,  $P < 0.001$ ;  $t = 8.9747$ ,  $P < 0.001$ ;  $t = 16.2291$ ,  $P < 0.05$ ) (Figure 2A). In addition, there was a significant difference in the IgM (+) and IgM (–) CD14<sup>+</sup>CD16<sup>+</sup> mononuclear cells among the groups ( $P < 0.05$ ) (Figure 2B). The TG, TC, HDL-C, LDL-C, Lp (a), hs-CRP, and Hcy levels in the myocardial infarction, angina pectoris, and control groups were also significantly different ( $P < 0.05$ ) (Table 2).

### ***HCMV Infection Is An Independent Factor that Contributes to the Development of CHD***

To clarify whether HCMV infection is an independent factor leading to CHD, we tested possible predisposing factors in all patients and controls and found that the factors, smoking ( $P < 0.05$ ), hypertension ( $P < 0.05$ ), diabetes ( $P < 0.001$ ), family history ( $P < 0.001$ ), HCMV-IgM ( $P < 0.001$ ),

and HCMV-IgG ( $P < 0.001$ ) showed significant association in the single-factor analysis (Figure 3), while age ( $P = 0.154$ ) and body mass index ( $P = 0.140$ ) did not (Table 3). Multivariate logistic regression analysis showed that hypertension, diabetes, family history, HCMV-IgM, and HCMV-IgG are independent factors affecting CHD.

## **DISCUSSION**

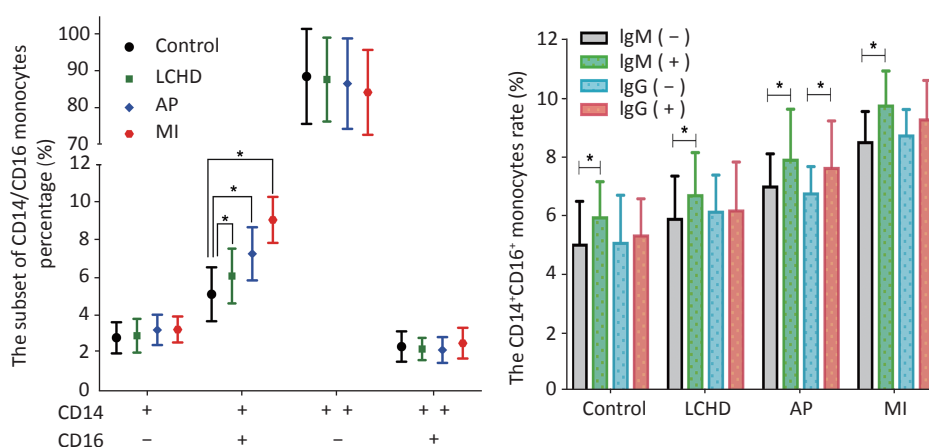
CHD is a progressive disease that is common in the elderly; it has a high mortality rate in the western countries. The incidence rate of CHD in China's population aged  $> 60$  years is  $\geq 80\%$ , and this rate is increasing every year. Our study showed that smoking, hypertension, diabetes, family history, and other risk factors are independent factors contributing to CHD. More importantly, this study also identified that the development of AS is closely associated with HCMV infection.

The serological epidemiology, related molecular biology of HCMV, as well as animal studies together

**Table 1.** Differences in IgM (+) and IgG (+) rates between the disease and control groups

Group (count)	IgM (+)			IgG (+)		
	Count	%	P Value	Count	%	P Value
Control (79)	15	19.0	/	35	44.3	/
LCHD (77)	26*	33.8	0.036	48*	62.3	0.024
AP (64)	26*	40.6	0.004	43*	67.2	0.006
MI (51)	27*	52.9	$< 0.001$	41*	80.3	$< 0.001$

**Note.** The tests were carried out in 4 groups: control, LCHD (latent coronary heart disease), AP (angina pectoris), and MI (myocardial infarction). Data are presented as the mean. Statistical significance of differences between groups was evaluated using the ANOVA test. \* $P < 0.05$  versus control.



**Figure 2.** Monocytes and subgroups in latent CHD. LCHD (latent coronary heart disease), AP (angina pectoris), MI (myocardial infarction), and control groups. \* $P < 0.05$  between groups.

indicate that the occurrence and development of AS is closely associated with HCMV infection. Adam et al.<sup>[39]</sup> were the first to demonstrate that HCMV infection is associated with AS. Adam et al. found that the HCMV infection rate was 90% higher in the AS population than in the non-AS group (90% and 74%, respectively). In this study our findings showed that, the positive rate of a high antibody titer was greater in the AS population than in controls ( $P < 0.001$ , 75% to 26%); the titer was not affected by TG, TC, and other risk factors. These results suggest that the occurrence and development of atherosclerotic CHD is associated with HCMV infection.

After HCMV infection, the IgM and IgG antibodies are generally not disturbed by other factors, and so can be used as direct evidence of infection and to predict the development of AS post-infection<sup>[40]</sup>. Our study showed that the positive rates of HCMV-IgM and HCMV-IgG were higher in the latent CHD (33.8% and 62.3%, respectively),

angina pectoris (40.6% and 67.2%, respectively), and myocardial infarction (52.9% and 80.3%, respectively) groups (Table 1) than in the controls (19.0% and 44.3%, respectively). Molecular biology-based research studies have also provided direct evidence for the hypothesis that HCMV infection can lead to AS<sup>[16,22]</sup>. Some animal experiments also support the hypothesis. Fabricant et al.<sup>[41]</sup> induced AS lesions in chickens with Marek disease virus and showed that their coronary, gastric, and celiac arteries displayed pathological changes.

Second, HCMV infection affects lipid metabolism and promotes immune and inflammatory responses<sup>[42]</sup>. Chronic, persistent, cyclically repeated inflammation may be an important factor affecting the development and pathogenesis of CHD. It has been reported that TG and TC levels rise markedly after HCMV infection, and are not affected by diet<sup>[43]</sup>. Comparison of the blood AS indexes [TG, TC, HDL-C, LDL-C, and Lp (a)] (Table 2) revealed

**Table 2.** Blood indexes of atherosclerosis ( $\bar{x} \pm SD$ )

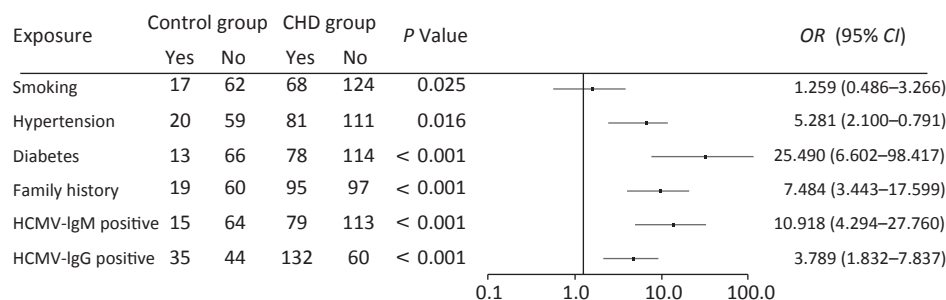
Group	TG (mmol/L)	TC (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	Lp (a) (nmol/L)	hs-CRP (mg/L)	Hcy ( $\mu$ mol/L)
Control	1.31 $\pm$ 0.47	3.70 $\pm$ 0.77	1.25 $\pm$ 0.33	1.86 $\pm$ 0.78	52.6 $\pm$ 9.34	3.78 $\pm$ 0.99	12.20 $\pm$ 2.60
LCHD	1.42 $\pm$ 0.52	3.88 $\pm$ 0.77	1.26 $\pm$ 0.45	3.11 $\pm$ 0.23	70.95 $\pm$ 8.45	5.29 $\pm$ 1.33	13.40 $\pm$ 2.60
AP	2.21 $\pm$ 0.21*	5.44 $\pm$ 0.54*	1.03 $\pm$ 0.25*	3.48 $\pm$ 0.35*	81.8 $\pm$ 8.44*	8.27 $\pm$ 1.39*	16.10 $\pm$ 3.60*
MI	2.62 $\pm$ 0.32*	6.01 $\pm$ 0.80*	0.93 $\pm$ 0.24*	3.90 $\pm$ 0.49*	95.45 $\pm$ 8.12*	11.87 $\pm$ 2.95*	19.50 $\pm$ 4.40*

**Note.** The tests were carried out in 4 groups: control, LCHD (latent coronary heart disease), AP (angina pectoris), and MI (myocardial infarction). Data are expressed as mean  $\pm$  standard deviation. \* $P < 0.05$  versus control. Triglyceride (TG), Cholesterol (TC), High- and low-density lipoprotein cholesterol (HDL-C, LDL-C), Lipoprotein A [Lp (a)], Hypersensitive C-reactive protein (hs-CRP) and Homocysteine (Hcy).

**Table 3.** Single-factor analysis of general clinical data (risk factors) in CHD and control groups

Risk factors	Control group (n = 79)	CHD group (n = 192)	t	P
Age	56.6 $\pm$ 4.2	57.5 $\pm$ 4.9	1.43	0.154
Body mass index (kg/m <sup>2</sup> )	23.3 $\pm$ 4.9	24.2 $\pm$ 4.4	1.48	0.140

**Note.** Data are expressed as mean  $\pm$  standard deviation. T-tests showed no significance.



**Figure 3.** Logistic regression analysis of risk factors (smoking, hypertension, diabetes, family history, HCMV-IgM, and HCMV-IgG) in CHD and control groups.

significant differences in these levels between the myocardial infarction, angina pectoris, and control groups ( $P < 0.05$ ). Significant differences were also noted between the IgM (+) and IgM (-), IgG (+), and IgG (-) rates in the latent CHD, angina pectoris, myocardial infarction, and control groups ( $P < 0.05$ ). However, the change in trend of HCMV infection and blood AS index could not be captured because of the low number of subjects in this study.

Third, the expression of CD14<sup>+</sup>CD16<sup>+</sup> mononuclear cells in the latent CHD, angina pectoris, and myocardial infarction groups showed increasing trends; correlations with acute HCMV infection were also found. Thus, assessment of HCMV antibodies and peripheral blood CD14<sup>+</sup>CD16<sup>+</sup> monocytes may provide clues for the occurrence and development of AS. Among the cells proliferating in AS endothelial injury, one third are mononuclear macrophages, of which CD14<sup>+</sup>CD16<sup>+</sup> monocytes have a strong pro-inflammatory effect<sup>[44]</sup>. Many cells, especially monocytes, macrophages, endothelial cells, and smooth muscle cells are susceptible to HCMV infection<sup>[26]</sup>; they play an important role in the development of atherosclerotic vascular diseases. It has been found that HCMV persists in the precursors of bone marrow mononuclear cells, which act as HCMV carriers<sup>[45]</sup>. Therefore, the virus can infect mononuclear macrophages in patients with CHD and promote its development. Monocytes and macrophages play important roles in the pathogenesis of CHD<sup>[46]</sup>. Several studies have found that different subgroups of monocytes play different roles<sup>[47]</sup>. There is evidence for a change in the proportion of monocyte subsets in the initial stage<sup>[48]</sup>. A potential new target for treatment is the chemotaxis of endothelial monocytes and their adherence to HCMV-infected endothelial cells. Increased CMV infection and adhesion molecule expression, induced by endothelial cell injury, may play a positive feedback role in the regulation of CD14<sup>+</sup>CD16<sup>+</sup> monocyte expression. HCMV increases CD36 mRNA transcription resulting in high expression of CD36 receptor in CD14<sup>+</sup>CD16<sup>+</sup> monocytes, which is a main mechanism to promote AS<sup>[49]</sup>. The results of our study show that an increase in IgM positivity in the CD14<sup>+</sup>CD16<sup>+</sup> monocyte subgroup is associated with the virus. As the HCMV antibody IgM represents a recent infection, an acute HCMV infection might affect the expression of CD14<sup>+</sup>CD16<sup>+</sup> monocytes<sup>[50]</sup>. Our findings revealed that the CD14<sup>+</sup>CD16<sup>+</sup> mononuclear cells displayed features that are typical of mature and inflammatory functions, and thus the high expression of

CD14<sup>+</sup>CD16<sup>+</sup> monocytes should not be overlooked.

Monocytes in the blood are induced by chemokines to migrate to the intima of blood vessels and change into macrophages, when their phagocytic lipid results in characteristic foam cells. This process is the initiation of AS<sup>[51]</sup>. Damage to the local endothelium and infiltration of lipids induces the self-proliferation of mononuclear macrophages and increases their number in the plaque<sup>[52]</sup>, resulting in the formation of more foam cells after lipid phagocytosis<sup>[53]</sup>. So mononuclear macrophages play an important role in the pathogenesis of CHD. In addition, we also found that different subgroups of mononuclear cells play different roles. Evidence has shown that regulation of the ratios of monocyte subsets and changes in the functions of these subsets are potential new targets for AS therapy<sup>[54]</sup>.

The results of the present study showed that the number of monocytes, proportion of mononuclear cells, and expression of CD14<sup>+</sup>CD16<sup>+</sup> mononuclear cells are higher in the latent CHD, angina pectoris, and myocardial infarction groups as compared to the control group, indicating the CD14<sup>+</sup>CD16<sup>+</sup> monocyte subgroup is an independent risk factor for CHD. Huang et al.<sup>[55]</sup> examined the expression of the CD14<sup>+</sup>CD16<sup>+</sup> monocyte subset in peripheral blood from different populations and found that the levels were significantly higher in acute coronary syndrome patients than in stable angina patients and controls. Further studies have shown that the number of CD14<sup>+</sup>CD16<sup>+</sup> monocytes is negatively correlated with the level of high-density lipoprotein, and positively correlated with AS lipid changes. This suggests that there may be some correlation between the abnormal changes in CD14<sup>+</sup>CD16<sup>+</sup> monocytes and blood lipids. Schlitt et al.<sup>[56]</sup> found that the proportion of CD14<sup>+</sup>CD16<sup>+</sup> cells is higher in the blood of patients with CHD than in normal controls, and that the concentration of CD14<sup>+</sup>CD16<sup>+</sup> cells is positively correlated with the concentration of TNF- $\alpha$ . People with increased CD14<sup>+</sup>CD16<sup>+</sup> cells display a 5 times higher risk of CHD than the normal population. The local immune response in AS plaques is also a key factor in the occurrence of malignant events such as myocardial infarction, and most of the inflammatory factors are produced by mononuclear macrophages. Based on the expression of CD14 (LpS receptor) and CD16 (Fc gamma-III receptor) on the cell surface, monocytes are divided into four subgroups (CD14<sup>++</sup>CD16<sup>-</sup>, CD14<sup>++</sup>CD16<sup>+</sup>, CD14<sup>+</sup>CD16<sup>+</sup>, and CD14<sup>+</sup>CD16<sup>-</sup>); of these, different monocyte subsets have different effects on AS and play different roles in myocardial infarction. We

observed in our study that the CD14<sup>+</sup>CD16<sup>+</sup> mononuclear cells are more mature and inflammatory than the classical mononuclear cells (CD14<sup>++</sup>CD16<sup>-</sup>). Induced by lipopolysaccharide, CD14<sup>+</sup>CD16<sup>+</sup> mononuclear cells increase the synthesis and secretion of TNF- $\alpha$  and matrix metalloproteinase, enhance the expression of inflammatory chemokine receptors on the membrane surface, promote the synthesis of cytokines IFN- $\gamma$  and IL-6, and inhibit collagen fibrinolysis in smooth muscle cells. Therefore, the role of highly expressed CD14<sup>+</sup>CD16<sup>+</sup> monocyte subsets in the pathogenesis of CHD cannot be ignored. During the development of AS, monocyte receptors and ligands also play important roles. For example, CD36 mediates the phagocytosis of OX-LDL by mononuclear cells to form foam cells<sup>[57]</sup>, while CD40 and the cell surface p-Selectin glycoprotein ligand-1 form platelet-monocyte aggregates (PMAs). PMAs use intracellular signaling pathways that cause monocytes to secrete and express IL-6, chemokine-1, and extracellular matrix enzymes; the activated platelets also enhance the phagocytosis of monocytes, thus increasing the possibility of cardiovascular risk events. PMAs are significantly higher in case of acute myocardial infarction, as well as in patients with potential AS thrombus formation and at risk for hypertension and diabetes<sup>[58]</sup>. Because AS is a dynamic process of CHD, static imaging has certain limitations<sup>[59]</sup>. Hematological assessment is a simple and quick way to predict dynamic changes in AS plaques by labeling related markers<sup>[60]</sup>. Continuous assessment can make up for the limitations of imaging. Therefore, the detection of monocyte subsets has special clinical significance for the dynamic assessment and prognosis of CHD. Targeted cell therapy for AS is a promising and valuable clinical strategy based on the specific immunoregulation of the expression of different subsets of mononuclear cells. In conclusion, it is likely that HCMV infection and monocyte subsets play important roles in the development of AS.

In summary, HCMV infection stimulates/inhibits lipid metabolism and increases activity of the immune and inflammatory systems. HCMV infection is correlated with the occurrence and development of CHD. Since the expression of peripheral CD14<sup>+</sup>CD16<sup>+</sup> mononuclear cells in the CHD group was increased and correlated with acute HCMV infection, both HCMV antibody and CD14<sup>+</sup>CD16<sup>+</sup> mononuclear cells can be used to monitor the occurrence and development of CHD. Thus, HCMV antibody and peripheral blood CD14<sup>+</sup>CD16<sup>+</sup> mononuclear cells can

be used to monitor the occurrence and development of CHD. However, it has to be noted that certain questions still remain and need to be explored. For example, if HCMV promotes coronary AS development, what is the breakthrough? What specific roles do the monocyte subsets play in the development of AS? Are there differences in the distribution of HCMV and monocytes in different parts of AS plaques? Are there different distributions at different stages of AS? Can the development of AS plaques be reduced by preventing HCMV infection or altering the function of specific types of monocytes? What is the relationship between HCMV infection and the expression of monocyte subsets? With the development of research and the emergence of new technologies, we will have a clearer understanding of HCMV infection and the types of monocytes, thus facilitating the development of a better strategy for the prevention and treatment of atherosclerotic diseases.

#### AUTHOR CONTRIBUTIONS STATEMENT

HL and QW performed the whole experiments, YZ responsible for the figures. JW, HW, MZ, and JL organized the content of the entire manuscript and wrote the whole sections. HL, QW and ZL contributed to the design of the work. All authors read and approved the final manuscript.

#### CONFLICT OF INTEREST STATEMENT

The authors report that they have no competing interests.

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