

Abnormal immunity of non-survivors with COVID-19: predictors for mortality

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Abstract

Background The outbreak of coronavirus disease 2019 (COVID-19) has rapidly spread all over the world. The specific information about immunity of non-survivors with COVID-19 is scarce. We aimed to describe the clinical characteristics and abnormal immunity of the confirmed COVID-19 non-survivors.

Methods In this single-centered, retrospective, observational study, we enrolled 125 patients with COVID-19 who were died between Jan, 13 and Mar 4, 2020 from Renmin Hospital of Wuhan University. 414 randomly recruited patients with confirmed COVID-19 who were discharged from the same hospital during the same period served as control. Demographic and clinical characteristics, laboratory findings and chest computed tomograph results at admission, and treatment were collected. The immunity-related risk factors associated with in-hospital death were detected.

Results Non-survivors were older than survivors. More than half of non-survivors was male. Nearly half of the patients had chronic medical illness. The common signs and symptoms at admission of non-survivors were fever. Non-survivors had higher white blood cell (WBC) count, more elevated neutrophil count, lower lymphocytes and platelete count, raised concentration of procalcitonin and C-reactive protein (CRP) than survivors. The levels of CD3⁺ T cells, CD4⁺ T cells, CD8⁺ T cells, CD19⁺ T cells, and CD16⁺56⁺T cells were significantly decreased in non-survivors when compared with survivors. The concentrations of immunoglobulins (Ig) G, IgA and IgE were increased, whereas the levels of complement proteins (C)3 and C4 were decreased in non-survivors when compared with survivors. Non-survivors presented lower levels of oximetry saturation at rest and lactate. Old age, comorbidity of malignant tumour, neutrophilia, lymphocytopenia, low CD4⁺ T cells, decreased C3, and low oximetry saturation were the risk factors of death in patients with confirmed COVID-19. The frequency of CD4⁺ T cells positively correlated with the numbers of lymphocytes and the level of oximetry saturation, whereas CD4⁺ T cells were negatively correlated with age and the numbers of neutrophils.

Conclusion

Abnormal cellular immunity and humoral immunity were considerable in non-survivors with COVID-19. Neutrophilia, lymphocytopenia, low CD4⁺ T cells, and decreased C3 were the immunity-related risk factors predicting mortality of patients with COVID-19.

Introduction

In December 2019, the outbreak of coronavirus disease 2019 (COVID-19) has rapidly spread all over the world. The pathogen has been identified as a novel enveloped RNA beta-coronavirus that has currently been known to be severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. Like severe acute respiratory syndrome (SARS) coronavirus and Middle East respiratory syndrome (MERS) coronavirus, SARS-CoV-2 is highly pathogenic coronavirus to human. The virus has high transmission capability as well as high morbidity and mortality[2-4]. Until Apr 22, 2020, the acute respiratory disease has affected

more than 200 countries around the world. Globally, 2553853 cases were confirmed, 176323 patients have died from this viral infection. However, little is still known about the coronavirus.

Recent report has showed that the severely ill patients with confirmed COVID-19 may develop dyspnea and hypoxemia within 1 week after onset of the disease, which may quickly progress to acute respiratory distress syndrome (ARDS) or end-organ failure[5, 6]. The general epidemiological features and clinical characteristics of patients with COVID-19 have been previously reported[2, 4, 7]. However, these studies were based on relatively small sample sizes, specific information about abnormal immunity of non-survivors with COVID-19 has not yet been well described. In this study, we aim to explore the clinical characteristics and abnormal immunity, and further evaluate the immunity-related risk factors associated with death in patients with COVID-19 from a single hospital in Wuhan, China.

Methods

Patients' involvement and data collection

In this retrospective, single center study, a total of 125 patients who died from confirmed COVID-19 between Jan, 13 and Mar 4, 2020 from Renmin Hospital of Wuhan University, the designated hospital to treat severe patients with COVID-19 in Wuhan, China. 414 randomly recruited patients with confirmed COVID-19 who were discharged from the same hospital and during the same period served as control. All patients were confirmed by at least two positive nucleic acid test for SARS-CoV-2. Patients with COVID-19 were diagnosed according to the World Health Organization interim guidance[8] and Diagnosis and Treatment Guideline for Novel Coronavirus Pneumonia (Trial Version 7.0)[9]. The confirmed COVID-19 patients were classified as severe or non-severe cases based on the American Thoracic Society guidelines for community-acquired pneumonia[10]. The patients who received systemic corticosteroid treatment before admission were excluded. The criteria for patient discharge were the absence of fever for at least 3 days, substantial improvement in both lungs in chest computed tomograph (CT), clinical remission of respiratory symptoms, and two throat-swab samples negative for SARS-CoV-2 RNA obtained at least 24 h apart. The study was approved by the Research Ethics Commission of Renmin Hospital of Wuhan University (No.WDRY2020-K143), and written informed consent was waived by the Research Ethics Commission.

Demographic and clinical characteristics (included comorbidities, signs and symptoms), laboratory findings, chest CT scan results and treatment were extracted from electronic medical records system of Renmin Hospital of Wuhan University and analyzed by three independent researchers. The access was granted by the director of the hospital. The date of disease onset and admission date, as well as the severity of COVID-19, were also recorded. None of these cases have been previously reported.

Laboratory testing

Patient pharyngeal swab specimens were collected for SARS-CoV-2 nucleic acid detection using real-time reverse transcriptase-polymerase chain reaction (RT-PCR) assay with a standard procedure recommended

by China Center for Disease Control and Prevention (CDC). The viral nucleic acid testing for all patients was performed by the clinical laboratory from Renmin Hospital of Wuhan University which was the certified laboratory for SARS-CoV-2 test. Other laboratory testing at admission, including white blood cell (WBC), neutrophil, lymphocyte, platelet, procalcitonin (PCT), C-reactive protein (CRP), CD3⁺ T cells, CD4⁺ T cells, CD8⁺ T cells, CD19⁺ T cells, CD16⁺ 56⁺ T cells, immunoglobulins (Ig) G, IgM, IgA, IgE, complement proteins (C)3, C4, oximetry saturation, and lactate, were performed in the same hospital and results were retrieved from electronic medical records.

Statistical Analysis

All statistical analyses were performed using SPSS 24.0 software. Categorical variables were expressed with frequencies and percentages. Continuous variables were presented as median (interquartile range (IQR)). Means for continuous variables were compared with independent-samples t tests when the data were normally distributed; if not, the Mann-Whitney U test was used. Proportions for categorical variables were compared with the chi-square and Fisher's exact test. Univariable logistic regression between basic disease or different parameter and demise was performed. Moreover, the main risks related with demise were examined using multivariable logistic regression models adjusted for potential confounders. Receiver Operating Characteristic (ROC) curve model was also used to predict death efficacy. Spearman's correlation test was used for calculation of correlation between different factors. Statistical significance was determined at $P \leq 0.05$.

Results

Demographic and clinical characteristics

539 patients with confirmed COVID-19 (125 non-survivors and 414 survivors) with important data in their medical records were included in this study. All patients had pneumonia with abnormal findings on chest CT scan. The median age of all patients was 58.0 years (IQR 43.0–69.0), ranging from 16 years to 97 years. However, non-survivors (70 years, IQR 61.5-80) were significantly older than survivors (54 years, IQR 37-65) ($P < 0.0001$). Of these patients, the majority was female (52.7%). However, more than half of non-survivors was male (56.8%). Nearly half of the patients (44.9%) had chronic medical illness. In non-survivors, hypertension (49.6%) was the most common comorbidity, followed by diabetes (20.0%) and coronary heart disease (16.0%). The median interval from illness onset to death was 7.0 days (IQR 4.0-11.0), which is markedly lower than the median time from illness onset to discharge 15 days (IQR 9.0 - 21.0). The common signs and symptoms at admission of non-survivors were fever (88%), followed by cough (64.8%), dyspnea (62.4%), fatigue (62.4%) and chest tightness (58.4%). As for clinical classification, the patients were divided into severe group and non-severe groups, nearly all of non-survivors were severe cases at admission. Severe patients (98.4%) were more likely to die compared with non-severe patients. Almost all the non-survivors received antibiotic drug (94.4%). Both the two group patients were given antiviral therapy or Chinese Medicine, such as Lianhua Qingwen, Oseltamivir, Arbidol,

and Lopinavir/ritonavir. Intravenous human immunoglobulin and corticosteroid were used more frequently in non-survivors than survivors (Table 1).

Blood routine examination, cellular immunity, humoral immunity, and other laboratory findings in non-survivors with COVID-19

Although WBC and platelet count of both non-survivors and survivors were in normal range, the non-survivors had higher WBC count (7.85 vs $5.07 \times 10^9/L$), more elevated neutrophil count (6.41 vs $3.08 \times 10^9/L$), smaller lymphocyte count (0.69 vs $1.20 \times 10^9/L$) and lower platelets count (172 vs $211 \times 10^9/L$) than survivors ($P < 0.001$). Raised infection-related biomarkers, including procalcitonin (0.21 vs 0.06 ng/mL) and CRP (70.5 vs 7.2 mg/L) were found in non-survivors when compared with these of survivors ($P < 0.001$). The levels of CD3⁺ T cells (277 vs 814 cells/ μ l), CD4⁺ T cells (172 vs 473 cells/ μ l), CD8⁺ T cells (84 vs 262.5 cells/ μ l, $P < 0.001$), CD19⁺ T cells (88 vs 141 cells/ μ l) and CD16⁺56⁺ T cells (79 vs 128.5 cells/ μ l) were significantly decreased in non-survivors when compared with survivors ($P < 0.001$). The serum concentrations of IgG (13.30 vs 11.95 g/L, $P < 0.001$), IgA (2.54 vs 2.21 g/L, $P = 0.012$), and IgE (71.30 vs 42.25 IU/ml, $P < 0.001$) were increased, whereas the serum levels of C3 (0.89 vs 0.99 g/L, $P < 0.001$) and C4 (0.22 vs 0.24 g/L, $P = 0.001$) were decreased in non-survivors when compared with survivors. The non-survivors presented lower levels of oximetry saturation (90 vs 97% , $P < 0.001$) at rest and lactate (2.40 vs 1.90 mmol/L, $P < 0.001$) (Table 2).

Associations between immunity-related indexes and death risk of COVID-19 patients

Univariable logistic regression analysis indicated that old age, comorbidity of malignant tumour, neutrophilia, lymphocytopenia, enhanced CRP, lower CD4⁺ T cells, and decreased C3 were associated with death (Table 3). Generalized linear model showed that old age, comorbidity of malignant tumour, neutrophilia, lymphocytopenia, lower CD4⁺ T cells, decreased C3 and lower oximetry saturation was positively with the risk of death (Table 4). ROC curve analysis was conducted to evaluate the role of these factors in predicting death efficacy. The area under the ROC curve (AUC) was 0.792 (confidence interval [CI], 0.764-0.837) for age, 0.761 (95% CI, 0.709-0.812) for neutrophils, 0.797 (95% CI, 0.753-0.840) for lymphocytes, 0.821 (95% CI, 0.782-0.860) for CRP, 0.848 (95% CI, 0.814-0.883) for CD4⁺ T cells, 0.630 (95% CI, 0.574- 0.685) for C3 and 0.808 (95% CI, 0.759-0.856) for oximetry saturation. Additionally, the cutoff value was 64.5 for age, 5.835 for neutrophils, 0.945 for lymphocytes, 31.4 for CRP, 380.5 for CD4⁺ T cells, 0.809 for C3, and 94.5 oximetry saturation (Table 5, Figure 1). The frequency of CD4⁺ T cells positively correlated with the numbers of lymphocytes ($r = 0.787$, $P < 0.001$) and the level of oximetry saturation ($r = 0.295$, $P < 0.001$), Whereas CD4⁺ T cells were negatively correlated with age ($r = -0.323$, $P < 0.001$) and the numbers of neutrophils ($r = -0.244$, $P < 0.001$). However, CD4⁺ T cells showed no significant correlation with C3 ($P = 0.78$) (Figure 2).

Discussion

In this report, we showed That the non-survivors with confirmed COVID-19 had higher WBC count, neutrophilia, lymphocytopenia, and lower platelet level when compared with survivors at admission. Remarkably abnormal cellular immunity and humoral immunity were found in non-survivors. Old age, comorbidity of malignant tumour, neutrophilia, lymphocytopenia, low CD4⁺ T cells, decreased C3, and low oximetry saturation were positively with the risk of death in patients with COVID-19.

In this study, we found that non-survivors with confirmed COVID-19 were older, especially above 64.5 years, and more than half of non-survivors was male. Additionally, nearly half of the patients had chronic medical illness, especially comorbidity of malignant tumour was the risk of death. Previous reports have showed that The age-dependent defects in T-cell and B-cell function and the excess production of type 2 cytokines could lead to a deficiency in control of viral replication and prolonged proinflammatory responses, potentially leading to poor outcome[11]. Furthermore, chronic comorbidities compromise immune system[12]. Therefore, our data suggested that COVID-19 is more likely to infect those elder men with chronic comorbidities, and age is one of the death risk factor of patients with COVID-19, as previously reported[13]. In this study, our findings showed that almost all non-survivors were severely illness at admission, who were more likely to die compared with the non-severe patients even if they were given integrated therapy. Meanwhile, the non-survivors had higher WBC count, neutrophilia, lymphocytopenia and lower platelet levels. Additionally, neutrophilia and lymphocytopenia were the markers of death risks of patients with COVID-19. This was consistent with the findings that severe patients with COVID-19 had a higher number of neutrophil count and a lower number of lymphocyte count during the period of diseases [12, 14]. It has been reported that neutrophilia and lymphocytopenia were significantly associated with higher risks of the development of ARDS[5]. Also, it has been showed that phagocytosis, release of granular contents, and secretion of cytokines are important effector functions of stimulated neutrophils, suggesting a protective immunity against the virus[15]. However, excessive elevated neutrophils can lead to cytokine storm and tissue damage, resulting in severe pneumonia and death[15], which have been found in patients with SARS[16, 17] and MERS[18]. As for lymphocytes, it reported that the functional exhaustion of cytotoxic lymphocytes is associated with SRAS-CoV-2 infection. Hence, SARS-CoV-2 infection may break down antiviral immunity at an early stage[19]. Lymphocytopenia was also found in the MERS cases. MERS-CoV can directly infect human primary T lymphocytes and induce T-cell apoptosis through extrinsic and intrinsic apoptosis pathways[20]. Therefore, it is possible that neutrophilia and lymphocytopenia at least partially be related to the development of critical illness, with a high mortality rate in the confirmed COVID-19 patients. However, future studies to identify the mechanisms of these features may help us to understand this disease.

It has been accepted that lymphocytes subsets play an important role in the maintenance of immune system function. Here, our results indicated that the numbers of CD3⁺ T cells, CD4⁺ T cells, CD8⁺ T cells, B cells (CD19⁺), and natural killer (NK) cells (CD16⁺CD56) were significantly decreased in non-survivors at admission, compared with survivors. Meanwhile, markedly increased serum concentrations of IgG, IgA, and IgE and noticeably decreased serum levels of C3 and C4 were found in non-survivors at admission

when compared with survivors. Our findings suggested that it was possible that SARS-CoV-2 infection may impair lymphocytes, especially T lymphocytes, and the humoral immune system on the onset of disorder. CD4⁺ T cells, B cell lymphopenia and NK cell count is depleted, probably as result of the rapidly propagating virus[21]. To mount an antiviral response, the innate immune system recognizes molecular structures that are produced by the invasion of the virus. Helper T (Th) cells orchestrate the overall adaptive response, while cytotoxic T cells are essential in killing of viral infected cells. Humoral immune response plays a protective role by limiting infection at later phase and prevents reinfection in the future. Therefore, SARS-CoV-2 infection can activate both cellular immune response and humoral immune responses in humans[22]. And then, uncontrolled inflammatory innate responses and impaired adaptive immune responses may lead to harmful tissue damage, both locally and systemically[23]. Patients who survived SARS-CoV and MERS-CoV infections usually had better immune responses than non-survivors and this decrease persisted until the recovery period of SARS-CoV pneumonia[24].

It has been reported that CD4⁺ T cells and CD8⁺ T cells play a key antiviral role by balancing the combat against pathogens and the risk of developing autoimmunity or overwhelming inflammation[25]. Depletion of CD4⁺ T cells, but not CD8⁺ T cells, leads to an enhanced immune-mediated interstitial pneumonitis and delayed clearance of SARS-CoV from the lungs in a SARS-CoV-infected mouse model, demonstrating the vital role of CD4⁺ T cells in SARS-CoV infection[26]. Previous report has showed that higher number of CD4⁺ T cell may protect patients from developing ARDS in SARS-CoV infection[27]. In our study, our data showed that the number of CD4⁺ T cells positively correlated with the numbers of lymphocytes and the level of oximetry saturation and negatively correlated with age and the numbers of neutrophils in non-survivors. Furthermore, low number of CD4⁺ T cells was the risk of death in patients with confirmed COVID-19. Therefore, our results provided a possible evidence that decreased number of CD4⁺ T cells is the immunity-related risk factor predicting death rate of patients with COVID-19. Similar to these findings, autopsy of patients with COVID-19 showed that the counts of peripheral CD4 and CD8 T cells were substantially reduced, accounts for, in part, the severe immune injury in this patient[28]. High levels of pro-inflammatory cytokines released from CD4⁺ T cells were observed in SARS-CoV and MERS-CoV infection, suggesting a potential cytokine storm-mediated disease severity[18]. Additionally, the recruitment of immune cell populations in the patient's blood was before the resolution of symptoms[29].

The importance of complement in SARS-CoV pathogenesis is controversial. The complement system is an essential component of the innate immune system, and complement plays an important role in the host antiviral response[30]. C3 is required for protection from pandemic 2009 H1N1 and highly pathogenic avian influenza (HPAI) H5N1 influenza virus infections by aiding in viral clearance and regulating lung inflammation[31]. However, activation of the complement system results in an immune reaction capable of destroying pathogens and their products. C3 was an important host mediator of SARS-CoV-induced disease and C3 regulates a systemic proinflammatory response to SARS-CoV infection[32]. Here, our data showed that markedly decreased serum levels of C3 and C4 were observed in non-survivors at admission when compared with survivors. Additionally, our results

suggested that decreased serum level of C3 is the immunity-related risk factor predicting mortality of patients with COVID-19.

Our study has some limitations. First, we mainly evaluated the number change of cellular immunity- and humoral immunity-related cell subsets; the function of these cells should to be elucidated. Second, due to the retrospective study design, not all laboratory tests, such as Interleukin (IL)-6, Interferon- γ , IL-10 and other cytokines, were performed in all patients, and the dynamic data after treatment were also incomplete.

Conclusions

In conclusion, abnormal cellular immunity and humoral immunity were considerable in non-survivors with COVID-19. Neutrophilia, lymphocytopenia, low number of CD4⁺ T cells, and decreased level of C3 were the immunity-related risk factors predicting mortality of patients with COVID-19. These may be helpful in evaluating prognosis of patients with COVID-19.

List Of Abbreviations

COVID-19: coronavirus disease 2019

WBC: white blood cell

CRP: C-reactive protein

PCT: procalcitonin

Ig: immunoglobulins

SARS-CoV-2: severe acute respiratory syndrome coronavirus 2

SARS: severe acute respiratory syndrome

MERS: Middle East respiratory syndrome

ARDS: acute respiratory distress syndrome

CT: computed tomograph

RT-PCR: reverse transcriptase-polymerase chain reaction

CDC: Center for Disease Control and Prevention

C: complement proteins

IQR : interquartile range

ROC: Receiver Operating Characteristic

CI: confidence interval

NK: natural killer

Th: Helper T

HPAI: highly pathogenic avian influenza

IL: Interleukin

Declarations

Ethical Approval and Consent to participate

The study was approved by the Research Ethics Commission of Renmin Hospital of Wuhan University (No.WDRY2020-K143), and written informed consent was waived by the Research Ethics Commission.

Consent for publication

Not applicable Consent for publication.

Availability of data and materials

The datasets generated and/or analysed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Competing interests

No conflicts of interest are declared by the authors.

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Authors' contributions

ZY had the idea for and designed the study and take responsibility for the integrity of the data and the accuracy of the data analysis. ZYT, WMM, WT, ZZS, LXC, ZSL and WXJ collected the data. ZY and NHX did the analysis. ZY, NHX and HK drafted the paper, and all authors critically revised the manuscript for important intellectual content and gave final approval for the version to be published. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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References

1. Coronaviridae Study Group of the International Committee on Taxonomy of V: **The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2.** *Nature microbiology* 2020, **5**(4):536-544.
2. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, Qiu Y, Wang J, Liu Y, Wei Y *et al*: **Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study.** *Lancet* 2020, **395**(10223):507-513.
3. Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y, Ren R, Leung KSM, Lau EHY, Wong JY *et al*: **Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus-Infected Pneumonia.** *The New England journal of medicine* 2020, **382**(13):1199-1207.

4. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X *et al*: **Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China.** *Lancet* 2020, **395**(10223):497-506.
5. Wu C, Chen X, Cai Y, Xia J, Zhou X, Xu S, Huang H, Zhang L, Zhou X, Du C *et al*: **Risk Factors Associated With Acute Respiratory Distress Syndrome and Death in Patients With Coronavirus Disease 2019 Pneumonia in Wuhan, China.** *JAMA internal medicine* 2020.
6. Zhou Y, Zhang Z, Tian J, Xiong S: **Risk factors associated with disease progression in a cohort of patients infected with the 2019 novel coronavirus.** *Annals of palliative medicine* 2020, **9**(2):428-436.
7. Chen T, Wu D, Chen H, Yan W, Yang D, Chen G, Ma K, Xu D, Yu H, Wang H *et al*: **Clinical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study.** *Bmj* 2020, **368**:m1091.
8. **Clinical management of severe acute respiratory infection (SARI) when COVID-19 disease is suspected**—Interim guidance [[https://www.who.int/publications-detail/clinical-management-of-severe-acute-respiratory-infection-when-novel-coronavirus-\(ncov\)-infection-is-suspected](https://www.who.int/publications-detail/clinical-management-of-severe-acute-respiratory-infection-when-novel-coronavirus-(ncov)-infection-is-suspected)]
9. **The Seventh Version of the COVID-19 Diagnosis and Treatment Guidance** [<http://health.people.com.cn/n1/2020/0304/c14739-31616706.html>]
10. Metlay JP, Waterer GW, Long AC, Anzueto A, Brozek J, Crothers K, Cooley LA, Dean NC, Fine MJ, Flanders SA *et al*: **Diagnosis and Treatment of Adults with Community-acquired Pneumonia. An Official Clinical Practice Guideline of the American Thoracic Society and Infectious Diseases Society of America.** *Am J Respir Crit Care Med* 2019, **200**(7):e45-e67.
11. Opal SM, Girard TD, Ely EW: **The immunopathogenesis of sepsis in elderly patients.** *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2005, **41** Suppl 7:S504-512.
12. Qin C, Zhou L, Hu Z, Zhang S, Yang S, Tao Y, Xie C, Ma K, Shang K, Wang W *et al*: **Dysregulation of immune response in patients with COVID-19 in Wuhan, China.** *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2020.
13. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, Xiang J, Wang Y, Song B, Gu X *et al*: **Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study.** *Lancet* 2020, **395**(10229):1054-1062.
14. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, Wang B, Xiang H, Cheng Z, Xiong Y *et al*: **Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China.** *Jama* 2020.
15. Lamichhane PP, Samarasinghe AE: **The Role of Innate Leukocytes during Influenza Virus Infection.** *Journal of immunology research* 2019, **2019**:8028725.
16. Wong CK, Lam CW, Wu AK, Ip WK, Lee NL, Chan IH, Lit LC, Hui DS, Chan MH, Chung SS *et al*: **Plasma inflammatory cytokines and chemokines in severe acute respiratory syndrome.** *Clin Exp Immunol* 2004, **136**(1):95-103.
17. Wong RS, Wu A, To KF, Lee N, Lam CW, Wong CK, Chan PK, Ng MH, Yu LM, Hui DS *et al*: **Haematological manifestations in patients with severe acute respiratory syndrome: retrospective analysis.** *Bmj* 2003, **326**(7403):1358-1362.

18. Mahallawi WH, Khabour OF, Zhang Q, Makhdoum HM, Suliman BA: **MERS-CoV infection in humans is associated with a pro-inflammatory Th1 and Th17 cytokine profile.** *Cytokine* 2018, **104**:8-13.
19. Zheng M, Gao Y, Wang G, Song G, Liu S, Sun D, Xu Y, Tian Z: **Functional exhaustion of antiviral lymphocytes in COVID-19 patients.** *Cell Mol Immunol* 2020.
20. Chu H, Zhou J, Wong BH, Li C, Chan JF, Cheng ZS, Yang D, Wang D, Lee AC, Li C *et al*: **Middle East Respiratory Syndrome Coronavirus Efficiently Infects Human Primary T Lymphocytes and Activates the Extrinsic and Intrinsic Apoptosis Pathways.** *The Journal of infectious diseases* 2016, **213**(6):904-914.
21. Giamarellos-Bourboulis EJ, Netea MG, Rovina N, Akinosoglou K, Antoniadou A, Antonakos N, Damoraki G, Gkavogianni T, Adami ME, Katsaounou P *et al*: **Complex Immune Dysregulation in COVID-19 Patients with Severe Respiratory Failure.** *Cell host & microbe* 2020.
22. Baruah V, Bose S: **Immunoinformatics-aided identification of T cell and B cell epitopes in the surface glycoprotein of 2019-nCoV.** *Journal of medical virology* 2020, **92**(5):495-500.
23. Cao X: **COVID-19: immunopathology and its implications for therapy.** *Nature reviews Immunology* 2020.
24. Arabi YM, Arifi AA, Balkhy HH, Najm H, Aldawood AS, Ghabashi A, Hawa H, Alothman A, Khaldi A, Al Raiy B: **Clinical course and outcomes of critically ill patients with Middle East respiratory syndrome coronavirus infection.** *Ann Intern Med* 2014, **160**(6):389-397.
25. Cecere TE, Todd SM, Leroith T: **Regulatory T cells in arterivirus and coronavirus infections: do they protect against disease or enhance it?** *Viruses* 2012, **4**(5):833-846.
26. Chen J, Lau YF, Lamirande EW, Paddock CD, Bartlett JH, Zaki SR, Subbarao K: **Cellular immune responses to severe acute respiratory syndrome coronavirus (SARS-CoV) infection in senescent BALB/c mice: CD4+ T cells are important in control of SARS-CoV infection.** *J Virol* 2010, **84**(3):1289-1301.
27. Li CK, Wu H, Yan H, Ma S, Wang L, Zhang M, Tang X, Temperton NJ, Weiss RA, Brenchley JM *et al*: **T cell responses to whole SARS coronavirus in humans.** *J Immunol* 2008, **181**(8):5490-5500.
28. Xu Z, Shi L, Wang Y, Zhang J, Huang L, Zhang C, Liu S, Zhao P, Liu H, Zhu L *et al*: **Pathological findings of COVID-19 associated with acute respiratory distress syndrome.** *The Lancet Respiratory medicine* 2020, **8**(4):420-422.
29. Thevarajan I, Nguyen THO, Koutsakos M, Druce J, Caly L, van de Sandt CE, Jia X, Nicholson S, Catton M, Cowie B *et al*: **Breadth of concomitant immune responses prior to patient recovery: a case report of non-severe COVID-19.** *Nat Med* 2020, **26**(4):453-455.
30. Stoermer KA, Morrison TE: **Complement and viral pathogenesis.** *Virology* 2011, **411**(2):362-373.
31. O'Brien KB, Morrison TE, Dundore DY, Heise MT, Schultz-Cherry S: **A protective role for complement C3 protein during pandemic 2009 H1N1 and H5N1 influenza A virus infection.** *PLoS One* 2011, **6**(3):e17377.
32. Gralinski LE, Sheahan TP, Morrison TE, Menachery VD, Jensen K, Leist SR, Whitmore A, Heise MT, Baric RS: **Complement Activation Contributes to Severe Acute Respiratory Syndrome Coronavirus**

Tables

Table 1: Demographics, clinical characteristics and treatment of patients with COVID-19

	Total (539)	Non-survivor (125)	Survivor (414)	P value
Age	58(43-69)	70(61.5-80)	54(37-65)	<0.0001
Sex				0.015
Male	255(47.3%)	71(56.8%)	184(44.4%)	
Female	284(52.7%)	54(43.2%)	230(55.6%)	
Chronic medical illness	242(44.9%)	88(70.4%)	154(37.2%)	<0.0001
Chronic obstructive lung disease	22(4.1%)	12(9.6%)	10(2.4%)	<0.0001
Diabetes	58(10.9%)	25(20.0%)	34(8.2%)	<0.0001
Hypertension	140(26.0%)	62(49.6%)	78(18.8%)	<0.0001
Coronary heart disease	37(6.9%)	20(16.0%)	17(4.1%)	<0.0001
Cerebrovascular diseases	19(3.5%)	15(12.0%)	4(1.0%)	<0.0001
Hepatitis	11(2.0%)	3(2.4%)	8(1.9%)	0.746
Malignant tumour	23(4.3%)	11(8.8%)	12(2.9%)	0.0042
Chronic kidney disease	8(1.5%)	6(4.8%)	2(0.5%)	0.0005
Immunodeficiency disease	2(0.4%)	0(0.0%)	2(0.5%)	0.436
Time from illness onset to death or discharge, days	13(7-20)	7(4-11)	15(9-21)	<0.0001
Signs and symptoms at admission				
Fever		110[88%]		0.001
Rhinorrhoea	420[77.9%]	2 [1.6%]	310[74.9%]	0.945
Cough	6[1.1%]	81[64.8%]	4[1.0%]	0.402
Expectoration	332[61.6%]	48[38.4%]	251[60.6%]	0.017
Chest tightness	161[29.9%]	73[58.4%]	113[27.3%]	<0.0001
Dyspnea	187[34.7%]	78[62.4%]	114[27.5%]	<0.0001
Fatigue	187[34.7%]	78[62.4%]	98[23.7%]	<0.0001
Nausea	261[48.4%]	5[4.0%]	183[44.2%]	0.02
Diarrhea	9[1.7%]	8[6.4%]	4[1.0%]	0.073
	58[10.8%]		50[12.1%]	
Clinical Classification				<0.0001
Non-severe	133(24.7%)	2(1.6%)	131(31.6%)	
Severe	406(75.3%)	123(98.4%)	283(68.4%)	
Treatment				
Antibiotic drug	374(69.4%)	118(94.4%)	256(61.8%)	<0.0001
Antiviral therapy or Chinese Medicine	225(41.7%)	58(46.4%)	167(40.3%)	0.228
Lianhua Qingwen	301(55.8%)	40(32.0%)	261(63.0%)	<0.0001
Oseltamivir	157(29.1%)	48(38.4%)	109(26.3%)	0.0092
Arbidol	370(68.6%)	65(52.0%)	305(73.7%)	<0.0001
Lopinavir/ritonavir	32(5.9%)	18(14.4%)	14(3.4%)	<0.0001
Human immunoglobulin	250(38.4%)	91(72.8%)	159(38.4%)	<0.0001
Alpha-interferon	170(31.5%)	34(27.2%)	136(32.9%)	0.233
Thymosin	59(10.9%)	20(16.0%)	39(9.4%)	0.039
Glucocorticoids	249(46.2%)	89(71.2%)	160(38.6%)	<0.0001

Data are median (IQR) and n (%). p values were calculated by Mann-Whitney U test, χ^2 test, or Fisher's exact test, as appropriate. Statistical significance was determined at $P \leq 0.05$.

Table 2: Laboratory findings of patients with COVID-19

	Normal Range	Total (539)	No-survivor (125)	Survivor (414)	P value
WBC ($\times 10^9/L$)	3.5-9.5	5.41(4.1-7.59)	7.85(4.74-12.01)	5.07(3.96-6.76)	<0.001
Neutrophils ($\times 10^9/L$)	1.8-6.3	3.54(2.42-5.72)	6.41(3.77-10.97)	3.08(2.29-4.63)	<0.001
Lymphocytes ($\times 10^9/L$)	1.1-3.2	1.04(0.76-1.5)	0.69(0.42-0.93)	1.20(0.88-1.63)	<0.001
Haemoglobin (g/L)	115-150	126(115-138)	127(113.5-139.5)	126(116-137)	0.761
Platelets ($\times 10^9/L$)	125-350	205(151-261)	172(118-235)	211(163-271)	<0.001
CRP (mg/L)	0-10	16.9(5-61.3)	70.5(39.95-133.5)	7.2(5-37.95)	<0.001
PCT (ng/mL)	<0.1	0.07(0.04-0.25)	0.21(0.11-0.95)	0.06(0.03-0.12)	<0.001
CD3(cells/ μl)	723-2737	667(356-1010)	277(163.5-430)	814(516-1088)	<0.001
CD4(cells/ μl)	404-1612	392(200-586)	172(99.5-267.5)	473(291-657.75)	<0.001
CD8(cells/ μl)	220-1129	221(104-366)	84(39.5-155.5)	262.5(163-405.25)	<0.001
CD19(cells/ μl)	80-616	132(82-202)	88(52-151)	141(96-219)	<0.001
CD16+56(cells/ μl)	84-724	114(70-190)	79(39-144)	128.5(79-210)	<0.001
IgG(g/L)	7.0-16.0	12.2(10.3-14.6)	13.30(10.75-16.95)	11.95(10.18-13.93)	<0.001
IgM(g/L)	0.4-2.3	0.95(0.70-1.3)	0.94(0.70-1.26)	0.95(0.69-1.31)	0.658
IgA(g/L)	0.7-4.0	2.26(1.74-3)	2.54(1.81-3.46)	2.21(1.73-2.89)	0.012
IgE(IU/ml)	<100	52.3(18.3-133)	71.30(30.2-214.5)	42.25(18.3-120)	<0.001
C3(g/L)	0.9-1.8	0.97(0.82-1.1)	0.89(0.74-1.04)	0.99(0.84-1.13)	<0.001
C4(g/L)	0.1-0.4	0.241(0.18-0.32)	0.22(0.159-0.30)	0.24(0.19-0.32)	0.001
Oximetry saturation (%)	95-100	97(92-98)	90(83-94)	97(95-98)	<0.001
Lactate (mmol/L)	0.5-1.5	2(1.5-2.53)	2.40(1.9-3.65)	1.90(1.43-2.38)	<0.001

Data are median (IQR). WBC: white blood cell. CRP: C-reactive protein. PCT: procalcitonin. Ig: immunoglobulins. C: complement proteins. p values were calculated by Mann-Whitney U test, χ^2 test, or Fisher's exact test, as appropriate. Statistical significance was determined at $P \leq 0.05$.

Table 3: Univariable logistic Regression of death risk of patients with COVID-19

	Wald	P	OR	95% CI
Age	27.347	0.001	0.912	0.881-0.944
Malignant tumour	4.563	0.023	0.176	0.036-0.866
Neutrophils	5.239	0.022	0.881	0.790-0.982
Lymphocytes	7.674	0.003	0.109	0.032-0.186
CRP	4.052	0.044	0.992	0.984-1.000
CD4	9.147	0.002	1.005	1.002-1.009
C3	8.033	0.005	40.209	3.326-528.035

OR: odds ratio. CI: confidence interval. CRP: C-reactive protein. C: complement proteins. Statistical significance was determined at $P \leq 0.05$.

Table 4: Generalised linear model of patients with COVID-19

	Wald Chi-Square	P	Standard error
Age	25.182	0.001	0.0009
Malignant tumour	24.070	0.001	0.0638
Neutrophils	9.813	0.002	0.0062
Lymphocytes	5.831	0.016	0.0357
CD4	3.602	0.048	0.00268
C3	6.928	0.008	0.0291
Oximetry saturation	9.555	0.002	0.0017

C: complement proteins. Statistical significance was determined at $P \leq 0.05$.

Table 5: ROC curve model of patients with COVID-19

	Area	Std.error	P	95%CI lower	95%CI upper	Cut off
Age	0.792	0.023	0.000	0.764	0.837	64.5
Neutrophils	0.761	0.026	0.000	0.709	0.812	5.835
Lymphocytes	0.797	0.22	0.000	0.753	0.840	0.945
CRP	0.821	0.020	0.000	0.782	0.860	31.4
CD4	0.848	0.018	0.000	0.814	0.883	380.5
C3	0.630	0.028	0.000	0.574	0.685	0.809
Oximetry saturation	0.808	0.25	0.000	0.759	0.856	94.5

ROC: Receiver Operating Characteristic. CI: confidence interval. CRP: C-reactive protein. C: complement proteins. Statistical significance was determined at $P \leq 0.05$.

Figures

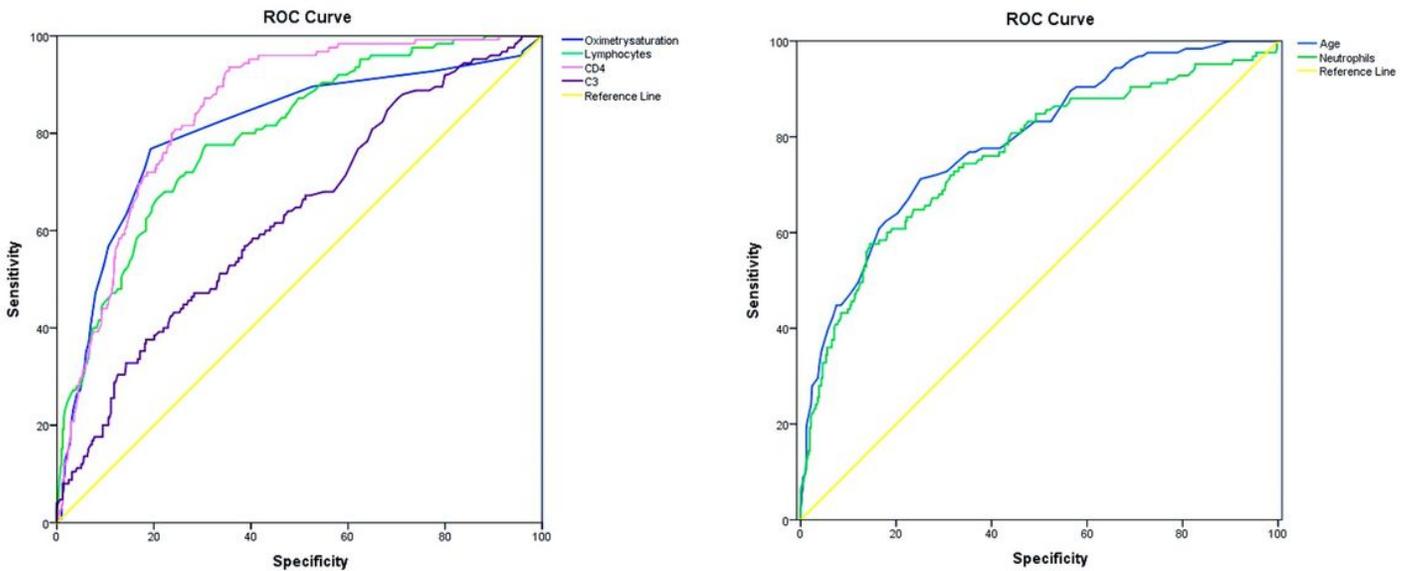


Figure 1

ROC curve model of age, neutrophils, lymphocytes, CD4, C3 and Oximetry saturation in patients with COVID-19.

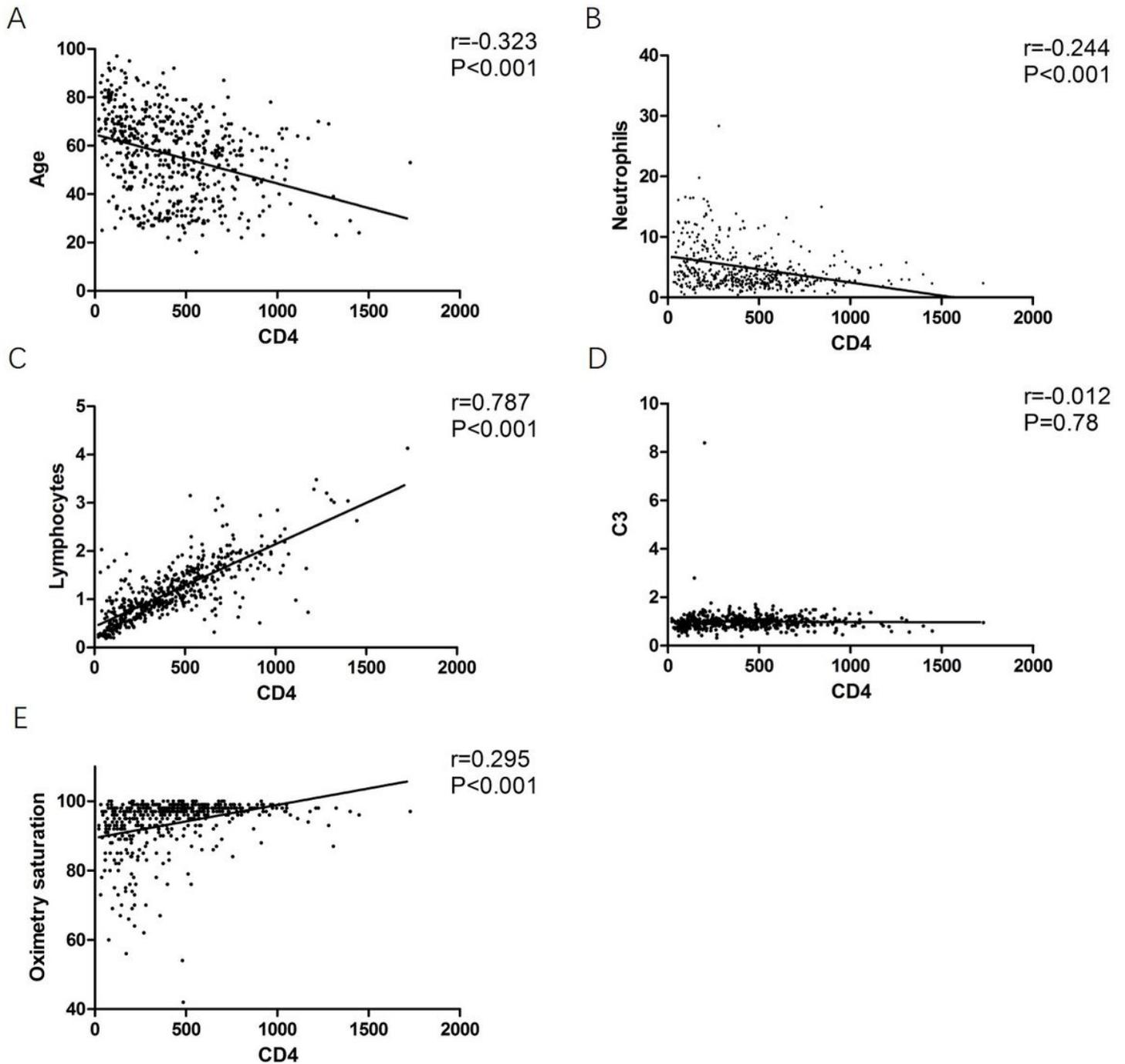


Figure 2

Correlation between CD4 and age (A); CD4 and neutrophils (B); CD4 and lymphocytes (C); CD4 and C3 (D); CD4 and Oximetry saturation (E) of COVID-19 patients. Spearman's test was used to evaluate the correlation. Statistical significance was determined at $P \leq 0.05$.