Overall Reduced Baseline Lymphocyte Subsets Closely Related to the Poor Prognosis and the Disease Severity in Patients With COVID-19 and Diabetes Mellitus

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Research

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Abstract

Introduction

Dysregulated host immune response was common in COVID-19. In this study we aimed at the characteristics of lymphocyte subsets and its relationship with the disease progression in COVID-19 patients with or without DM.

Methods

The baseline peripheral lymphocyte and subsets were compared between 55 healthy cases (control group) and 95 confirmed cases with COVID-19 (COVID-19 group), and between COVID-19 patients with and without DM.

Results

The prevalence of DM in COVID-19 group was 20%, and severe cases had higher the prevalence of DM than non-severe cases (P=0.006), moreover significantly poor prognosis and higher severe rate were found in those with DM relative to those without DM (P=0.001,0.003, respectively). In COVID-19 group overall and significant reduced lymphocyte and subsets, especially B and T lymphocytes were found, especially in those with DM. Partial decreased lymphocyte subsets, age and DM closely related with the disease progression and the prognosis.

Conclusions

These findings provide a reference for clinicians that immunomodulatory treatment maybe improve disease progression and prognosis of COVID-19 patients, especially those with DM.

Introduction

Though as a world pandemic [1-6] a good prognosis was found in most coronavirus disease 2019 (COVID-19) patients, but a few patients with rapidly disease progression had the poor prognosis, [7-11] the mortality rate in China was about 3.5~5.56%. [6,12] diabetes mellitus (DM) as a comorbidity could promote disease progression and worsen prognosis. [8-9]

Host immune dysregulated response especially reduced cellular immune played an important role in the pathophysiology of disease of COVID-19[8,13-14] SARS, [15]MERS-COV [16].DM could lead to dysfunctional cellular immune.[17]

But the effects of coexist of COVID-19 and DM on lymphocyte and subsets, and the characteristics of lymphocyte subsets and its relationship with the disease progression in COVID-19 patients with or without DM are unknown and worth study.

Methods

Objects

A cross-sectional study with a sample size of 150 patients was conducted in the Public and Health Clinic Centre of Chengdu from January 16, 2020 to March 16, 2020. Among them, 95 patients with COVID-19 from the hospital isolation ward were assigned to the COVID-19 group (the source of the cases has been previously explained in the literature),[14] and 55 healthy people from medical examination clinic were assigned to the control group.The

Ethics Committee of the Public and Health Clinic Centre of Chengdu approved the study and the ethical approval number was PJ-K2020-26-01. Written informed consent was waived by the Ethics Commission of the designated hospital because of emerging infectious diseases.

The clinical typing, disease diagnosis and cured criteria of COVID-19 were applied according to the seventh Trial Version of the Novel Coronavirus Pneumonia Diagnosis and Treatment Guidance. [7]

The prognosis of COVID-19 inclued cured, unhealed and death.[7]

The discharge criteria were as follows: normal body temperature over three days; obvious improvement of respiratory symptoms; obviously improved lung imaging; negative nucleic acid in two consecutive respiratory specimens at least 24hours interval.[7]

The viral negative conversion time was the time from onset to the first negative nucleic acid of meeting discharge criteria. [7]

The DM diagnosis criteria were applied accorded to the corresponding guidelines. [18]

The participants in the COVID-19 group were divided into the non-DM subgroup (patients without DM), the DM subgroup (patients with DM), respectively.

The participants in each subgroup were also divided into the non-severe subgroup (the clinical type belonged to light and common type) and the severe subgroup (the clinical type belonged to severe and critically illness type).

Collection of data

Demographic data, clinical data, lymphocyte and subsets of all 150 cases were collected, then established databases. Researcher strictly control the accuracy, authenticity and completeness of data.

Statistical analyses

Statistical analysis was done through GraphPad Prism 8(GraphPad, CA, the USA). x±SD was expression for the measurement data, and ANOVA was used for a multi-group comparison with variance homogeneity and normal distribution data, and least significant difference (LSD) *t* test was used for further comparison between the two groups. While without variance homogeneity and normal distribution data independent sample Kruskal-Wallis H(K) test was used for a multi-group comparison, and Mann-Whitney U test was used for further comparison between the two groups. An independent-sample *t*-test were compared between two groups. Percentage or proportion was expression for enumeration data, and Chi-square test was used for comparison of this data. Spearman correlation analysis was used for two-factor correlation analysis, multiple stepwise regression was used for multi-factor correlation analysis. Statistically significant defined as $P<0.05$.

Results

Baseline conditions

Patients in the COVID-19 group were significantly younger than those in the control group, and those in the non-DM subgroup were younger than those in the DM subgroup ($P=0.0097$, 0.0022, respectively) (Table 1). But there was no

significant difference of age between the control group and the COVID-19 DM subgroup, of gender between the control group and the COVID-19 group, between the non-DM subgroup and the DM subgroup (all P>0.05) (Table 1).

Patients in the non-severe non-DM subgroup was significantly younger than the other three subgroups (P=0.000), and similar age was found in the other three subgroups (P>0.05) (Table 2). There was no significant difference of gender between four subgroups (P>0.05) (Table 2).

The non-DM subgroup had lower HbA1c level and FPG level than the DM subgroup (all P=0.000) (Table 3), moreover the non-severe non-DM subgroup had lower FPG level than the severe non-DM subgroup (P=0.0336), while similar HbA1c level were found between severe subgroup and non-severe subgroup whether with or without DM (all P>0.05), and similar FPG level were found between severe DM subgroup and non-severe DM subgroup (P>0.05) (Table 3).

The prevalence of DM and the severe rate in COVID-19 patients

The prevalence of DM in the COVID-19 group was 20% (19/95), and those severe patients had higher the prevalence of DM than those non-severe patients (P=0.006), moreover the severe rate in the non-DM subgroup was lower than that in the DM subgroup (P=0.003), there were all significant differences.

The characteristics of baseline lymphocyte and subsets in COVID-19 patients

In the COVID-19 group at baseline lymphocyte count level and percentage value (Figure 1A, 1B), CD3+ count level (Figure 2A),CD3+CD4+ count level and percentage value (Figure 2C, 2D),CD3+CD8+ count level (Figure 2E), B(CD19+) count level and percentage value (Figure 3A, 3B), and NK (CD56+) count level and percentage value (Figure 4A, 4B) were all lower than that in control group (all P<0.05). Moreover in the COVID-19 DM subgroup lymphocyte count level and percentage value (Figure 1A, 1B), CD3+ count level (Figure 2A), CD3+CD4+ count level (Figure 2C), CD3+CD8+ count level (Figure 2E), B(CD19+) count level and percentage value (Figure 3A, 3B) were lower, but NK (CD56+) count level and percentage value (Figure 4A, 4B) were higher than that in the COVID-19 non-DM subgroup, especially lymphocyte percentage value, B(CD19+) count level and percentage value, NK (CD56+)count level and percentage value (all P>0.05).

The disease progression and prognosis of COVID-19 patients

In DM group the prognosis was worse and the severe rate was higher (P=0.001, 0.003, respectively), moreover the non-severe DM subgroup and the severe DM subgroup all had longer the virus negative conversion time than those two non-DM subgroups, and the longest in-hospital time was found in the severe DM subgroup (P=0.000, 0.009, respectively) (Table 4).

The relationship of lymphocyte subsets and DM with the disease progression and prognosis in COVID-19 patients

Through spearman correlation analysis, age and DM were positively correlated, while lymphocyte count level and percentage value, CD3+ count level and percentage value, CD3+CD4+ count level and percentage value, B(CD19+) count level and CD3+CD8+ count level were all negatively correlated with the disease severity (Table 5); simultaneously the factors positively correlated with the viral negative conversion time included age and DM (Table 5); furthermore the disease severity, the coronavirus negative conversion time, DM and age were positively correlated, but lymphocyte percentage value was negatively correlated with the prognosis (Table 5).

By multiple step wise regression analysis for the disease severity the indicating factors included CD3+CD4+ percentage value, lymphocyte percentage value, age and DM (Table 6); moreover for the virus negative conversion time those contained B(CD19+) percentage value and lymphocyte percentage value (Table 6); furthermore for the prognosis those consisted of the coronavirus negative conversion time, the disease severity and age (Table 6).

Discussion

In this study we found that the prevalence of DM in the COVID-19 group was 20% (19/95), and those severe patients had higher the prevalence of DM than those non-severe patients. As a comorbidities for COVID-19 patients, [8-9,20]the 20%(19/95) prevalence of DM in this cohort was consistent with one literature reported that 20%(8/41), [8]higher than other literature reported that 13% (13/99).[9] And the 36.67%(11/30) prevalence of DM in severe patients relative to 12.31%(8/65) in non-severe patients were not consistent with literature reported that no significant difference of DM prevalence was found between severe and non-severe patients.[13]The reasons may be that there were 8 newly diagnosed DM cases after admission in this cohort , of them 3 non-severe cases and 5 severe cases. This discovery prompt that closely monitoring plasma glucose, testing glycated hemoglobin and performing glucose tolerance test for COVID-19 patients are necessary in order to find people with diabetes in time.

Further analysis we found that the severe rate in patients with COVID-19 and DM coexisting was lower than those Only COVID-9 and no DM, moreover DM was positively correlated with the viral negative conversion time and the disease severity, simultaneously it was an essential influencing factor for the disease severity, these findings was consistent with those literature reports that in patients with acute respiratory distress syndrome (SARS) and DM in 2003, the mortality rate ,the rate of check in the intensive care unit sand mechanical ventilation were 3.0~3.3 times of those no DM,[21]the intensive care units rate of patients withH1N1 influenza and DM was 4.29 times of those no DM,[22]in 2014a high-risk factor of the Middle East Respiratory Syndrome coronavirus infection severe cases was also DM.[22]

We also found that overall and significantly reduced lymphocyte and subsets existed in COVID-19 patients, especially those with DM. Moreover the severe rate was the highest in those with DM, the prognosis was worst, and lymphocyte and subsets especially CD4+, CD8+ T and B cells was the lowest in those severe cases with DM, these findings was consistent with literature.[13] But in the literature there was no hierarchical analysis performed between COVID-19 patients with and without DM .[13]

Further analysis we found that factors negatively correlated the disease severity were lymphocyte and subsets, negatively correlated the prognosis was lymphocyte. The indicating factors for the disease severity were lymphocyte percentage value, CD4+ percentage value, age and DM, moreover for the prognosis included the disease severity, age and the virus negative conversion time.

In this study we also found that severe patients without DM were significantly older than non-severe patients without DM, this was consistent with that a poor prognosis was found in elderly COVID-19 patients.[8-9]Study found that between old and young mice CD4 T cell subsets were markedly different, exhausted three cell subsets, cytotoxic, and activated regulatory T cells (a Tregs) rarely appeared in young mice, but with age that gradually accumulated. Extreme anti-inflammatory and pro-inflammatory phenotypes of cytotoxic CD4 T cells and a Tregs were most unexpected.[23] It was found that the relative frequency and total number of B cells will decrease with age. Plasma blasts, memory cell types and transitional B cells decrease in the older than 70 years group.[24] Lymphocytes and their subsets (including NK (CD56 +) cells, B (CD19 +) cells and T (CD3 +) cells) were mainly

responsible for regulating host immunity. T cells played an important role in promoting or maintaining inflammation by producing inflammatory cytokines.[22,25-28]A subtype of CD4+ effector T cells was activated Th1 cells, which triggered phagocyte-dependent inflammation and cell-mediated immunity through the production of Interferon-γ (IFN-γ), interleukin 2 (IL-2) and tumor necrosis factor β (TNF-β).[22]In contrast, another subtype of CD4+ effector T cells, activated Th2 cells, modulated the antibody response by producing IL-13, IL-10, IL-9,IL-6, IL-5 and IL-4.[25]Viral infection played a major role in disease progression by inducing indirect host immune response and direct cytopathic effects.[13,26]A fast and well-coordinated innate host immune response was the first line of defense against viral infections, but a dysregulated immune response could lead to excessive inflammation and even death.[13]

Non-severe non-DM patients were younger than severe non-DM ones, and age was positively correlated with the prognosis, the viral negative conversion time and the disease severity, moreover age was an essential indicating factor for the prognosis and the disease severity, which was consistent with that the elderly had a poor prognosis.[8-9]However similar age was found between non-severe DM and severe DM patients, that is to say, age was not a factor in the disease severity for DM patients, which was inconsistent with those reports.[8-9]Type 2 diabetes mellitus (T2DM) is a systemic chronic low-grade inflammatory disease. The function of specific T lymphocyte subsets (including T regulatory (Treg) cells) has changed, leading to the following hypothesis: partial inflammation exacerbate T2D autoimmunity.[29]T cells play an important role in promoting or maintaining insulin resistance and inflammation by inducing the production of pro-inflammatory cytokines in metabolic organs (such as pancreas, muscle, adipose tissue and liver).[28,30-32] In adipose tissue the major inflammatory cells was macrophages.[28,30-32]Proinflammatory M1 macrophages releasing proinflammatory cytokines such as IL-6, TNF-a and IL-1contribute to the local and systemic inflammation.[32]On the contrary, anti-inflammatory M2 macrophages secreting IL-10inhibit the activity of most pro-inflammatory cell types including M1 macrophages.[32]IL-10 by interacting with the p38/MAPK pathway suppress TNF-a.[32]Th1 cells producing TNF-a,IL-2,IFN-γpromote M1 polarization and enhance its pro-inflammatory functions. In contrast, Th2 cells skew the differentiation of macrophage towards M2 by producing anti-inflammatory IL-4, and IL-13. [28,30-32] Therefore, Th1 and Th2 responses, which are closely related to M1/M2 polarization, may also have a critical role in T2DM. [28,30-32]

Previous research found that in COVID-19 patient higher expression of proinflammatory cytokines and chemokines, especially in the severe cases, the consumption of CD4+ and CD8+ T cells, and the decrease of regulatory T cells, might result in aggravated inflammatory responses, the production of cytokine storm and make damaged tissue worse. Maybe lower number of lymphocytes suggested a role for dysregulated immune responses in COVID-19 pathogenesis.[13-14]Our research discoveries suggested that the coexistence of viral infection and DM result in more dysregulated host immune responses thus worsen the already aggravated inflammatory process, more susceptible to bacterial infections, more severe organ damage and worse prognosis, simultaneously the coexistence of viral infection and DM can reduce or delay antibody production by decreasing B(CD19+) count level and percentage value, thereby delaying the removal of the virus, worsening prognosis.

In this work, we evaluated that the characteristics of lymphocyte subsets and its relationship with the disease progression in COVID-19 patients with or without DM, and found that the coronavirus disease 2019 (COVID-19) patients had overall reduced lymphocyte and subsets. Partial decreased lymphocyte subsets, age and DM as factors closely related with the disease severity, the viral negative conversion time and the prognosis. Our study demonstrated several novel information that the coexistence of viral infection and DM result in more dysregulated host immune responses thus worsen the already aggravated inflammatory process, more susceptible to bacterial infections, more severe organ damage and worse prognosis, simultaneously the coexistence of viral infection and

DM can reduce or delay antibody production, thereby delaying the removal of the virus, worsen prognosis. Combination immunomodulatory therapy based on comprehensive treatment might improve prognosis of COVID-19 patients, especially those severe cases or with DM.

Our study had several limitations. It was a retrospective, single center and small sample study. Despite that, our study demonstrated several novel information that the coexistence of viral infection and DM result in more dysregulated host immune responses thus worsen the already aggravated inflammatory process, more susceptible to bacterial infections, more severe organ damage and worse prognosis, simultaneously the coexistence of viral infection and DM can reduce or delay antibody production, thereby delaying the removal of the virus, worsen prognosis.

Conclusions

The COVID-19patients had overall reduced lymphocyte and subsets and overall decreased lymphocyte subsets and DM maybe aggravated the prognosis by aggravating the disease severity and prolonging the viral negative conversion time. Combination immunomodulatory therapy based on comprehensive treatment might improve disease progression and prognosis of COVID-19 patients, especially those severe cases or with DM.

Declarations

Ethics approval and consent to participate

The Ethics Committee of the Public and Health Clinic Centre of Chengdu approved the study and the ethical approval number was PJ-K2020-26-01. Written informed consent was waived by the Ethics Commission of the designated hospital because of emerging infectious diseases.

Consent for publication

All of participants understand that the information will be published without their child or ward's/their relative's (circle as appropriate) name attached, but that full anonymity cannot be guaranteed.All of participants understand that the text and any pictures or videos published in the article will be freely available on the internet and may be seen by the general public. The pictures, videosand text may also appear on other websites or in print, may be translated into other languages or used for commercial purposes.All of participants have been offered the opportunity to read the manuscript.

Availability of data and material

All data, models, or code generated or used during the study are available from the corresponding author by request: Dafeng Liu,E-mail:liudf312@126.com

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Concept and design: Dafeng Liu, Yong Wang, Bennan Zhao, Lijuan Lan, Yaling Liu, Lei Bao, Hong Chen, Min Yang, Qingfeng Li; Data acquisition: Dafeng Liu, Yong Wang, Bennan Zhao, Lijuan Lan, Yaling Liu, Lei Bao, Hong Chen, Min Yang, Qingfeng Li; data analysis and interpretation: Dafeng Liu, Yong Wang, Bennan Zhao, Lijuan Lan, Yaling Liu, Lei Bao, Hong Chen, Min Yang, Qingfeng Li; Drafting the manuscript: Dafeng Liu, Yong Wang, Bennan Zhao; administrative, technical, or material support: Dafeng Liu, Yong Wang, Bennan Zhao, Lijuan Lan, Yaling Liu, Lei Bao, Hong Chen, Min Yang, Qingfeng Li; study supervision: Yilan Zeng,Yalin Liu.

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Tables

Table 1. Comparison of baseline conditions between three groups（*n*=150）

variable	Control group （*n*=55）	COVID group（*n*=95）			χ^2 or *F* score	*P* score
		Total （*n*=95）	non-DM subgroup （*n*=76）	DM subgroup(*n*=19)		
age（year）	55.54±7.79	49.13±17.13**	46.75±17.16***	60.22±11.88##	*F*=6.272	0.0004
male（case） %	25(45.45)	46(48.42)	36(47.37)	10(52.63)	χ^2=0.290	0.865

Abbreviations: DM, diabetes mellitus; non-DM, without diabetes mellitus. Unpaired one ANOVA were used for intergroup comparison of age. Chi-square test was used for comparison of gender. Unpaired t-tests were used for comparison of age between control group and COVID-19 group, COVID-19 non-DM subgroup, COVID-19 DM subgroup, **P<0.001, ***P<0.001. Unpaired t-tests were used for comparison of age between the latter two subgroups, ##P<0.01.

Table 2. Comparison of baseline conditions between COVID-19 four subgroups（*n*=95）

variable	non-DM group（*n*=76）		DM group(*n*=19)		χ^2or *F* score	*P* score
	non-severe subgroup(*n*=57)	severe subgroup(*n*=19)	non-severe subgroup (*n*=8)	severe subgroup(*n*=11)		
age （year）	42.67±14.71	58.00±19.24**	61.50±11.12***###	59.36±12.31***	*F*=8.663	0.000
male （case） %	25(43.86)	11(57.89)	3(37.50)	7(63.64)	χ^2=2.532	0.469

Abbreviations: DM, diabetes mellitus; non-DM, without diabetes mellitus. Unpaired one ANOVA were used for intergroup comparison of age. Chi-square test was used for comparison of gender. Unpaired t-tests were used for comparison of age between two of four subgroups. Comparison of age between the non-severe non-DM subgroup and severe non-DM subgroup, non-severe DM subgroup, severe DM subgroup, **P<0.01, ***P<0.001; Comparison of age between the non-severe non-DM subgroup and non-severe DM subgroup,###P<0.001;Comparison of age between the severe non-DM subgroup and severe DM subgroup, P>0.05.

Table 3. Comparison of glucose metabolic parameters between COVID-19 four subgroups（*n*=95）

variable	non-DM group（*n*=76）		DM group(*n*=19)		χ^2or *F* score	*P* score
	non-severe(*n*=57)	severe (*n*=19)	non-severe (*n*=8)	severe (*n*=11)		
FPG （mmol/L）	5.38±0.69	5.81±0.91*	7.48±4.63**	7.35±1.19****####	*F*=8.621	0.000
HbA1c (%)	5.46±0.73	5.58±0.48*	6.88±2.75****####	6.89±1.12****####	*F*=33.485	0.000

Abbreviations: FPG, fasting plasma glucose; HbA1c, glycated hemoglobin A_1c; DM, diabetes mellitus; non-DM, without diabetes mellitus. Unpaired one ANOVA were used for intergroup comparison of age. Chi-square test was used for comparison of gender. Unpaired t-tests were used for comparison of age between two of four subgroups. Comparison of FPGandHbA1cbetween the non-severe non-DM subgroup and severe non-DM subgroup, non-severe DM subgroup, severe DM subgroup,*P<0.05, **P<0.01, ****P<0.0001; Comparison of FPGandHbA1c between the severe non-DM subgroup and severe DM subgroup, ####P<0.0001;Comparison of FPG and HbA1c between the non-severe DM subgroup and severe DM subgroup, P>0.05.

Table 4. Comparison of the disease severity, the virus negative conversion time and the prognosis between four subgroups（n=95）

variable	non-DM group（*n*=76）		DM group(*n*=19)		χ^2or *F* score	*P* score
	non-severe subgroup(*n*=57)	severe subgroup(*n*=19)	non-severe subgroup(*n*=8)	severe subgroup(*n*=11)		
virus negative conversion time	18.49±10.02	20.53±9.25	28.00±12.84	27.73±9.57	4.097	0.009
In-hospital time	14.25±8.72	17.79±12.33	19.38±8.12	29.27±16.59	6.704	0.000
severe（case（%）	19(25.00)		11(61.11)		-2.940	0.003
prognosis					-3.394	0.001
cured(case（%）	53（71.05）		5(26.32)			
unhealed	21(26.32)		13(68.42)			
death	2(2.63)		1(5.26)			

Abbreviations: FPG, fasting plasma glucose; HbA1c, glycated hemoglobin A1c; DM, diabetes mellitus; non-DM, without diabetes mellitus.

Table 5.Spearman correlation analysis of the disease severity, virus negative conversion time, prognosis, baseline lymphocyte subsets, age and DM（*n*=95）

variable	disease severity (1= common,2= severe,3=critically illness)		virus negative conversion time(days)		prognosis(1=cure,2=unheal,3=death)	
	r	p	r	p	r	p
DM (1=without,2=with)	0.320	0.000	0.337	0.001		
Age (year)	0.361	0.000	0.264	0.010	0.263	0.010
LY (cells/ul)	-0.341	0.001				
LY% (%)	-0.371	0.000			-0.209	0.042
CD3+(cells/ul)	-0.379	0.000				
CD3+CD4+(cells/ul)	-0.388	0.000				
CD3+CD8+(cells/ul)	-0.351	0.000				
CD+3% (%)	-0.302	0.003				
CD3+CD4+%	-0.219	0.033				
CD19+(cells/ul)	-0.266	0.033				
disease severity (1=common,2= severe,3=critically illness)					0.331	0.001
virus negative conversion time(days)					0.299	0.003

Abbreviations: DM, diabetes mellitus. LY, lymphocytes.

Table 6.Multiple stepwise regression analysis of influencing factors of the disease severity, the coronavirus negative conversion time and the prognosis（*n*=95）

independent variable		B	Std. Error	Beta	t	p
the disease severity (1= common,2= severe,3=critically illness)	constant	1.471	0.384	-	3.828	0.000
	DM (1=without,2=with	0.537	0.185	0.266	2.897	0.005
	age	0.013	0.004	0.275	3.007	0.003
	CD3+CD4+%	-0.023	0.007	-0.287	-3.236	0.002
	LY%	-0.025	0.012	-0.200	-2.173	0.032
the coronavirus negative conversion time	constant	18.421	3.173	-	5.805	0.000
	CD19+% (%)	0.394	0.184	0.255	2.134	0.037
	LY% (%)	-0.413	0.171	-0.290	-2.422	0.019
the prognosis	constant	0.33	0.161	-	2.067	0.042
	coronavirus negative conversion time	0.022	0.006	-0.354	4.005	0.000
	disease severity	0.220	0.063	0.316	3.481	0.001
	age	0.007	0.003	0.204	2.141	0.035

Abbreviations: DM, diabetes mellitus. LY, lymphocytes count.

Figures

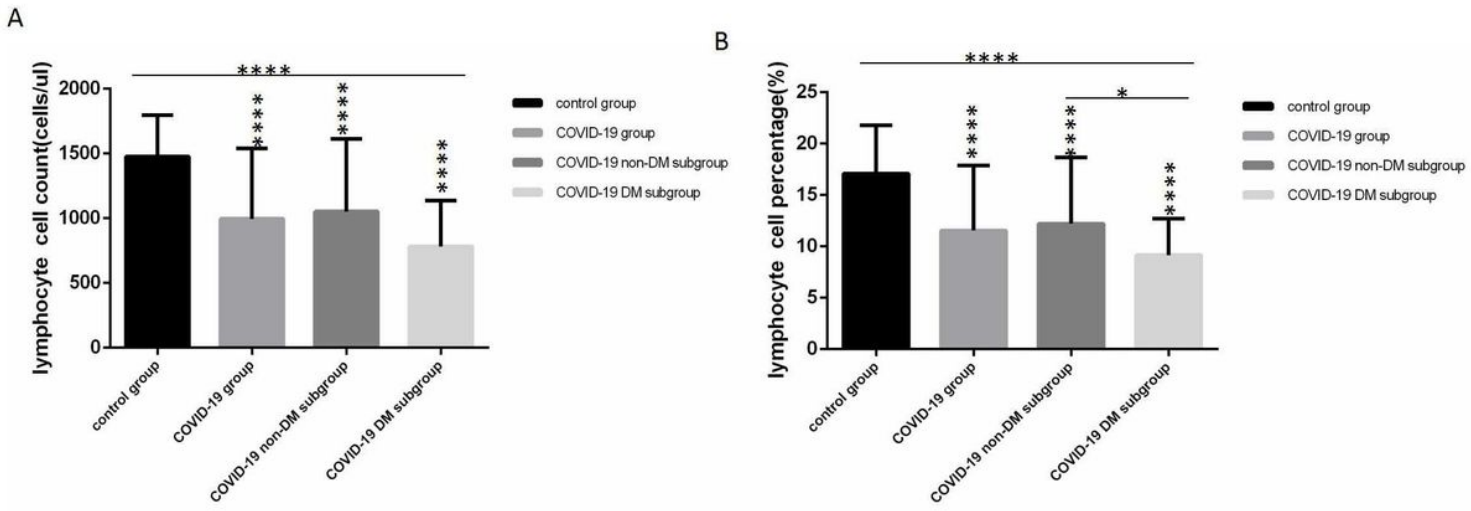

Figure 1

Comparison of lymphocyte count levels and percentage values between four groups. Abbreviations: DM, diabetes mellitus; non-DM, without diabetes mellitus; COVID-19, coronavirus disease 2019. A. lymphocyte count. B. lymphocyte percentage. Unpaired one ANOVA were used for intergroup comparison (A, B, p all<0.0001). Unpaired t-tests were used for comparison with the control group or between two groups, *P<0.05, ****P<0.0001.

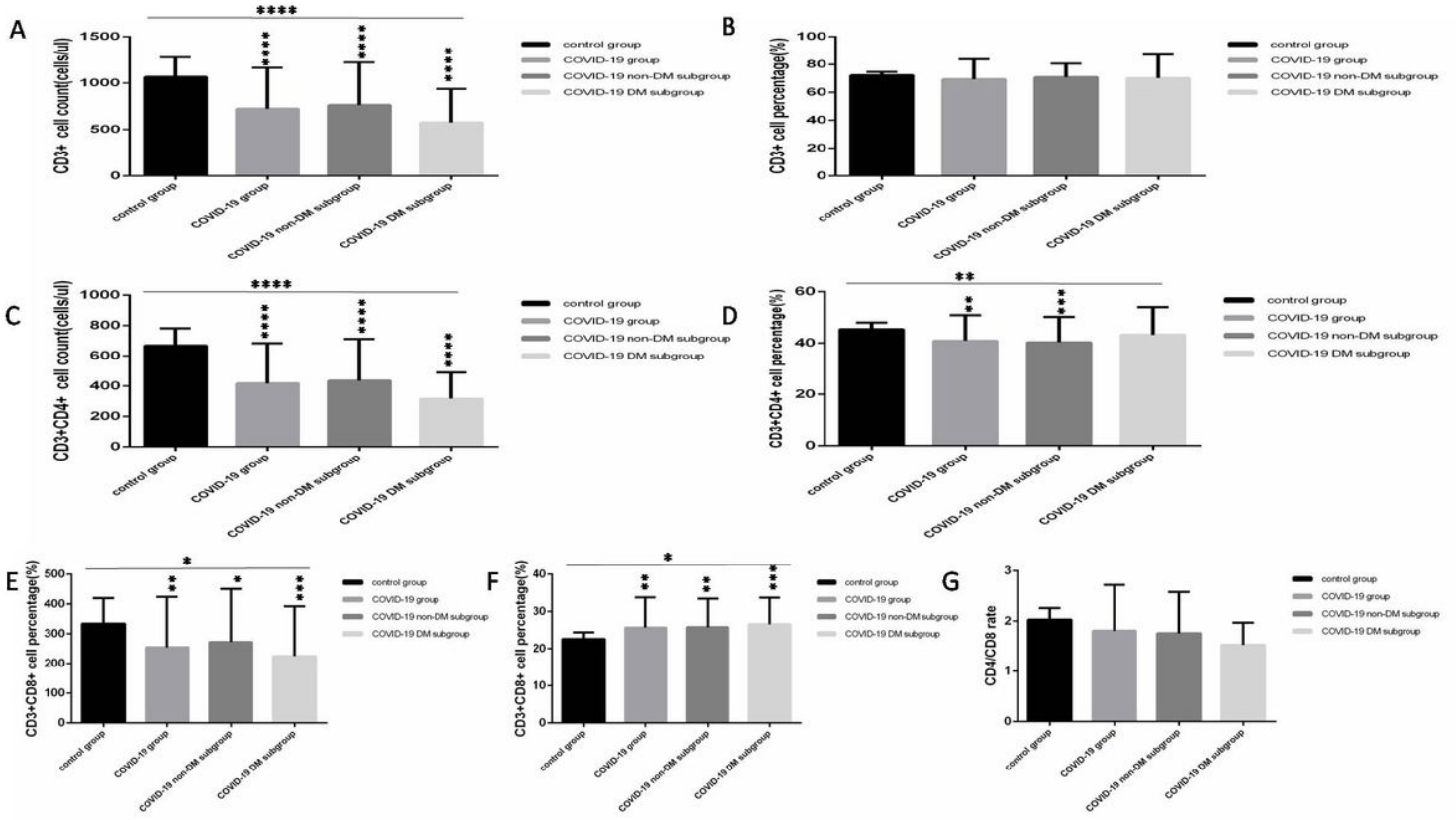

Figure 2

Comparison of T lymphocytes and subsets count levels and percentage values between four groups. Abbreviations: DM, diabetes mellitus; non-DM, without diabetes mellitus; COVID-19, coronavirus disease 2019. A.CD3+ cell count. B.CD3+ cell percentage. C.CD3+CD4+ cell count. D.CD3+CD4+ cell percentage. E.CD3+CD8+ cell count. F.CD3+CD8+ cell percentage. G. Ratio of CD4+/CD8+. Unpaired one ANOVA were used for intergroup comparison (A, C, P all<0.0001; D, P<0.01; E, F, P all<0.05). Unpaired t-tests were used for comparison with the control group, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

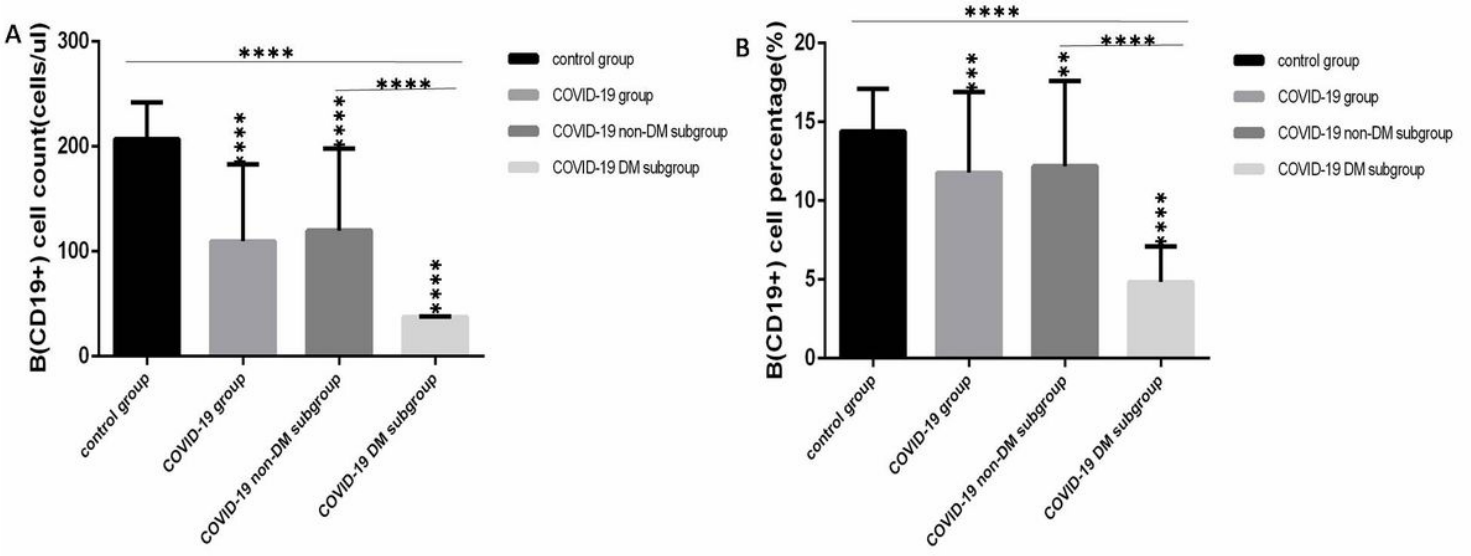

Figure 3

Comparison of B lymphocytes count levels and percentage values between four groups. Abbreviations: DM, diabetes mellitus; non-DM, without diabetes mellitus; COVID-19, coronavirus disease 2019. A.B lymphocytes count. B. B lymphocytes percentage. Unpaired one ANOVA were used for intergroup comparison (A, B, P all<0.0001). Unpaired t-tests were used for comparison with the control group or between two groups, **P<0.01, ***P<0.001, ****P<0.0001.

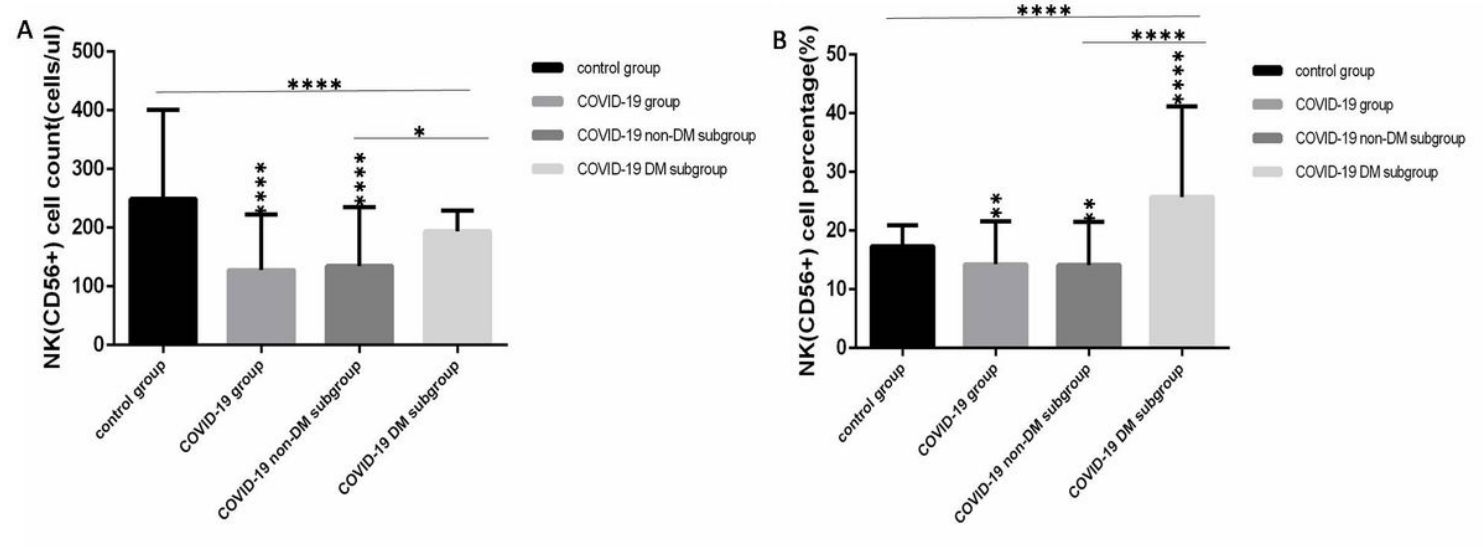

Figure 4

Comparison of NK lymphocytes count levels and percentage values between four groups. Abbreviations: DM, diabetes mellitus; non-DM, without diabetes mellitus; COVID-19, coronavirus disease 2019. A. NK lymphocyte count. B. NK lymphocytes percentage. Unpaired one ANOVA were used for intergroup comparison (A, B, P all<0.0001). Unpaired t-tests were used for comparison with the control group or between two groups, *P<0.05, **P<0.01, ****P<0.0001.

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Figure 5

Comparison of lymphocytes count levels and percentage values between four subgroups. Abbreviations: DM, diabetes mellitus; non-DM, without diabetes mellitus; COVID-19, coronavirus disease 2019. A. lymphocytes count. B. lymphocytes percentage. Unpaired two ANOVA were used for interaction comparison(A, B,P all>0.05).Unpaired one ANOVA were used for intergroup comparison (A, B,P all <0.01).Unpaired t-tests were used for comparison with the non-severe non-DM subgroup,*P<0.05,**P<0.01.Unpaired t-tests were used for comparison with the severe non-DM subgroup, #P<0.05,##P<0.01.

Figure 6

Comparison of T lymphocytes count levels and percentage values between four subgroups. Abbreviations: DM, diabetes mellitus; non-DM, without diabetes mellitus; COVID-19, coronavirus disease 2019. A.CD3+ cell count. B.CD3+ cell percentage. C.CD3+CD4+ cell count. D.CD3+CD4+ cell percentage. E.CD3+CD8+ cell count. F.CD3+CD8+ cell percentage. G. Ratio of CD4+/CD8+. Unpaired two ANOVA were used for interaction comparison(B,P<0.05;A, C, D, E, F, G,P all>0.05).Unpaired one ANOVA were used for intergroup comparison (A, D,P all <0.01; C,P<0.001;B, E, F, G,P all>0.05).Unpaired t-tests were used for comparison with the non-severe non-DM subgroup,*P<0.05,**P<0.01, ***P<0.001.Unpaired t-tests were used for comparison with the severe non-DM subgroup, #P<0.05,##P<0.01.

Figure 7

Comparison of B lymphocytes count levels and percentage values between four subgroups. Abbreviations: DM, diabetes mellitus; non-DM, without diabetes mellitus; COVID-19, coronavirus disease 2019.A. B lymphocytes count. B. B lymphocyte percentage. Unpaired two ANOVA were used for interaction comparison(A,B,P all>0.05).Unpaired one ANOVA were used for intergroup comparison (A, P<0.05; B,P>0.05).Unpaired t-tests were used for comparison with the non-severe non-DM subgroup or between two groups,*P<0.05, ***P<0.001.Unpaired t-tests were used for comparison with the severe non-DM subgroup,##P<0.01.

Figure 8

Comparison of NK lymphocytes count levels and percentage values between four subgroups. Abbreviations: DM, diabetes mellitus; non-DM, without diabetes mellitus; COVID-19, coronavirus disease 2019. A. NK lymphocyte count. B. NK lymphocyte percentage. Unpaired two ANOVA were used for interaction comparison(A,B,P all>0.05).Unpaired one ANOVA were used for intergroup comparison (A, B,P all>0.05).Unpaired t-tests were used for comparison with the non-severe non-DM subgroup or between two groups, P all>0.05.Unpaired t-tests were used for comparison with the severe non-DM subgroup, P all>0.05.