Heliyon 7 (2021) e08412

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Superiority of lymphocyte ratio over total leukocyte count in detecting the severity of COVID- 19 pneumonia

Rawaa Hadi Shareef [a,*], Zinah Dhiaa Zwain [b], Wadhah Abdulhussian Mahbuba [c]

[a] Department of Pharmacology and Toxicology, College of Pharmacy, University of Kufa, Najaf, Iraq
[b] Medical Education Unit, College of Medicine, University of Kufa, Najaf, Iraq
[c] Department of Surgery, College of Medicine, University of Kufa, Najaf, Iraq

ARTICLE INFO

Keywords:
Coronavirus
Lymphocytes
Monocytes
White blood cells

ABSTRACT

Background: Coronavirus disease (COVID-19) is an infectious disease caused by a recently discovered coronavirus. Blood test including complete blood count is crucial in diagnosing of several viral and bacterial infection.
Aims: This study aimed to assess the association between lymphocyte ratio and other WBC types and severity of COVID-19 pneumonia.
Methods: The design of this study was a cross-sectional study. A complete blood count and erythrocyte sedimentation rate (ESR) was done for one hundred twenty-six COVID-19 patients (76 males and 50 females; aged 20–70 years). Patients were randomly recruited from multicenter in Al-Najaf Governorate, Iraq.
Results: The study had revealed an inverse correlation between severity of COVID-19 infection and both lymphocytes and monocytes ratio even in patients with normal WBC count. Additionally, there was a direct correlation between platelets and leukocyte count. The relation between leukocyte count and ESR level was significant in a patient with elevated WBC only.
Conclusion: Lymphocytes and monocyte ratios inpatient with COVID-19 infection can be used as predictors for the severity of infection. Increased leukocyte count resulted in increases in platelets inpatient with COVID-19.

1. Introduction

Coronaviruses are positive-sense RNA virus, it has a broad range of natural host and they can influence on many systems [1]. In humans, coronavirus can cause many diseases that extend from the common cold to severe respiratory tract infection, such as, severe acute respiratory syndrome (SARS), and middle east respiratory syndrome (MERS) [2].

At the end of 2019, an outbreak of acute respiratory infection was recorded in Wuhan business city of China. At first, the virus is named as (2019-nCoV) or COVID-19 [3]. Then, this virus is named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by the International Committee on Taxonomy of Virus (ICTV) [4].

The SARS-CoV-2 is a member of the order Nidovirales, from the family Coronaviridae and sub-family Orthocoronavirinae. This family can be divided into four kinds: Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronavirus. Based on molecular characterization, the SARS-CoV-2 is considered a new Beta coronavirus that originates from bats and belongs to the subgenus Sarbecovirus [5]. The transmission is thought to occur mainly by respiratory droplets and by contact with contaminated surfaces directly.

The period of incubation of COVID-19 is from 3-6 days [6, 7, 8, 9]. The most common symptoms of SARS-CoV-2 are cough, fever, and fatigue, while headache, hemoptysis, and diarrhea are considered less common symptoms [10, 11]. SARS-COV-2 have a noticeable impact on the immune system. It affects the adaptive immune system by stopping the production of antibodies and the T-cell response. Consequently, it results in inflammation [12]. Additionally, affects lymphocyte count and causing lymphopenia. Cytokine storm syndrome (CSS) could be the sequelae if the adaptive immune system did not control the inflammation within 7–10 days. CSS could be defined as abnormal secretion of inflammatory specific markers, for example, IL-1, IL-2, IL-8, IL-12, IL-18, TNF-α, GM-CSF, TNF-γ in addition to IL-6, which has a suppressive effect on the immune system [13]. Patients with CSS have continuing high fever, liver dysfunction and coagulation system disorder [14].

Hematological parameters and blood biochemical indices as erythrocyte sedimentation rate (ESR), differential WBC count and ratios can

* Corresponding author.
E-mail address: za4389452@gmail.com (R.H. Shareef).

https://doi.org/10.1016/j.heliyon.2021.e08412
Received 7 August 2021; Received in revised form 2 November 2021; Accepted 14 November 2021

give an idea about the severity of COVID-19 disease [15]. Many patients with COVID-19 were found to have either leukocytosis or leukopenia [16].

This study aims to investigate the relationship between COVID-19 and changes in different blood parameters.

2. Materials and methods

2.1. Study design and setting

A cross-sectional study that involved 126 patients (50 females and 76 males, aged between 20-70 years old). All patients had been confirmed COVID-19 by polymerase chain reaction (PCR) for throat and nasal swab. The patients were randomly recruited from multicenter (Al-Hakeem Hospital and Al-Sadder Teaching Hospital) in Al-Najaf governorate, Iraq from July 2020 to September 2020.

2.2. Data collection

From each individual, five mls of venous blood was collected, were placed in two separated tubes. Blood parameters were measured by hematology analyzer (Abbott, USA). The hematology analyzer contains three technologies: electrical impedance, flow cytometry, and fluorescent flow cytometry. The electrical impedance technology gives information about blood cell count and the volume of these cells. The flow cytometry gives information about the blood cells morphology and differentiation of white blood cells. The fluorescent flow cytometry is useful in platelet analysis. Also, we measured erythrocyte sedimentation rate (ESR).

2.3. Ethical approval and patient informed consent

Ethical approval for this study was granted from the Al-Hakeem Hospital and Al-Sadder Teaching Hospital in Al-Najaf City (No. 1105A in June 2020). Informed consent was obtained from all individual participants included in the study.

2.4. Statistical analysis

Statistical analysis was performed by using (Prism 7). Using, percentage, mean, standard error, and correlation analysis. The correlation coefficient "*r*" was used to evaluate the strength of the association between two variables. When the P-value was <0.05 it was considered significant.

3. Results

Table 1 showed the demographic and baseline characteristics of patients infected with COVID-19. Table 2 showed the distribution of white blood cells among the population study. Figure 1a showed that there is a significant positive correlation between white blood cells and ESR (ml/hour) only when the count of white blood cells more than $11,000 \times 10^3$/μL ($r = 0.2$; $P = 0.019$). Figure 1b, showed that there is a highly significant negative correlation between lymphocytes and ESR ($r = 0.36$; $P = 0.22$). In addition, figure 1c showed that there is a significant negative correlation between monocytes and ESR ($r = 0.19$; $P = 0.02$). In figure 1d, the white blood cells showed direct proportion with platelet counts $x10^3$/μL ($r = 0.35$, $p < 0.0001$).

Table 1. Baseline characteristics of patients infected with COVID-19.

Baseline variables		Number	%
Age	18–38	60	47.62
	39–59	53	42.06
	60–80	13	10.32
Sex	Male	76	60.32
	Female	50	39.68
WBC	Normal	81	64.28
	Low	19	15.08
	High	26	20.64
Lymphopenia		47	37.30
Neutrophilia		34	26.98
Monocytosis		20	15.87
Anemia		34	26.95

Table 2. Distribution of white blood cells ($x10^3$ μ/L) in the population study.

White Blood cells	Range	Mean	SE	95% CI
Neutrophils	50.8	65.76	1.147	63.4–68.03
Lymphocytes	45.7	25.2	0.995	23.23–27.17
Monocytes	20.50	7.1	0.298	6.51–7.69
Eosinophil	3.5	0.651	0.069	0.515–0.788
Basophil	3.45	0.90	0.052	0.798–1.007

SE, standard error.

4. Discussion

After rhinoviruses, infection by coronavirus is considered as the second leading cause of the common cold, this infection is seasonal, but the incidence is elevated in the spring and winter [17]. The incubation period of the virus is about 2–5 days, and the infection by the virus occurs primarily by direct person-to-person contact. Many studies have shown, that the binding ability of COVID-19 to an angiotensin-converting enzyme-2 receptor in vivo is 10–20 times that of SARS-COV, which determines that it is transmitted from person to person more easily and causes a global pandemic [17].

The current study shows that there is a relation between corona patients and the presence of lymphopenia (37.7%) with neutrophilia (26.9%) in their blood. These results are probably because most viruses when infect humans cause lymphocytosis as lymphocytes considered as virus-fighting effector cells [18]. All the coronavirus families like SARS-CoV, MERS-CoV, and SARS-CoV-2 cause a decrease in lymphocytes in infected patients [19, 20]. The mechanism behind that may be either attributed to the direct attack of the virus on lymphocytes or by immune-mediated apoptosis of lymphocytes [21, 22, 23]. In addition to that, other mechanisms can back up this result, one of these that angiotensin-converting enzyme 2 receptors (ACE2 receptors) are likely the cell receptor of COVID-19 as well as a receptor for SARS-CoV [24], and for this reason, the cells that exhibit ACE2 receptors are susceptible to SARS-CoV-2 infection. A study done by Xu et al. showed that ACE2 receptors were expressed in different parts of the body such as the lungs, digestive system, and oral mucosa [24].

The direct effect of COVID-19 on the lymphocytes could lead to their lysis. Additionally, infection by COVID-19 induces the production of many cytokines like interleukin-7, interleukin-2, interleukin-6, tumor necrosis factor-alpha, and interferon-gamma that are recognized as a cytokine storm and can lead to lymphocyte apoptosis in addition to atrophy of lymphoid organs [25]. These results were agreed with other studies like [11, 21, 26].

Neutrophils are the most numerous type of circulating white blood cells and are considered an important component of the immune system. In innate immunity, they act as the first line of defense during bacterial and fungal infection. By phagocytosis as well as neutrophil extracellular trap (NET) formation, these cells play an important protective role in killing these microorganisms. However, the role of it in viral infections is still unclear. It has been found that neutrophils are not necessary for viral clearance from pulmonary cells and host survival in mice infected by SARS-CoV [27]. However, an inhuman, affected person by COVID-19 exhibited extensive infiltration of neutrophils in pulmonary capillaries with extravasation into the alveolar space. So, the presence of acute

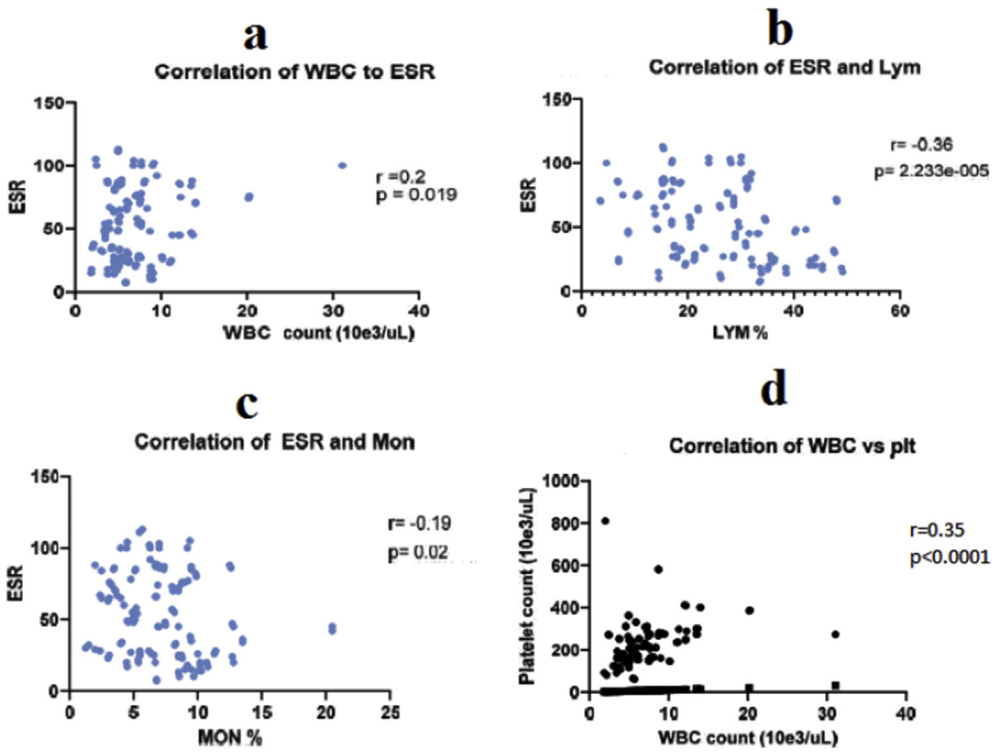

Figure 1. a) Correlation between WBC and erythrocyte sedimentation rate (ESR); b) Correlation between ESR and lymphocytes; c) Correlation between ESR and monocytes; d) Correlation between WBC vs. platelet count.

capillaritis, as well as tracheal neutrophilic mucositis, confirms the extent of inflammation across the airways [27].

Moreover, also found that the count of red blood cell of COVID-19 patients was low in comparison with the normal reference value. This result may be due to the infection of COVID-19 caused inhibition of hematopoiesis in the bone marrow of patients. A study by Andrey Prilutskiy et al. state autopsy results in four affected patients. They found that within the pulmonary lymph nodes, three of them had histologic evidence of hemophagocytosis, while the other one showed no hemophagocytosis in the liver or bone marrow, but only in the spleen, there is a hemophagocytosis. This anemia could be due to hemophagocytosis [28].

As a results the lymphocytes ratio in COVID-19 pneumonia can be a better predictor for the severity of infection than WBC count.

In COVID-19 pneumonia, there are an excessive releasing of many inflammatory cytokines and chemokine such as tumor necrosis factor-α, interleukin-1, interleukin-6, and interleukin-8 [29]. Macrophage, endothelial cells, and neutrophils are activated by the release of these inflammatory molecules and results in the expression of tissue factors within the lungs, which is resulting in initiation and enhancing the pulmonary coagulopathy and microvascular thrombosis [30]. Interleukin-6 is considered as a key cytokine in severe COVID-19 infection as well as is a key activator of coagulopathy by inducing the expression of tissue factors and increasing production of fibrinogen and platelets [31, 32]. Data revealed that there is a significant positive correlation between WBC and platelet count and this confirm that the more severe infection mean higher platelet count. Interestingly, the monocytes ratio was inversely related to ESR level. Accordingly, the monocytes ratio could be a good indicator of infection severity in those patients. The monocytes cells consider as cells of innate immunity [33], that have an important role in phagocytosis, inflammatory responses, antigen presentation, and other immune functions. Additionally, during sterile and non-sterile inflammation, the monocytes can extravasate into peripheral tissues and differentiate into macrophages or dendritic cells. Also, based on their relative expression of CD14 and CD16, these cells are consisting of three subsets; these are commonly identified as classical (CD14 + CD16−), intermediate (CD14 + CD16+), and non-classical (CD14dimCD16+) subtypes [34]. A study by Zhou et al. showed that in peripheral blood of patients diagnosed with COVID-19, there is a significant increase in the circulating proportions of CD14 + CD16 + monocytes. Also, noted that there is a significant increase in monocytes producing interleukin-6 in patients with mild COVID19, and in patients with severe infection, the monocytes increased and this result suggests that monocytes may be a key contributor to cytokine storm in COVID-19 [35].

One limitations in this study is the used of general classification of WBC. However, our monitoring guideline which used routinely in our hospitals rely on CBC, ESR, Interleukin and D-dimer, but not routinely done for patients with COVID. So, we depend on what routinely cheap and easy to be done which is CBC. We believe that, our paper is to get a routine marker that be used in our Iraqi hospitals and in the middle to low income countries. So, we are implemented that lymphocytes counting can be a good predictor for COVID-19 and our results can confirm that. However, the sub classification of lymphocytes can be done for further evaluation in another study. Another limitations is small sample size. At time of data collection, the total number of confirmed cases of COVID-19 was limited. So, the number of patients that included in our study can be considered sufficient in comparison to the total numbers. Additionally, the statistic that have been used resulted in a significant P value. From the above, we believe the sample size can be considered enough to conclude the finding.

5. Conclusion

Lymphocytes and monocyte ratios inpatient with COVID-19 infection can be used as predictors for the severity of infection. Increased leukocyte count resulted in increases in platelets inpatient with COVID-19.

6. Limitation

The study has many of limitations which are:

- Low budget for our health system because our country is part of middle income-countries.
- Outdated health system (health system is an old fashion system in Iraq).
- Absence of large centers of intensive care in Iraq.
- Lack of proper registration and follow up (electronic registration of patient's data not found in Iraq).
- Lack of research teams inside hospital and highly sophisticated investigations.
- Difficulty in accessing and long term follow up to the majority of the patients after discharge from hospital because most of them never return to hospital or going to the private clinic or refuse follow up.

Declarations

Author contribution statement

Wadhah Mahbuba: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Rawaa Shareef: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Zinah Zwain: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

[1] S.R. Weiss, J.L. Leibowitz, Coronavirus pathogenesis, Adv. Virus Res. 81 (2011) 85–164.
[2] R. Lu, X. Zhao, J. Li, P. Niu, B. Yang, H. Wu, et al., Genomic characterization and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding, Lancet 395 (10224) (2020 Feb 22) 565–574.
[3] W.J. Guan, Z.Y. Ni, Y. Hu, W.H. Liang, C.Q. Ou, J.X. He, et al., China medical treatment expert group for covid-19. Clinical characteristics of coronavirus disease 2019 in China, N. Engl. J. Med. 382 (18) (2020 Apr 30) 1708–1720.
[4] Coronaviridae Study Group of the International Committee on Taxonomy of Viruses., A.E. Gorbalenya, S.C. Baker, et al., The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2, Nat. Microbiol. 5 (2020) 536–544.
[5] N. Zhu, D. Zhang, W. Wang, X. Li, B. Yang, J. Song, et al., China novel coronavirus investigating and research team. A novel coronavirus from patients with pneumonia in China, 2019, N. Engl. J. Med. 382 (8) (2020 Feb 20) 727–733.
[6] J.F. Chan, S. Yuan, K.H. Kok, K.K. To, H. Chu, J. Yang, et al., A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster, Lancet 395 (10223) (2020 Feb 15) 514–523.
[7] Sonia Mor, Prem Saini, Subhash Kumar Wangnoo, T. Bawa, Worldwide spread of COVID-19 Pandemic and risk factors among Co-morbid conditions especially Diabetes Mellitus in India, Res. J. Pharm. Technol. 13 (5) (2020) 2530–2532.
[8] Sarfaraz Ahmad, Ambreen Shoaib, Sajid Ali, Sarfaraz Alam, Nawazish Alam, Maksood Ali, Mujtaba Ali, Ayaz Ahmad, Salahuddin Ansari, Mohammad Daud Ali, Epidemiology, risk, myths, pharmacotherapeutic management and socio economic burden due to novel COVID-19: a recent update, Res. J. Pharm. Technol. 13 (9) (2020) 4435–4442.
[9] Abbas Kinbar Kuser, Sagban Marja Tarar, Omar Mansib Kassid, Nael Hussein Zayer, CNS and COVID-19: neurological symptoms of Hospitalized Patients with Coronavirus in Iraq: a surveying case sequences study, Res. J. Pharm. Technol. 13 (12) (2020) 6291–6294.
[10] K. Dhama, S. Khan, R. Tiwari, S. Sircar, S. Bhat, Y.S. Malik, et al., Coronavirus disease 2019–COVID-19, Clin. Microbiol. Rev. 33 (2020) e00028, 20.
[11] C. Huang, Y. Wang, X. Li, L. Ren, J. Zhao, Y. Hu, et al., Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China, Lancet 395 (10223) (2020 Feb 15) 497–506.
[12] B.G. Chousterman, F.K. Swirski, G.F. Weber, Cytokine storm and sepsis disease pathogenesis, in: Seminars in Immunopathology 5, Springer, 2017, pp. 517–552.
[13] Y. Zhou, C. He, L. Wang, B. Ge, Post-translational regulation of antiviral innate signaling, Eur. J. Immunol. 47 (2017) 1414–1426.
[14] S. Shabbir, M.H. Raza, M. Arshad, et al., The interplay between the immune system and SARS-CoV-2 in COVID-19 patients, Arch. Virol. 166 (2021) 2109–2117.
[15] Y. Liu, Y. Yang, C. Zhang, F. Huang, F. Wang, J. Yuan, et al., Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury, Sci. China Life Sci. 63 (3) (2020 Mar) 364–374.
[16] A.J. Rodriguez-Morales, et al., Clinical, Laboratory and Imaging Features of COVID-19: A Systematic Review and Meta-Analysis', Travel Medicine and Infectious Disease 34, Elsevier, 2020, p. 101623 (February).
[17] X. Yuan, W. Huang, B. Ye, C. Chen, R. Huang, F. Wu, et al., Changes of hematological and immunological parameters in COVID-19 patients, Int. J. Hematol. 112 (4) (2020 Oct) 553–559.
[18] D. Wrapp, N. Wang, K.S. Corbett, J.A. Goldsmith, C.L. Hsieh, O. Abiona, et al., Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation, Science 367 (6483) (2020) 1260–1263.
[19] Y. Zhu, X. Cao, G. Tao, W. Xie, Z. Hu, D. Xu, The lymph index: a potential hematological parameter for viral infection, Int. J. Infect. Dis. 17 (7) (2013), e490 e493493.
[20] J.A. Al-Tawfq, K. Hinedi, S. Abbasi, M. Babiker, A. Sunji, M. Eltigani, Hematologic, hepatic, and renal function changes in hospitalized patients with Middle East respiratory syndrome coronavirus, Int. J. Lab. Hematol. 39 (3) (2017) 272–278.
[21] A.A. Rabaan, S.H. Al-Ahmed, S. Haque, R. Sah, R. Tiwari, Y.S. Malik, et al., SARS-CoV-2, SARS-CoV, and MERS-COV: a comparative overview, Infezioni Med. Le 28 (2) (2020) 174–184.
[22] N.S. Panesar, What caused lymphopenia in SARS and how reliable is the lymphokine status in glucocorticoid-treated patients? Med. Hypotheses 71 (2) (2008) 298–301.
[23] H. Chu, J. Zhou, B.H. Wong, C. Li, J.F. Chan, Z.S. Cheng, et al., Middle East respiratory syndrome coronavirus efficiently infects human primary T lymphocytes and activates the extrinsic and intrinsic apoptosis pathways, J. Infect. Dis. 213 (6) (2016) 904–914.
[24] H. Xu, L. Zhong, J. Deng, J. Peng, H. Dan, X. Zeng, et al., High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa, Int. J. Oral Sci. 12 (2020) 8.
[25] E. Terpos, I. Ntanasis-Stathopoulos, I. Elalamy, E. Kastritis, T.N. Sergentanis, M. Politou, et al., Hematological findings and complications of COVID- 19, Am. J. Hematol. 95 (2020) 834–847.
[26] Al-Naqeeb OM. Analysis of COVID-19 outbreak in Iraq from the perspective of doctors working in Iraqi hospitals. Cough.;60:55-56.
[27] B. Tomar, H.J. Anders, J. Desai, S.R. Mulay, Neutrophils and neutrophil extracellular traps drive necroinflammation in COVID-19, Cells 9 (6) (2020 Jun 2) 1383.
[28] A. Prilutskiy, M. Kritselis, A. Shevtsov, I. Yambayev, C. Vadlamudi, Q. Zhao, et al., SARS-CoV-2 Infection-Associated Hemophagocytic Lymphohistiocytosis: an Autopsy Series with Clinical and Laboratory Correlation, medRxiv, 2020.
[29] C. Qin, L. Zhou, Z. Hu, S. Zhang, S. Yang, Y. Tao, et al., Dysregulation of immune response in patients with COVID-19 in Wuhan, China, Clin. Infect. Dis. 71 (15) (2020) 762–768.
[30] D. McGonagle, J.S. O'Donnell, K. Sharif, P. Emery, C. Bridgewood, Immune mechanisms of pulmonary intravascular coagulopathy in COVID-19 pneumonia, Lancet Rheumatol. 2 (2020) e437–e445.
[31] M. Ranucci, A. Ballotta, U. Di Dedda, E. Bayshnikova, M. Dei Poli, M. Resta, et al., The procoagulant pattern of patients with COVID-19 acute respiratory distress syndrome, J. Thromb. Haemostasis 18 (2020) 1747–1751.
[32] B. Liu, M. Li, Z. Zhou, X. Guan, Y. Xiang, Can we use interleukin-6 (IL-6) blockade for coronavirus disease 2019 (COVID-19)-induced cytokine release syndrome (CRS)? J. Autoimmun. (2020) 102452.
[33] C.V. Jakubzick, G.J. Randolph, P.M. Henson, Monocyte differentiation and antigen-presenting functions, Nat. Rev. Immunol. 17 (2017) 349–362.
[34] L. Ziegler-Heitbrock, P. Ancuta, S. Crowe, M. Dalod, V. Grau, D.N. Hart, et al., Nomenclature of monocytes and dendritic cells in the blood, Blood 116 (2010) e74–80.
[35] Y. Zhou, B. Fu, X. Zheng, D. Wang, C. Zhao, Y. qi, et al., Pathogenic T cells and inflammatory monocytes incite inflammatory storm in severe COVID-19 patients, Natl. Sci. Rev. (2020 Mar 13) nwaa041.