LUPINE PUBLISHERS

Current Trends on Biotechnology & Microbiology

DOI: 10.32474/CTBM.2020.01.000121

ISSN: 2641-6875

Research Article

Thoughts on the Reference Range of Laboratory Tests

Shi-min Zhang[1] and Ying Lan[2]*

[1]*Department of Laboratory Medicine, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College China*

[2]*State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences, China*

***Corresponding author:** Ying Lan, State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences, Beijing, 100101, China

Received: February 17, 2020 **Published:** June 17, 2020

Abstract

This article discusses the value research data in Guan's paper published on medRxiv, February 9, 2020. In terms of experimental data, the preprinted paper is by far the largest sample number of 2019-new coronavirus (SARS-CoV-2) infected patients. Including 552 hospitals from 31 provinces in China as of January 29, 2020, a total of 1,099 cases. Laboratory evaluations include multiple blood cell counts, blood chemistry, coagulation tests, liver and kidney function, electrolytes, C-reactive protein, calcitonin, lactate dehydrogenase, and creatine kinase. We will focus on the reference value determination of blood routine data.

Keywords: Reference range; 2019-nCoV, SARS-CoV-2, 2019-nCoV ARD, COVID-19

Introduction

Professor Guan et al. [1] recently published an article "clinical characteristics of 2019 novel coronavirus infection in china". It is a preprinted version of medRxiv on BMJ publishing platform. The authors of the article covered all frontline anti-epidemic frontlines including Guangzhou Institute of Respiratory Disease, Wuhan Jin-yintan Hospital, Institute of Hematology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, The Central Hospital of Wuhan, Huanggang Central Hospital, Department of Medicine and Therapeutics, et al. The Chinese University of Hong Kong. In this retrospective study, the authors extracted the data on 1,099 patients with laboratory-confirmed 2019-nCoV ARD (COVID-19) from 552 hospitals in 31 provinces/provincial municipalities through January 29th, 2020. So far, it is the largest number of cases collected, the clinical characteristics of the patients with the most complete distribution, and the most comprehensive Chinese experience of SARS-CoV-2 treatment are released to the world.

In the preprinted paper, the authors found that fever occurred in only 43.8% of patients with 2019-nCoV ARD (COVID-19) but developed in 87.9% following hospitalization. Demographic data showed a median age of 47.0 years (IQR, 35.0-58.0 years). 2.09% were healthcare workers. The median incubation period was 3.0 days (range, 0 to24.0). Patients who underwent chest computed tomography on admission, 76.4% manifested as pneumonia. There are normal radiological manifestations in some infected patients. In addition, the researchers obtained a series of laboratory findings, such as 82.1% and 36.2% of patients had lymphopenia and thrombocytopenia, respectively. Overall, leukopenia was observed in 33.7% of patients. Most patients demonstrated elevated levels of C-reactive protein, rare increases in D-dimer levels, and so on. The standards used by the authors in analyzing laboratory data do not meet the standards of the People's Republic of China's health industry (WS/T 405-2012). We will take this article "Clinical Characteristics of New Coronavirus Infections in China 2019"as an example to explore the reference scope of laboratory test.

Methods and Results

The report delineated 1,099 patients with 2019-nCoV ARD (COVID-19) from 552 hospitals in 31 provinces/province-level municipalities. Cases were diagnosed based on the WHO interim guidance [2]. Patients were classified into severe and non-serious 2019-nCoV ARD (COVID-19) based on the guideline of the American Thoracic Society and Infectious Disease Society of America [3]. Radiologic and laboratory findings was also based on the grouping of severe and non-severe case patients. Table 1 showed lymphopenia

was observed in 82.1% of patients on admission (79.3% in non-severe cases; 95.5% in severe cases), and thrombocytopenia in 36.2% (31.6% in non-severe cases; severe cases 57.7%). Overall, leukopenia was observed in 33.7% of patients (28.1% in non-severe cases; 61.1% in severe cases).

Hemoglobin (Hb) showed almost no significant decrease. There are significant differences of Hb level between non-serious cases and severe cases. It should be noted that only 978 of the 1099 cases provided complete blood routine test data, while the data of absolute value of lymphocytes and platelet counts were less than 978 cases. This indicating that there is no uniform requirement for the integrity of test data.

Most patients (60.7%) demonstrated elevated levels of C-reactive protein levels (56.4% in non-severe cases; 81.5% in severe cases), rare increases in alanine aminotransferase, aspartate aminotransferase, creatine kinase, and D-dimer. The authors only provided 560 cases of the D-dimer detection value, in which 46.4% patients manifest the increase tendency (43.2% in non-severe cases; 59.6% in severe cases). Severe cases had more prominent laboratory abnormalities (i.e., leukopenia, lymphopenia, thrombocytopenia, elevated C-reactive protein levels) as compared with non-severe cases (all P<0.05).

Discussion

In terms of experimental data, the preprinted paper is by far the largest sample number of 2019-new coronavirus (SARS-CoV-2) infected patients. We noticed that the author analyzed laboratory data, especially blood routine test data, and reached conclusions such as leukopenia, lymphopenia, thrombocytopenia, and elevated C-reactive protein in COVID-19 patients according to certain criteria, which is not conform to the standards of the People's Republic of China's health industry document WS/T405-2012.

For example, the leukopenia defined in the article is based on less than 4.0×109/L; the lymphocyte reduction is based on less than 1.5×109/L; the thrombocytopenia is based on less than 150×109/L; and the C-reactive protein level is based on be equal or greater than 10mg/L was abnormal (Table 1).

Table 1: Radiographic and laboratory findings of 1,099 patients with 2019-nCoV ARD.

Radiologic and Laboratory Findings	All Patients (n=1099)	Disease Severity			Composite Endpoint		
		Non-severe (n=926)	Severe (n=173)	P value	Yes (n=67)	No (n=1032)	P value
Radiologic Findings							
Abnormalities on chest X-ray-No./ total No. (%) Ground-glass opacity Local patchy shadowing Bilateral patchy shadowing Interstitial abnormalities	162/1099 (14.7) 55/1099 (5.0) 77/1099 (7.0) 100/1099 (9.1) 12/1099 (1.1)	116/926 (12.5) 37/926 (4.0) 56/926 (6.0) 65/926 (7.0) 7/926 (0.8)	46/173 (26.6) 18/173 (10.4) 21/173 (12.1) 35/173 (20.2) 5/173 (2.9)	<0.001 <0.001 0.007 <0.001 0.028	30/67 (44.8) 9/67 (13.4) 13/67 (19.4) 27/67 (40.3) 6/67 (9.0)	132/1032 (12.8) 46/1032 (4.5) 64/1032 (6.2) 73/1032 (7.1) 6/1032 (0.6)	<0.001 0.005 <0.001 <0.001 <0.001
Abnormalities on chest CT-No./total No. (%) Ground-glass opacity Local patchy shadowing Bilateral patchy shadowing Interstitial abnormalities	840/1099 (76.4) 550/1099 (50.0) 409/1099 (37.2) 505/1099 (46.0) 143/1099 (13.0)	682/926 (73.7) 449/926 (48.5) 317/926 (34.2) 368/926 (39.7) 99/926 (10.7)	158/173 (91.3) 101/173 (58.4) 92/173 (53.2) 137/173 (79.2) 44/173 (25.4)	<0.001 0.021 <0.001 <0.001 <0.001	50/67 (74.6) 30/67 (44.8) 22/67 (32.8) 40/67 (59.7) 15/67 (22.4)	790/1032 (76.6) 520/1032 (50.4) 387/1032 (37.5) 465/1032 (45.1) 128/1032 (12.4)	0.833 0.445 0.525 0.028 0.03
Laboratory Findings							
Median PaO_2: FiO_2 (interquartile range)	3.9 (2.9-4.7)	3.9 (2.9-4.5)	4.0 (2.8-5.2)	0.386	2.9 (2.2- 5.4)	4.0 (3.1- 4.6)	0.15
Blood leukocyte count $>10*10^9$/L $<4*10^9$/L	4.7 (3.5- 6.0) 58/978 (5.9) 330/978 (33.7)	4.9 (3.8-6.0) 39/811 (4.8) 228/811 (28.1)	3.7 (3.0-6.2) 19/167 (11.4) 102/167 (61.1)	<0.001 0.002 <0.001	6.1 (4.9- 11.1) 15/58 (25.9) 8/58 (13.8)	4.7 (3.5- 5.9) 43/920 (4.7) 322/920 (35.0)	<0.001 <0.001 0.002
Lymphocyte count $< 1.5*10^9$/L	1.0 (0.7- 1.3) 731/890 (82.1)	1.0 (0.8-1.4) 584/736 (79.3)	0.8 (0.6-1.0) 147/154 (95.5)	<0.001 <0.001	0.7 (0.6- 0.9) 50/54 (92.6)	1.0 (0.7- 1.4) 684/836 (81.5)	<0.001 0.059

Platelet count <150*10⁹/L	168.0(132.0-207.0) 315/869 (36.2)	172.0 (139.0-212.0) 225/713 (31.6)	137.5 (99.0-179.5) 90/156 (57.7)	<0.001 <0.001	156.5 (114.2-195.0) 27/58 (46.6)	169.0 (133.0-207.0) 288/811 (35.5)	0.067 0.122
Haemoglobin level-g/dl	134.0 (119.0-148.0)	135.0 (120.0-148.0)	128.0 (111.8-141.0)	<0.001	125.0 (105.0-140.0)	134.0 (120.0-148.0)	0.012
C-reactive protein level ≥10 mg/liter-No./total No. (%)	481/793 (60.7)	371/658 (56.4)	110/135 (81.5)	<0.001	41/45 (91.1)	440/748 (58.8)	<0.001
Procalcitonin level ≥0.5 ng/ml-No./total No. (%)	35/633 (5.5)	19/516 (3.7)	16/117 (13.7)	<0.001	12/50 (24.0)	23/583 (3.9)	<0.001
Lactose dehydrogenase ≥250 U/liter-No./total No. (%)	277/675 (41.0)	205/551 (37.2)	72/124 (58.1)	<0.001	31/44 (70.5)	246/631 (39.0)	<0.001
Aspartate aminotransferase >40 U/liter-No./total No. (%)	168/757 (22.2)	112/615 (18.2)	56/142 (39.4)	<0.001	26/52 (50.0)	142/705 (20.1)	<0.001
Alanine aminotransferase >40 U/liter-No./total No. (%)	158/741 (21.3)	120/606 (19.8)	38/135 (28.1)	0.043	20/49 (40.8)	138/692 (19.9)	0.001
Total bilirubin 17.1 μmol/liter-No./total No. (%)	76/722 (10.5)	59/594 (9.9)	17/128 (13.3)	0.337	10/48 (20.8)	66/674 (9.8)	0.03
Creatinine kinase ≥200 U/liter-No./total No. (%)	90/657 (13.7)	67/536 (12.5)	23/121 (19.0)	0.083	12/46 (26.1)	78/611 (12.8)	0.021
Creatinine ≥133 μmol/liter-No./total No. (%)	12/752 (1.6)	6/614 (1.0)	6/138 (4.3)	0.012	5/52 (9.6)	7/700 (1.0)	<0.001
D-dimer ≥0.5 mg/liter-No./total No. (%)	260/560 (46.4)	195/451 (43.2)	65/109 (59.6)	0.003	34/49 (69.4)	226/511 (44.2)	0.001
Sodium-mmol/liter	138.2 (136.1-140.3)	138.4 (136.6-140.4)	138.0 (136.0-140.0)	0.09	138.3 (135.0-141.2)	138.2 (136.1-140.2)	0.997
Potassium-mmol/liter	3.8 (3.5- 4.2)	3.9 (3.6-4.2)	3.8 (3.5-4.1)	0.044	3.9 (3.6- 4.1)	3.8 (3.5- 4.2)	0.854
Chloride-mmol/liter	102.9 (99.7-105.6)	102.7 (99.7-105.3)	103.1 (99.8-106.0)	0.206	103.8 (100.8-107.0)	102.8 (99.6-105.3)	0.092

Plus-minus values are means ± SD. Lymphopenia denoted the lymphocyte count of less than 1,500 per cubic millimeter. Thrombocytopenia denoted the platelet count of less than 150,000 per cubic millimeter. PaO_2:FiO_2 was defined as the ratio of the partial pressure of arterial oxygen to the fraction of inspired oxygen.

P values denoted the comparison between mild-moderate cases and severe cases.

As for D-dimer, the author took greater than or be equal 0.5mg/L as the standard for abnormal. However, the different hospitals in China, the difference in methods and reagents used, it is generally considered that the reference interval is less than 0.3mg/L or less than 0.5mg/L. The article uniformly took be equal or greater than 0.5mg/L as the standard for abnormal elevation. Obviously, the 552 hospitals as the participating units are unlikely to use exactly the same methods and reagents.

Table 2: Reference ranges for blood cell analysis in Chinese adult population.

Item1	Item2	Unit	Sexual	Reference range
白细胞计数 （WBC）	White Blood Cell	×10⁹/L	M/F	3.5~9.5
中性粒细胞绝对值（Neut#）	Neutrophil#	×10⁹/L	M/F	1.8~6.3
淋巴细胞绝对值（Lymph#）	Lymphocyte#	×10⁹/L	M/F	1.1~3.2
嗜酸性粒细胞绝对值（Eos#）	Eosinophil#	×10⁹/L	M/F	0.02~0.52
嗜碱性粒细胞绝对值（Baso#）	Basophil#	×10⁹/L	M/F	0~0.06
单核细胞绝对值（Mono#）	Monocyte#	×10⁹/L	M/F	0.1~0.6
中性粒细胞百分数（Neut%）	Neutrophil%	%	M/F	40~75
淋巴细胞百分数（Lymph%）	Lymphocyte%	%	M/F	20~50
嗜酸性粒细胞百分数（Eos%）	Eosinophil%	%	M/F	0.4~8.0
嗜碱性粒细胞百分数（Baso%）	Basophil%	%	M/F	0~1
单核细胞百分数（Mono%）	Monocyte%	%	M/F	3~10
红细胞计数（RBC）	Red Blood Cell	×10¹²/L	M	4.3~5.8
			F	3.8~5.1

血红蛋白（Hb）	Hemaglubin	g/L	M	130~175
			F	115~150
红细胞比容（Hct）	Hematocrit	L/L	M	0.40~0.50
			F	0.35~0.45
平均红细胞容积（MCV）	Mean corpuscular volume	fL	M/F	82~100
平均红细胞血红蛋白量（MCH）	Mean corpuscular hemoglobin	Pg	M/F	27~34
平均红细胞血红蛋白浓度（MCHC）	Mean corpuscular hemoglobin concentration	g/L	M/F	316~354
血小板计数（PLT）	Platelet	$\times 10^9$/L	M/F	125~350
注：此参考区间适用于静脉血的仪器检测方法 Note: This reference interval is suitable for instrumental detection of venous blood				

The China national industry standard blood routine test reference range was released in 2012, and it has been nearly eight years. The following table lists the reference range for the analysis of venous blood in the Chinese adult population for instrumental detection methods (Table 2).The preprinted article did not adopt this health industry standard, which shows that the criterion has not been accepted and recognized by Chinese clinicians. It is a very important topic whether national industry standards, especially reference ranges, are recognized by the majority of Chinese clinicians. Reference range is the basis for clinicians diagnosing diseases.

In view of the fact that the research object is Chinese, we suggest that researchers should try to adopt the domestic recommended standards instead of using the previous concepts and foreign standards. If domestic industry standards are used in this article, their statistical results and proportions may be quite different. In particular, the absolute value of lymphocytes should be significantly different, which may lead to large differences in conclusions. As we all know, the new coronavirus epidemic recently occurred in Wuhan city, Hubei Province, China has spread to other parts of China and abroad. As an acute respiratory infectious disease, the disease has been included in the Class B infectious diseases stipulated in the Law of the People's Republic of China on the Prevention and Control of Infectious Diseases and is managed as a Class A infectious disease. The National Health Committee of the People's Republic of China has continuously revised the "Diagnosis and Treatment Program for Pneumonia of New Coronavirus Infection", which is currently in its fifth edition on a trial basis. The guidelines point out that the diagnosis of suspected cases of COVID-19 should be combined with a comprehensive analysis of epidemiological history and clinical manifestations.

The clinical manifestations include

1. Fever and / or respiratory symptoms
2. The imaging characteristics of pneumonia described above (abbreviated in this article)
3. The total number of white blood cells is normal or decreased in the early stage of onset, or the lymphocyte count is decreased.

Any case with epidemiological history that meets any two of the clinical manifestations can be determined as a suspected case. Once a misdiagnosis occurs due to a misjudgment of the reference value range, it is likely to cause severe unforeseen consequences.

The biochemical test results involved in the preprint version of the article also did not adopt domestic standards, and the determination of many biochemical indicators is also related to gender, which are issues that clinicians should pay attention to. If the author team includes medical laboratory experts, I believe that the aforementioned laboratory data and reference ranges, and methodological flaw may be avoided. As stated by the authors, some cases had incomplete documentation of the exposure history, symptoms and laboratory testing given the variation in the structure of electronic database among different participating site and the urgent timeline for data extraction. Some cases were diagnosed in out-patient settings where medical information was briefly documented, and incomplete laboratory testing was applied.

On the other hand, in clinical laboratory practice, we recommend that laboratory technician communicate with clinicians to scientifically explain the clinical significance and reference range of laboratory data. Only in this way, a new reference range can be adopted and applicated in the hospital. Secondly, whether it is fully adopted or partially adopted, it depends on how technicians connect closely with the clinician and perform corresponding verification. The national industry standard is only a recommended standard, not a mandatory standard. Clinicians are generally rigorous when selecting a standard for disease diagnosis. The preprint article illustrates exactly this problem. When clinicians judge the test data, they have not adopted our health industry standards as the judgment standard. It is a typical example of the disconnection between laboratory and clinical. This is a problem worthy of laboratory physician's reflection and deep thought.

Acknowledgement

This work was supported by the National Key Research and Development Program of China 2016YFE0205800, and Open Research Fund of State Key Laboratory of Bioelectronics, Southeast University.

References

1. Guan W, Ni Z, Hu Y, Liang W, Ou C, et al. (2020) Clinical characteristics of 2019 novel coronavirus infection in China. medRxiv.
2. Metlay JP, Waterer GW, Long AC, Anzueto A, Brozek J, et. al. (2019) Diagnosis and treatment of adults with community-acquired pneumonia: An official clinical practice guideline of the American Thoracic Society and Infectious Disease Society of America. Am J Respir Crit Care Med 200(7): e45-e67.
3. World Health Organization (2020) Clinical management of severe acute respiratory infection when Novel coronavirus (nCoV) infection is suspected: interim guidance, Switzerland.

DOI: 10.32474/CTBM.2020.01.000121