

## RESEARCH ARTICLE

# The Increase in CD14<sup>+</sup>CD16<sup>+</sup> Monocytes is Correlated with Cardiovascular Disease Risk Marker in Type 2 Diabetes

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## Abstract

**BACKGROUND:** Type 2 Diabetes (T2D) impairs the innate immune system including monocytes. Monocytes are divided into two subgroups depending on the expression of cluster of differentiation (CD)14 and CD16 receptors, namely CD14<sup>+</sup>CD16<sup>-</sup> and CD14<sup>+</sup>CD16<sup>+</sup>. CD14<sup>+</sup>CD16<sup>+</sup> are proinflammatory monocytes and develop into M1 type macrophages, which contribute to foam cell production, a risk factor for cardiovascular disease (CVD). Therefore, it is important to determine the influence of T2D conditions on changes in monocyte subsets and whether these changes correlate with CVD risk markers.

**METHODS:** Peripheral blood mononuclear cell (PBMC) was obtained from 10 T2D subjects and 10 healthy donors. Subsequently, PBMC was incubated for 24 hours with and without 10 µL lipopolysaccharide. Flow cytometry was used to evaluate CD14 and CD16 expression, while multiplex

immunoassays were applied to measure interleukin (IL)-1β and IL-10 concentrations in supernatants.

**RESULTS:** In T2D, the percentage of CD14<sup>+</sup>CD16<sup>+</sup> monocytes increased ( $p=0.07$ ), and an increase in CD14<sup>+</sup>CD16<sup>+</sup> monocytes more than 6.8% was linked with CVD risk markers ( $r=10.146$ ,  $p=0.002$ ). Meanwhile, inflammatory mediators released by monocytes shown an increase in IL-1β ( $p=0.041$ ) but not in IL-10 ( $p=0.082$ ) in T2D subjects. Fasting blood glucose levels were also found to be substantially linked with an increase in CD14<sup>+</sup>CD16<sup>+</sup> monocytes ( $r=0.530$ ,  $p=0.016$ ).

**CONCLUSION:** T2D patients had a higher percentage of CD14<sup>+</sup>CD16<sup>+</sup> monocytes and IL-1β levels than healthy donors. An increase in CD14<sup>+</sup>CD16<sup>+</sup> monocytes above 6.8% associated with CVD risk markers in T2D patients.

**KEYWORDS:** type 2 diabetes, monocytes, CD14, CD16, cardiovascular disease risk marker

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## Introduction

Type 2 diabetes (T2D) is a chronic inflammatory disease characterized by glucose metabolism abnormalities resulting from impaired insulin secretion and resistance.(1) Diabetes is projected to affect 422 million adults globally by 2020, leading to 1.5 million deaths from complications,

and will keep increasing.(2,3) Insulin resistance (IR) has long been considered an important factor in the etiology and pathogenesis of T2D, especially metabolic syndrome leading to atherosclerosis and cardiovascular disease (CVD).(4-6)

Recent research suggested that innate immune cells, T cells, macrophages, and natural killer cells play a role in the pathogenesis of T2D and the complications, particularly

CVD.(7,8) Monocytes, as one of the immune cells included in the inflammatory response, have an uncertain relationship with diabetes environment. Furthermore, monocytes can be divided into two subgroups based on the expression of cluster of differentiation (CD)14 and CD16 receptors. These include classical (CD14<sup>+</sup> CD16<sup>-</sup>) accounting for 90% of monocytes circulation in the blood, while non-classical (CD14<sup>+</sup>CD16<sup>+</sup>) constitutes 10%.(9) Although the composition of non-classical monocytes subset is 10%, the increase is relatively related to the occurrence of infection and inflammation.(10) According to a study, monocyte differentiation is not transcriptionally imprinted, but can be influenced by external cues such as cytokines, retinoic acid, and pathogenderived products.(11,12)

Monocytes can develop into macrophages in both acute and chronic inflammatory conditions through the Monocyte-derived macrophages (mo-Mac) pathway.(13) As one of the innate cells, macrophages are recognized to have a significant role in the production of macrophage foam cells, which are a risk factor for CVD.(14) T2D, which is regarded as an inflammatory disorder (increased pro-inflammatory cytokines and decreased anti-inflammatory cytokines), may result in increased inflammatory monocyte (CD14<sup>+</sup>CD16<sup>+</sup>) differentiation. The transformation of CD14<sup>+</sup>CD16<sup>+</sup> monocytes into macrophages results in the development of type M1 macrophages, which play a role in the formation of macrophage foam cells.(15,16) However, the influence of diabetic conditions on monocyte differentiation and its association with CVD risk markers has not been investigated. Therefore, it is necessary to conduct research to explore the influence of the T2D environment on changes in monocyte subsets and whether these changes are associated with CVD risk markers. In this study, we also measured interleukin (IL)-1 $\beta$  and IL-10 levels to characterize monocyte-mediated pro/anti-inflammatory responses.

## Methods

### Study Subjects and Study Design

The study protocol was authorized by the Independent Ethics Committee (IEC) of the Faculty of Medicine, University of Indonesia-Cipto Mangkunkusomo Hospital (No. KET-459/UN2.F1/ETIK/PPM.00.02/2022). Before being included, all participants consisting of Outpatient Clinic patients from the Ministry of Energy and Mineral Resources provided written informed permission. Healthy donors over the age of 18 with fasting blood glucose (FBG) of 100 mg/dL, body mass index of 25 kg/m<sup>2</sup>, and glycated hemoglobin (HbA1c)

of 5.7%, were included. Doctors identified T2D patients with FBG>126 mg/dL, body mass index (BMI)>25 kg/m<sup>2</sup>, and HbA1c>6.4% using American Diabetes Association guidelines. However, pregnant women, cancer patients, people on steroid immunosuppressants at the time of the sample, and people with blood clotting issues were excluded.

### Metabolic Marker Analysis

Blood sample as much as 3 mL was withdrawn from subjects using EDTA tubes for the measurement of FBG, low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, and triglyceride. These markers were tested after a 12 hours fast using an enzymatic method (Architect C8000, Abbott, Chicago, IL, USA). The samples were also used for sensitivity C-reactive protein (hsCRP) assessment using the enzyme immunoassay method (Architect C8000, Abbott). Meanwhile, serum isolated from venous blood in 3 mL serum vacutainer tubes was used to determine the hemoglobin A1c (HbA1c) levels using the high pressure liquid chromatography method (Variant II Turbo, Bio-Rad Laboratories, Hercules, CA, USA).

### Peripheral Blood Mononuclear Cell (PBMC) Culture

Whole blood was drawn from 10 T2D subjects and 10 healthy donors, followed by cells culturing for 24 hours to obtain PBMC. In both subject groups, the treatment consisted of lipopolysaccharide (LPS) stimulation and no LPS stimulation. In brief, PBMC were isolated from heparinized blood in PBS using Ficoll-Paque Plus (GE Healthcare Life Science, Chicago, IL, USA) at a 1:1 v/v ratio. The sample was then centrifuged at 400 g for 20 minutes at room temperature without stopping to produce three layers. The buffy coat-containing middle layer was pipetted and transferred to a fresh conical tube. PBS was used to wash the cell suspense twice. PBMC were cultivated at  $2 \times 10^6$  cells/mL in a 24 well plate using complete medium Roswell Park Memorial Institute (RPMI)-1640 (Gibco, Oxford, UK), 1% penicillin-streptomycin (Gibco), 1% Amphotericin B (Gibco), and 10% fetal bovine serum (FBS) (Gibco) overnight at 37°C in 5% CO<sub>2</sub>. At harvest, the supernatant was removed for IL-1 $\beta$  and IL-10 analysis, while the pellet containing PBMC was eradicated with triple select for flow cytometer analysis.

### Monocytes Subset Analysis

Flow cytometry was used to analyze monocytes subset using FITC Mouse Anti-Human CD14 (Cat No. 555573, BD Biosciences, Franklin Lakes, NJ, USA) and FITC Mouse

Anti-Human CD16 (Cat No. 555406, BD Biosciences). Monocytes subset was identified through gating on a flow cytometer (BD FACSCanto II, BD Biosciences). The process entailed the initial separation of cells with debris, followed by segregating cells containing CD14<sup>+</sup>. Finally, cells were grouped into CD14<sup>+</sup>CD16<sup>-</sup> (classical monocytes) and CD14<sup>+</sup>CD16<sup>+</sup> (non-classical monocytes).

### Cytokine IL-1 $\beta$ and IL-10 Analysis

The supernatant from PBMC culture was analyzed with Mouse Anti-Human IL-1 $\beta$  and IL-10 antibodies using bead-based multiplex assay (R&D Systems, Minneapolis, MN, USA). Briefly, the analysis process was as follows: 20  $\mu$ L supernatant and standard samples were mixed individually with previous beads, placed in a well plate, and shaken for 2 hours. At each stage, the plate was washed three times with 200  $\mu$ L washing buffer. Approximately 20  $\mu$ L IL-1 $\beta$  and IL-10 detection antibodies were added to each well and incubated for 1 hour on a plate shaker. Subsequently, 20  $\mu$ L streptavidin-phycoerythrin was added and incubated on a plate shaker for 30 minutes. In the final stage, the plate was rinsed three times, 150  $\mu$ L Sheat Fluid Plus was added, and the Luminex 200 was used to read the plates.

## Results

### Characteristics of Subjects

A total of 20 respondents with complete data and meeting the inclusion criteria were collected, consisting of 10 T2D subjects and 10 healthy donors, with a gender ratio of men (50%) and women (50%). The average age of T2D subjects and healthy donors was 49.00 $\pm$ 6.85 years and 47.50 $\pm$ 4.8 years, respectively. Table 1 provided an overview of the data collected from both group of subjects, including FBG, HbA1c, LDL cholesterol, HDL cholesterol, triglyceride, and hsCRP of the healthy donor and T2D subjects.

**Table 1. Characteristics of subjects.**

Parameter	Healthy Donor (n=10)	T2DM Subjects (n=10)
Age (Years)	49.00 $\pm$ 6.85	47.50 $\pm$ 4.88
FBG (mg/dL)	88.30 $\pm$ 7.57	172.80 $\pm$ 75.74
HbA1c (%)	5.40 $\pm$ 0.21	8.65 $\pm$ 1.85
LDL (mg/dL)	148.60 $\pm$ 43.32	154.60 $\pm$ 32.65
HDL (mg/dL)	55.70 $\pm$ 15.55	46.30 $\pm$ 8.72
Triglycerides (mg/dL)	98.50 $\pm$ 43.78	234.90 $\pm$ 165.53
hsCRP (mg/L)	1.73 $\pm$ 2.44	5.32 $\pm$ 5.06

### PBMC Culture with LPS Stimulation and Non-LPS Stimulation in T2D and Non-T2D Subjects

The monocytes analysis was performed using LPS-stimulated and non-LPS-stimulated PBMC cultures in T2D subjects and healthy donors. The microscopic result fo PBMC culture after 24 hours of incubation were shown in Figure 1.

Monocytes of 14-20  $\mu$ m in size were observed using a 400x magnification microscope. Based on the results, there were no morphological differences between LPS and non-LPS stimulation in the two groups of subjects. The main variation was observed in LPS stimulation, where the cells appeared more clustered, as shown by red circles.

### Characteristics of Monocyte Cell Subsets with Flowcytometer Using CD14 and CD16 Markers

An overview of the flow cytometer showed differences in the characteristics of monocytes subset in T2D and non-T2D subjects, with T2D having a higher proportion of CD14<sup>+</sup>CD16<sup>+</sup> subset. Under LPS stimulation, monocytes subset polarizes towards CD14<sup>+</sup>CD16<sup>-</sup> subset, causing a decrease in the percentage of CD14<sup>+</sup>CD16<sup>+</sup> monocytes and increasing CD14<sup>+</sup>CD16<sup>-</sup> (Figure 2).

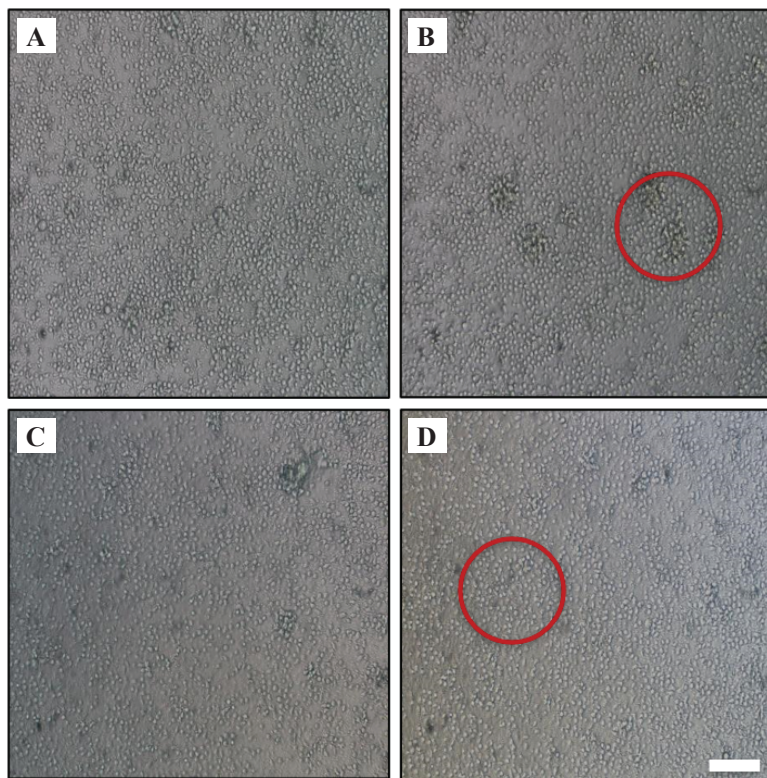
### Differences in the Proportion of CD14<sup>+</sup> and CD16<sup>+</sup> Monocytes in T2D Subjects and Healthy Donors

This test was carried out by categorizing the percentage of CD14<sup>+</sup> and CD16<sup>+</sup> monocytes as high (>6.8%) or low (<6.8%). The cut-off value of 6.8% corresponded to receiver operating characteristic (ROC) curve value with 90% sensitivity and 50% specificity. The percentage of high-category CD14<sup>+</sup>CD16<sup>+</sup> monocytes in T2D subjects was higher (90%) compared to the healthy donors (50%) ( $p=0.07$ ) (Figure 3). A cut-off value of 6.8% was used to examine the data distribution of CD14<sup>+</sup>CD16<sup>+</sup> monocytes in T2D, showing a median of 12.5%, meanwhile in healthy donor the median was 8.45%.

### The Proportion of IL-1 $\beta$ and IL-10 Cytokine Expression Distinctions between T2D Subjects and Healthy Donors

In T2D subjects and healthy donors, the ratio of IL-1 $\beta$  with and without LPS stimulation was significantly different ( $p=0.041$ ). Compared to healthy donors, the majority of T2D subjects had IL-1 $\beta$  levels higher than the median. There was no relationship between diabetic status and the ratio of IL-10 ( $p=0.082$ ). Although it was not statistically significant, there was a pattern of increased IL-10 with LPS stimulation in T2D, but the pattern of increase that occurred could not compensate for the increase in IL-1 $\beta$ .





**Figure 1. Microscopic image of PBMC culture results after 24 hours of incubation.** A: healthy donor without LPS stimulation; B: healthy donor with LPS stimulation; C: T2D patient without LPS stimulation; D: T2D patient with LPS stimulation. White bar: 150  $\mu$ m. Red circles: clustered cells.

### Increased CD14<sup>+</sup>CD16<sup>+</sup> Monocytes and the Risk of CVD in T2D Subjects

Table 2 showed a strong correlation between a high risk of CVD and an elevated percentage of CD14<sup>+</sup>CD16<sup>+</sup> monocytes without LPS stimulation ( $r=10.146$ ,  $p=0.002$ ).

The result showed that subjects with a higher percentage of CD14<sup>+</sup>CD16<sup>+</sup> monocytes (>6.8%) were 9.1 times more prone to develop CVD than those at <6.8%. The cut-off value of 6.8% corresponded to ROC curve value with 90% sensitivity and 50% specificity. Scatterplot was also used to determine the pattern of data distribution between DM status, hsCRP, and CD14<sup>+</sup>CD16<sup>+</sup> monocytes (Figure 4). An increasing pattern of hsCRP and CD14<sup>+</sup>CD16<sup>+</sup> monocytes in T2D subjects was also seen in Figure 4. The results showed that 7 out of 10 subjects had hsCRP values above 3 mg/L and CD14<sup>+</sup>CD16<sup>+</sup> levels were more than 6.8%.

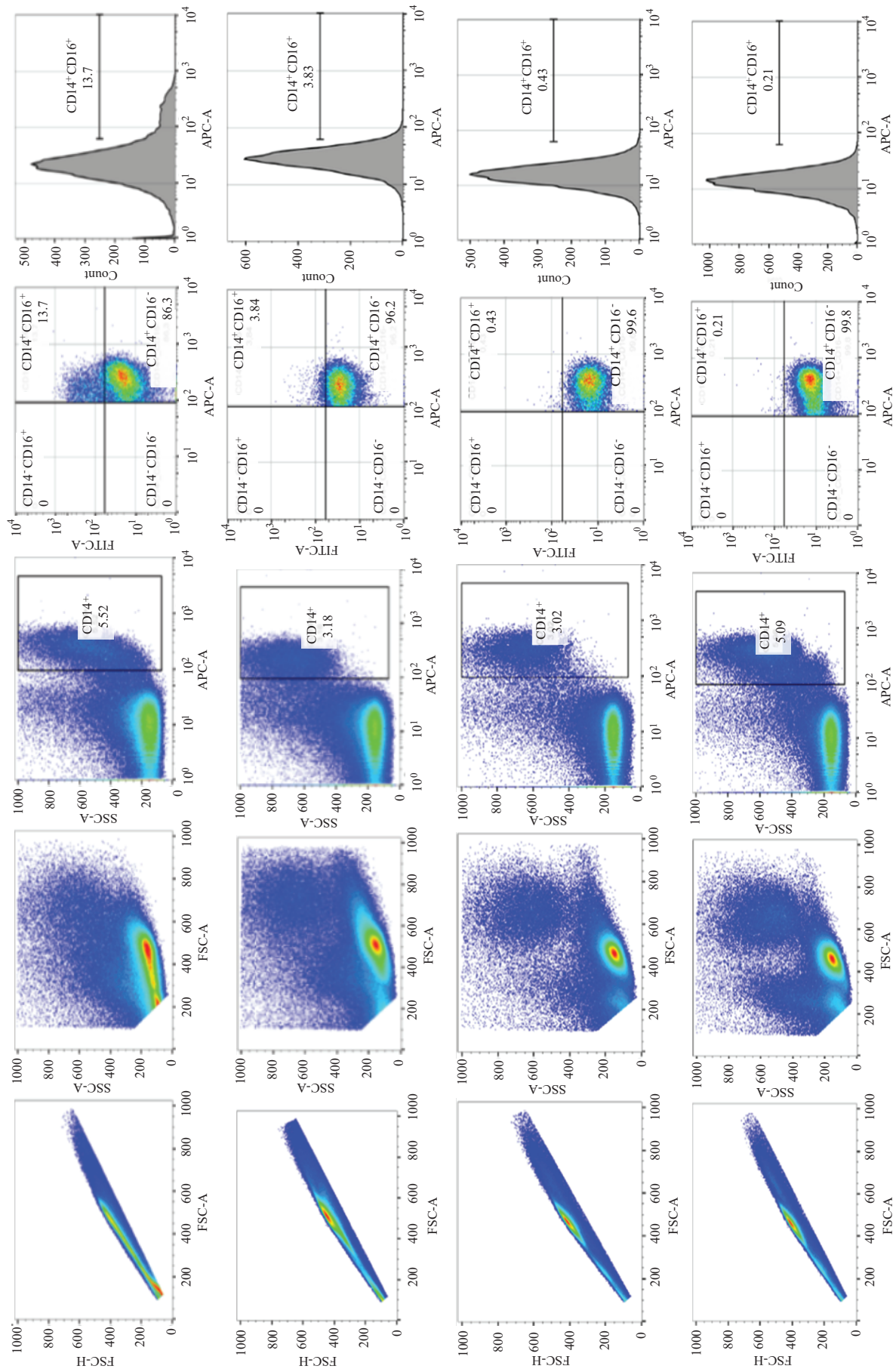
### Correlation Test of CD14<sup>+</sup>CD16<sup>+</sup> Monocytes with Metabolic Markers

Table 3 showed the relationship between the percentage of non-classical monocytes (CD14<sup>+</sup>CD16<sup>+</sup>) without LPS stimulation and age, fasting blood glucose, HbA1C, LDL cholesterol, HDL cholesterol, and triglycerides. Age and BMI had no significant relationship with CD14<sup>+</sup>CD16<sup>+</sup> monocytes, with values of ( $r=0.199$ ,  $p=0.401$ ) and ( $r=0.076$ ,  $p=0.750$ ), respectively. Meanwhile, a strong positive

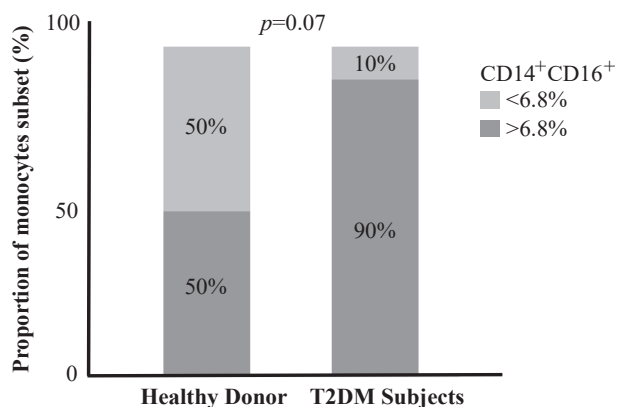
relationship was found between fasting blood glucose and CD14<sup>+</sup>CD16<sup>+</sup> monocytes ( $r=0.530$ ,  $p=0.016$ ).

The results showed no significant relationship between non-classical monocytes and HbA1C ( $r=0.340$ ,  $p=0.143$ ), LDL ( $r=-0.028$ ,  $p=0.907$ ), HDL ( $r=0.198$ ,  $p=0.403$ ), as well as triglycerides ( $r=0.052$ ,  $p=0.828$ ). Figure 5 represented the distribution pattern of CD14<sup>+</sup>CD16<sup>+</sup> monocytes in the high (>6.8%) and low (>6.8%) based on HbA1c and fasting blood glucose levels. Blood glucose value above 100 mg/dL showed a category of non-classical monocyte percentage (CD14<sup>+</sup>CD16<sup>+</sup>) at a high level (> 6.8%). Meanwhile, HbA1c values below 6% showed an even distribution between high and low categories of CD14<sup>+</sup>CD16<sup>+</sup> monocytes. An increase in the high category >6.8% was observed when HbA1C value was above 6% (Figure 5). When LDL and HDL concentrations were made into LDL/HDL ratio, there was no apparent pattern of increase in LDL/HDL ratio with high category CD14<sup>+</sup>CD16<sup>+</sup> monocytes.

The distribution pattern of CD14<sup>+</sup>CD16<sup>+</sup> monocytes was classified into the high and low categories, regarding LDL and hsCRP concentrations. The results showed an increase in CD14<sup>+</sup>CD16<sup>+</sup> monocytes with LDL values above 100 mg/dL and hsCRP concentrations surpassing 3 mg/L. Furthermore, CD14<sup>+</sup>CD16<sup>+</sup> monocytes were found in high concentrations in T2D subjects with elevated LDL, hsCRP, and IL-1 $\beta$  levels (Figure 6).



**Figure 2. Characteristics of monocyte cells by flow cytometer analysis in T2D subjects and healthy donor with and without LPS stimulation.** A: Characteristics of monocytes of monocytes cells without LPS stimulation in healthy donor; B: Characteristics of monocytes cells with LPS stimulation in T2D subject; C: Characteristics of monocytes cells with LPS stimulation in healthy donor; D: Characteristics of monocytes cells with LPS stimulation in healthy donor.



**Figure 3. The proportion of CD14<sup>+</sup>CD16<sup>+</sup> monocytes subset in T2D subjects and healthy donors.** Chi-square Fisher exact test was used to determine the difference between T2D subjects and healthy donor, significant if  $p < 0.05$ .

## Discussion

The initial report in 1970 showed that the proliferative activity of monocytes in response to inflammation was clinically characterized by a significant increase. T2D, which was defined as a chronic inflammatory condition, showed a tendency to increase the more inflammatory monocytes subset (CD14<sup>+</sup>CD16<sup>+</sup>). (1) Based on statistical analysis, the mean of CD14<sup>+</sup>CD16<sup>+</sup> monocyte was higher in T2D. The inflammatory condition in T2D changed the proportion of monocytes subset that was more inflammatory, as presented by an increase in CD14<sup>+</sup>CD16<sup>+</sup> monocytes. Several studies stated that there were differences in the proportion of monocytes subset between diabetes and non-diabetes. (17) Based on the results, the decrease in CD14<sup>+</sup>CD16<sup>+</sup> monocytes subset in T2D can be used as an indicator of improvement in inflammatory conditions. This is related to the function and mediators released by each subset, with CD14<sup>+</sup> and CD16<sup>+</sup> monocytes releasing more IL-1 $\beta$  and TNF- $\alpha$ . (7) CD14<sup>+</sup>CD16<sup>-</sup> monocytes, on the other hand, produced more of the anti-inflammatory cytokine IL-10. Furthermore, CD14<sup>+</sup> and CD16<sup>+</sup> proinflammatory features

are linked to the chemokine motif chemokine receptor 1 (CX3CR1), complement and FcR-mediated phagocytic activity, and transendothelial migration. (18)

Multiplex Immunoassays method was used to measure IL-1 $\beta$  and IL-10 in this study using PBMC culture supernatant samples. The results showed a significant correlation between an increase in monocytes and a decrease IL-10. A significant change was observed in the proportion of monocytes in T2D, showing a tendency toward a more inflammatory. Cytokines serving as inflammatory mediators play a crucial role as communicators between cells in the development of further inflammation. The results showed that there was no relationship between the expression of IL-1 $\beta$  and IL-10 between T2D patients and healthy donors. However, a significant difference in response was observed between both groups to LPS stimulation. In subjects without LPS stimulation, IL-1 $\beta$  and IL-10 were higher in non-T2D, but in conditions with LPS stimulation, IL-1 $\beta$  and IL-10 in T2D were higher. The results showed differences in the immune response to the presence of pathogens, where T2D patients were more inflammatory than healthy donors. This condition occurred due to a low-grade chronic inflammatory condition in T2D patients in response to pathogens causing several adverse clinical conditions such as the occurrence of a cytokine storm in SARS-CoV2 infection. (19)

A commonly used marker, namely hsCRP was applied in this study to measure the risk measurement of CVD. CVD risk based on hsCRP level is categorized into low (>1 mg/L), moderate (1-3 mg/L), and high (>3 mg/L). The analysis results showed that the percentage of CD14<sup>+</sup>CD16<sup>+</sup> monocytes at 6.8% increased the risk of CVD, occurring at an early stage in the development stage. Monocytes, as immune cells that play an important role in phagocytosis function, tend to increase polarization towards CD14<sup>+</sup>CD16<sup>+</sup> monocytes in T2D. An increase in the proportion of CD14<sup>+</sup>CD16<sup>+</sup> monocytes promotes the development process into M1-type macrophages. (17) Generally, M1 macrophages in the vascular endothelium have been shown to increase phagocytosis of oxidized LDL, contributing to foam cell formation and increasing the risk of plaque

**Table 2. Percentage of CD14<sup>+</sup>CD16<sup>+</sup> monocytes and CVD risk.**

CVD Risk	Non-classical Monocytes (%)		p-value
	<6.8%	>6.8%	
Low (hsCRP <1 mg/L)	5 (62.5%)	3 (37.5%)	0.006*
Average (1-3 mg/L)	2 (66.7%)	1 (33.3%)	
High (>3 mg/L)	0 (0%)	9 (100%)	

\* $p < 0.05$  is considered to be significant, Chi-square Fisher exact test.



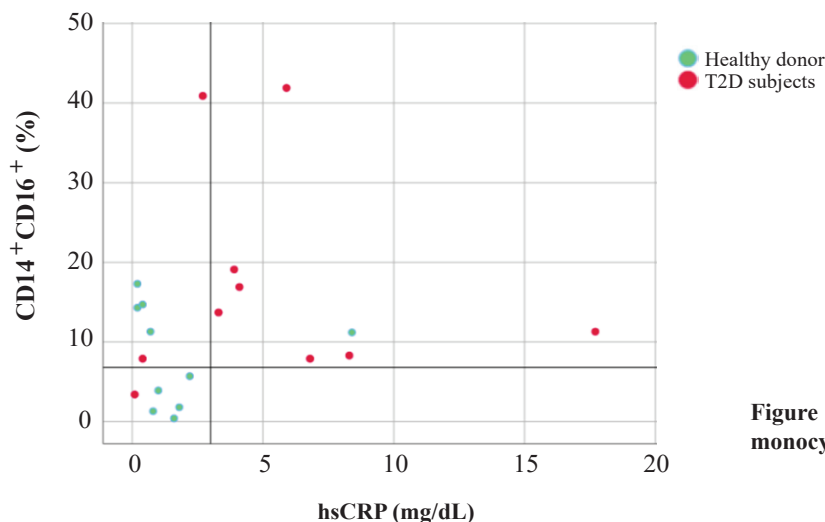


Figure 4. Scatterplot of hsCRP and CD14<sup>+</sup>CD16<sup>+</sup> monocyte based on diabetic status.

development in blood vessels as well as atherosclerosis.(20) Furthermore, the association of CD14<sup>+</sup>CD16<sup>+</sup> with T2D shows an increased risk of CVD.

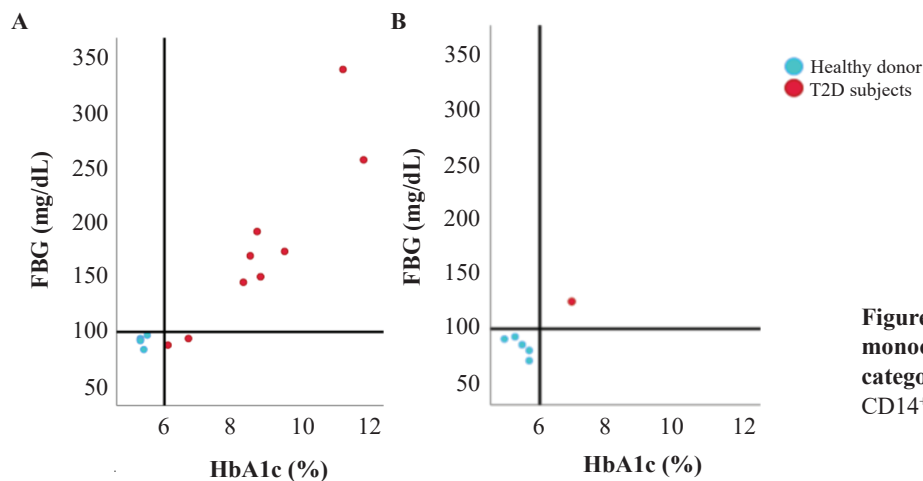
Spearman non-parametric correlation test was used to determine the relationship between CD14<sup>+</sup>CD16<sup>+</sup> monocytes and metabolic markers. The results showed a strong correlation between increased monocytes subset in T2D and fasting glucose levels, but not with HDL, LDL, triglycerides, or HbA1c. CVD risk was closely related to glucose as an indicator of clinical conditions of T2D.(21) The results showed that glucose was a metabolic indicator of an increased risk of CVD, while CD14<sup>+</sup>CD16<sup>+</sup> monocytes specified inflammatory conditions.(22)

In this study, HbA1c as an indicator of controlled and uncontrolled diabetic condition did not show a significant relationship with an increase in CD14<sup>+</sup>CD16<sup>+</sup> monocytes. Despite the absence of statistical significance, the pattern of the relationship formed suggested increasing HbA1c resulted in elevated CD14<sup>+</sup>CD16<sup>+</sup> monocytes.(23) Therefore, this observation confirmed that uncontrolled T2D altered the configuration of CD14<sup>+</sup>CD16<sup>+</sup> monocytes, increasing CVD risk. To further explore the current results, a potential strategy includes expanding the number of volunteers to determine a more comprehensive pattern might be necessary. Additionally, there is also a need for extensive exploration of CD14 and CD16 monocytes

Table 3. Correlation between monocytes subset and metabolic parameters.

Parameter		CD14 <sup>+</sup> CD16 <sup>+</sup>		CVD Risk (hsCRP)
		Without LPS	With LPS	
Age	r	0.199	-0.043	-0.213
	p	0.401	0.857	0.366
FBG	r	0.530*	0.425	0.593**
	p	0.016	0.062	0.006
HbA1c	r	0.340	0.199	0.560
	p	0.143	0.401	0.010
LDL	r	-0.028	-0.148	0.151
	p	0.907	0.534	0.526
HDL	r	0.198	0.174	-0.354
	p	0.403	0.463	0.126
TG	r	0.052	-0.119	0.124
	p	0.828	0.617	0.602
BMI	r	0.076	-0.022	0.022
	P	0.750	0.928	0.928

Correlation between parameters was tested with Spearman correlation test, \*\* $p < 0.01$ .



**Figure 5. Distribution of CD14<sup>+</sup>CD16<sup>+</sup> monocytes in high (>6.8%) and low (<6.8%) categories based on HbA1c and FBG. A: CD14<sup>+</sup> CD16<sup>+</sup>>6.8%; B: CD14<sup>+</sup> CD16<sup>+</sup><6.8%.**

including the association with CVD risk in non-diabetic subjects, T2D subjects without and with obesity, as well as obese subjects. Further investigation is also recommended to determine the impact of glucagon-like peptide-1 receptor agonists (GLP-1RA) and sodium-glucose cotransporter-2 (SGLT-2) on CD14 and CD16 monocytes, as well as the relevance to CVD risk.

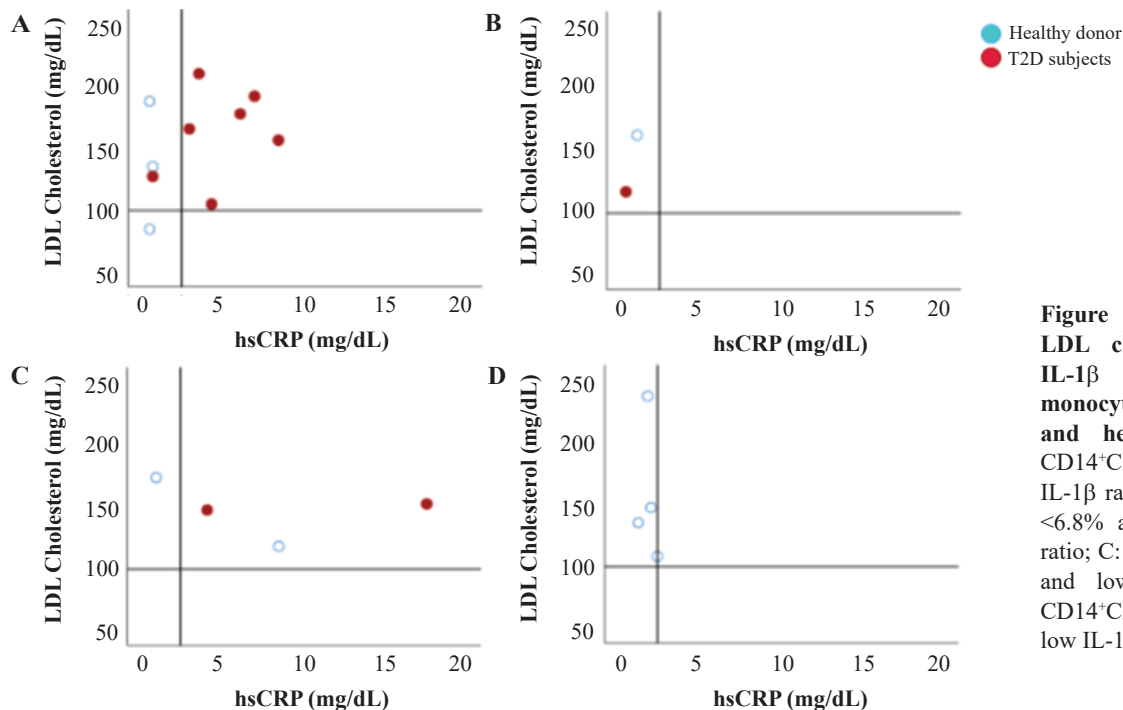
in an increase in the CD14<sup>+</sup>CD16<sup>+</sup> monocyte subset as well as an increase in the production of the proinflammatory mediator IL-1 $\beta$ . The CD14<sup>+</sup>CD16<sup>+</sup> monocyte subset can be utilized as an alternate marker for hsCRP in evaluating CVD risk, with cut off value >6.8% (sensitivity: 90%; specificity: 50%). Furthermore, hyperglycemia in T2D was reported to lead to an increase in CD14<sup>+</sup>CD16<sup>+</sup> monocytes.

### Conclusion

In conclusion, this study showed inflammatory conditions in T2D affect the configuration of monocyte subsets, resulting

### Acknowledgments

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**Figure 6. Scatterplot of LDL cholesterol, hsCRP, IL-1 $\beta$  and CD14<sup>+</sup>CD16<sup>+</sup> monocytes in T2D subjects and healthy donors. A: CD14<sup>+</sup>CD16<sup>+</sup>>6.8% and high IL-1 $\beta$  ratio; B: CD14<sup>+</sup>CD16<sup>+</sup><6.8% and high IL-1 $\beta$  ratio; C: CD14<sup>+</sup>CD16<sup>+</sup>>6.8% and low IL-1 $\beta$  ratio; D: CD14<sup>+</sup>CD16<sup>+</sup><6.8% and low IL-1 $\beta$  ratio.**



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### Authors Contribution

USH was involved in the concepting of the study and carried out the experiments. HW and ARP validated the design and analysis techniques, and were accountable as supervisors to ensure the work was done with integrity and accuracy. IRS and DLT gave critical reviews and suggestions for the improvement of the study. All authors have considered the results of the study, reviewed and approved the manuscript draft as well as the final publication.

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