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Routine blood tests as a potential diagnostic tool for COVID-19

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Abstract

Objectives: The outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) to date, the epidemic has gradually spread to 209 countries worldwide with more than 1.5 million infected people and 100,000 deaths. Amplification of viral RNA by rRT-PCR serves as the gold standard for confirmation of infection, yet it needs a long turnaround time (3–4 h to generate results) and shows false-negative rates as large as 15%–20%. In addition, the need of certified laboratories, expensive equipment and trained personnel led many countries to limit the rRT-PCR tests only to individuals with pronounced respiratory syndrome symptoms. Thus, there is a need for alternative, less expensive and more accessible tests.

Methods: We analyzed the plasma levels of white blood cells (WBCs), platelets, C-reactive protein (CRP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (GGT), alkaline phosphatase and lactate dehydrogenase (LDH) of 207 patients who, after being admitted to the emergency room of the San Raffaele Hospital (Milan, Italy) with COVID-19 symptoms, were rRT-PCR tested. Of them, 105 tested positive, whereas 102 tested negative.

Results: Statistically significant differences were observed for WBC, CRP, AST, ALT and LDH. Empirical thresholds for AST and LDH allowed the identification of 70% of either COVID-19-positive or -negative patients on the basis of routine blood test results.

Conclusions: Combining appropriate cutoffs for certain hematological parameters could help in identifying false-positive/negative rRT-PCR tests. Blood test analysis might be used as an alternative to rRT-PCR for identifying COVID-19-positive patients in those countries which suffer from a large shortage of rRT-PCR reagents and/or specialized laboratory.

Keywords: aspartate aminotransferase; blood test; COVID-19; lactate dehydrogenase; RT-PCR; WBC.

Introduction

At present the world is overwhelmed by a pandemic disease caused by a novel coronavirus which emerged in Wuhan, Hubei, China at the end of December 2019, named as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. The disease is sustained by a novel coronavirus named COVID-19 by the World Health Organization. To date, the epidemic has gradually spread to 208 countries worldwide with almost 1.5 million infected people and more than 70,000 deaths [2], both of which are rapidly increasing.

The disease urged governments to take drastic measures like the quarantine of hundreds of millions of residents worldwide. However, because of the COVID-19 symptomatology, which showed a large number of asymptomatics [3], these efforts are limited by the problem of differentiating between COVID-19-positive and -negative individuals.

The nucleic acid test serves as the gold standard method for the etiological diagnosis of SARS-CoV-2 infection. However, the large demand for rRT-PCR tests due to the worldwide extension of the virus is highlighting the limitations of this type of diagnosis on a large scale such as the long turnaround times (on average over 2–3 h to generate results) and the need of certified laboratories, expensive equipment and trained personnel [4]. In addition, rRT-PCR includes general analytical and preanalytical issues which may jeopardize the diagnostic accuracy of the test [5]. Yet several recent studies have reported as much as 20% false-negative results for this type of test [6–8]. These limitations make rRT-PCR unsuitable for a fast and large-scale screening aiming to a rapid diagnosis of patients. Such limitations become even more emphasized in those countries with limited resources like developing countries. Thus, the urgent need for alternative tests

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to quickly identify infected SARS-CoV-2 patients in order to prevent virus transmission and guarantee a prompt treatment for patients.

In Italy, where the infected people are over 100,000 with more than 10,000 deaths, the shortage of reagents and specialized laboratory forced the government to limit the swab test to those people who clearly show symptoms of severe respiratory syndrome. Thus, the over 100,000 infected people is a largely underestimated number.

Recent studies showed that a few hematologic parameters were clearly altered in COVID-19 patients [9, 10]. For instance, Liu et al. [11] showed a high level of transaminases and LDH in Chinese COVID-19 patients.

In our study, we analyzed the blood test results of 207 patients who, after being admitted to the San Raffaele Hospital (Milan, Italy) emergency room with COVID-19 symptoms, were tested for rRT-PCR. Of them, 105 tested positive whereas 102 tested negative. We analyzed the plasma levels of white blood cells (WBCs), platelets, C-reactive protein (CRP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) in the two groups, with the aim of highlighting statistically significant differences which could be useful for the identification of positive and negative COVID-19 patients. Thus, a simple blood test might help in identifying false-positive/negative rRT-PCR tests as well as play a crucial role in the mass screening of potential COVID-19-infected individuals in those countries which suffer from a large shortage of rRT-PCR reagents and/or specialized laboratory.

Materials and methods

The WBC, platelets, CRP, AST, ALT, GGT, ALP and LDH plasma levels were retrospectively analyzed and related to their corresponding rRT-PCR tests in 207 patients (80 females and 127 males), who were admitted to the San Raffaele Hospital (Milan, Italy) emergency room between the 20th of February and the 20th of March 2020 as suspected COVID-19 patients. The patients were randomly chosen (alphabetical order) to have a similar number of individuals in the positive (105) and negative (102) rRT-PCR test groups. The average age was 60.5 ± 19.2 years (59.3 ± 21.9 years and 61.2 ± 17.3 years for the female and male groups, respectively). Blood samples were collected, the same day of the rRT-PCR test, as described elsewhere [12, 13]. CRP, AST, ALT, GGT, ALP and LDH were measured on a Roche Cobas 8000 device (Roche Diagnostic, Basel, Switzerland) using either a spectrophotometric assay (AST, ALT and LDH), a colorimetric assay (ALP and GGT) or an immunoturbidimetric assay (CRP). WBC, platelets and the leukocyte formula were measured on Sysmex XE 2100 (Sysmex, Japan) [14].The rRT-PCR was performed on a Roche Cobas Z480 thermocycler (Roche Diagnostic, Basel, Switzerland) using the Roche provided Tib-Molbiol's 2019-nCoV Real-Time Reverse Transcription PCR Kit. RNA purification was performed using the Roche Magna pure system.

Individuals signed an informed consent authorizing the use of their anonymously collected data for retrospective observational studies (article 9.2.j; EU general data protection regulation 2016/679 [GDPR]), according to the San Raffaele Hospital policy (IOG075/2016).

Statistical analyses

Statistical analyses were performed using the software Excel (Microsoft, Redmond, WA, USA). Comparisons of the analyte plasma levels between the COVID-19-positive and -negative groups were performed using a two-tailed, unequal variances t-test (Welch test). Differences between the COVID-19-positive and -negative groups were considered statistically significant if the p-value was lower than 0.05.

Positive predictive value (PPV) was calculated as the ratio between the true-positive patients and those having both LDH and AST above the proposed cutoff. Negative predictive value (NPV) was calculated as the ratio between the true-negative patients and those having AST below the proposed cutoff.

Results

The 207 patients enrolled in this study were all from the emergency room. We selected 105 patients who showed positive rRT-PCR results (positive group) and 102 patients with a negative rRT-PCR result (negative group). Because of the large number of patients, we were not able to retrieve their clinical signs and symptoms. However, because in Italy there are currently strict directives suggesting to perform an rRT-PCR test only if patients show at least three acute respiratory syndrome symptoms, we might assume that most, if not all, of the individuals enrolled in this study went to the hospital emergency room with fever, cough and fatigue. The positive group was composed of 74 males and 31 females (average age 61.6 ± 15.6 and 61.8 ± 16.4 years for the male and female groups, respectively) (Table 1), whereas the negative group was composed of 53 males and 49 females (average age 60.7 ± 19.6 and 57.6 ± 23.8 years for the male and female groups, respectively) (Table 1).

WBCs were measured in all of the 207 patients and were significantly different between the two groups (Table 2). The p-value as low as <0.001 indicated a strong association between COVID-19-positive patients and a low WBC count (Table 2). The leukocyte formula also showed a significant association between the five components (neutrophils, eosinophils, basophils, lymphocytes and monocytes) and COVID-19 patients (Table 2). The most

Table 1: Demographic characteristics of the study population.

	COVID-19 positive		COVID-19 negative	
	n (%)	Age, years (STD)	n (%)	Age, years (STD)
Females	31 (29.5)	62.1 (18.6)	49 (48.0)	57.6 (23.8)
Males	74 (70.5)	61.6 (15.6)	53 (52.0)	60.7 (19.6)
Total	105 (100%)	61.8 (16.4)	102 (100%)	59.2 (21.7)

represented neutrophils and, to a lesser extent, lymphocytes, were lower in the positive group.

No association (p-value: 0.072) was observed between platelets and the disease (Table 2), whereas CRP was significantly higher in the positive group (Table 2). High values of the pyridoxal phosphate-dependent enzymes AST and ALT were significantly associated with the COVID-19 disease (Table 2). In contrast, ALP and GGT showed a p-value higher than 0.05 indicating no association between these two analytes and the pandemic disease (Table 2). A strong association was observed for LDH as well (p-value: <0.001) (Table 2). Unfortunately not all patients underwent a complete blood test analysis and a few analytes were measured in a limited number of patients (Table 2). For instance, AST was measured in all of the patients while LDH was measured in 141 only. Such patients were called the "LDH group" (78 and 63 from the positive and negative groups, respectively) (Table 2).

In order to tentatively develop a COVID-19 diagnostic method based on routine blood test analysis, we empirically adopted cutoff levels of 210 U/L and 35 U/L for LDH and AST, respectively, for COVID-19 positivity. In the LDH group, 66 patients had both LDH and AST above the cutoffs and 55 of them (PPV: 83.3%) tested positive at the rRT-PCR test (Table 3). In contrast, by considering patients with an AST level lower than 25 U/L as a marker of COVID-19 negativity, regardless of the LDH measurement, of the 141 patients, 32 fell in this group and 29 of them (NPV: 90.6%) were indeed rRT-PCR negative (Table 3). The remaining 43 patients having AST between 25 and 35 U/L could not be classified. Thus, in the LDH group, on the basis of their blood test results, 98 patients (69.5%) could be identified as either COVID-19 positive or negative with a PPV and an NPV of 83.3% and 90.6%, respectively. When extending the AST <25 U/L to the whole group (207 patients), we identified 54 patients and 48 of them tested negative (NPV: 88.9%) at the rRT-PCR test (Table 3).

Interestingly, even though the patients were randomly selected, a gender discrepancy was observed between the two groups: the negative group had a similar number of males and females (52% and 48%, respectively), whereas the positive group was mostly composed of males (70.5%).

Discussion

The low WBC count was associated with COVID-19 patients (Table 2). A similar finding was observed previously by Cheng et al. [15]; however, their p-value was much larger.

Table 2: Averaged laboratory finding levels and corresponding standard deviation obtained for patients positive for COVID-19 (according to rRT-PCR) and patients negative for COVID-19 (according to rRT-PCR).

Analyte	Unit	COVID-19 positive	COVID-19 negative	p-Value
WBC	$\times 10^9$ cells/L	6.47 ± 2.61 (105)	9.79 ± 5.25 (102)	**<0.001**
Neutrophils	$\times 10^9$ cells/L	4.76 ± 2.41 (94)	7.18 ± 5.30 (62)	**0.001**
Lymphocytes	$\times 10^9$ cells/L	1.13 ± 0.81 (94)	1.50 ± 0.89 (62)	**0.010**
Monocytes	$\times 10^9$ cells/L	0.52 ± 0.27 (94)	0.77 ± 0.57 (62)	**0.001**
Eosinophils	$\times 10^9$ cells/L	0.02 ± 0.06 (94)	0.09 ± 0.20 (62)	**0.003**
Basophils	$\times 10^9$ cells/L	0.00 ± 0.00 (94)	0.02 ± 0.04 (62)	**<0.001**
Platelets	$\times 10^9$ cells/L	208.1 ± 92.1 (105)	232.8 ± 88.0 (102)	0.072
CRP	mg/L	87.1 ± 81.2 (105)	63.1 ± 79.8 (101)	**0.034**
AST	U/L	56.2 ± 40.8 (105)	38.1 ± 53.2 (102)	**0.007**
ALT	U/L	47.9 ± 40.9 (103)	33.1 ± 53.2 (102)	**0.006**
ALP	U/L	91.1 ± 85.3 (53)	99.1 ± 121.3 (42)	0.727
GGT	U/L	104.8 ± 154.1 (58)	71.4 ± 136.1 (50)	0.814
LDH	U/L	388.0 ± 154.5 (78)	276.4 ± 118.3 (63)	**<0.001**

The number of data analyzed is given in brackets. The p-value of analytes showing a statistically significant difference (<0.05) between positive and negative patients is highlighted in bold.

Such a discrepancy might be reasonably associated with the relatively low number of patients enrolled in the Chinese study (11 positive and 22 negative). The leukocyte formula also showed a significant association between the five components and COVID-19-positive patients (Table 2). However, although basophils, eosinophils and monocytes showed a significant decrease with relatively low p-values (Table 2), their count, even in healthy individuals, is rather low and might be affected by a large variability regardless of the pathological situation. Thus, they might not have a clinical implication in the COVID-19 diagnosis.

We observed no association between platelets and the disease (Table 2); this was in contrast to the study by Cheng et al. [15], which showed a significant association between a low number of platelets and COVID-19 patients. Again, the reason for the discrepancy is likely to be associated with the low number of patients enrolled in the Chinese study.

CRP was also significantly different between the two groups (Table 2); however, the relatively high p-value indicates that this laboratory finding might not be very useful in discriminating between patients with or without COVID-19.

Transaminase and LDH were strongly associated with the COVID-19 disease (Table 2). High levels of AST and ALT have been observed previously in hospitalized COVID patients [16]; however, to the best of our knowledge, they were never compared with patients affected by pulmonary diseases but COVID-19 negative. The strong association between LDH and the COVID-19 disease (Table 2) might be explained by the facts that this enzyme is known to be a marker of lung damage [17] and that COVID-19 primarily infects the lower respiratory tracts.

We also observed a gender discrepancy between the two groups which cannot be attributed to a bias in the selection of patients. However, due to the limited number of patients enrolled in the study, we need more data in order to verify whether the large difference between males and females in the positive group was just a causality or could be an important factor to be considered when discriminating between positive and negative COVID-19 patients.

By empirically using cutoff levels for LDH and AST, we were able to identify, with an error rate similar to that observed for rRT-PCR [15], the COVID-19 positivity/negativity in almost 70% of the patients. We believe that by using a larger dataset and appropriate software to combine multiple variables (including gender) it should be possible to identify a panel of analytes with appropriate cutoffs, able to identify, with high accuracy, patients infected by COVID-19.

It must be noted that our study suffers from a few limitations like the relatively limited number of patients and the absence of recorded patients' clinical signs which can help in discriminating between positive and negative COVID-19 patients.

Conclusions

By comparing the routine blood analysis of 207 patients who were rRT-PCR tested, after being admitted to the emergency room with COVID-19 symptoms, we found statistically significant differences in the plasma levels of WBC, CRP, AST, ALT and LDH between those who were positive at the genetic test and those who were negative. Using rRT-PCR as the gold standard, almost 70% of the patients could be classified as COVID-19 positive or negative on the basis of their hematological parameters. Thus, a simple blood test might help in identifying false-positive/negative rRT-PCR tests but also might be used in developing countries and in those countries suffering from a shortage of rRT-PCR reagents and/or specialized laboratories as an inexpensive and available alternative to identify potential COVID-19 patients.

Table 3: Number of patients identified as COVID-19 positive or negative on the basis of the cutoff levels empirically obtained by routine blood analysis.

	Total patients	COVID-19 positive			COVID-19 negative			Unclassified
		LDH > 210[a] AST > 35[a]	TP[b]	PPV	AST < 25[a]	TN[b]	NPV	
LDH group	141	66	55	83.3%	32	29	90.6%	43
Whole group	207	66[c]	55[c]	83.3%[c]	54	48	88.9%	87[d]

Positive predictive value (PPV) and negative predictive value (NPV) were calculated as described in the Materials and methods. [a]LDH and AST levels are in U/L. [b]TP and TN are true positive and true negative according to the RT-PCR test. [c]The value is underestimated because LDH was missing in 76 patients. [d]The value is overestimated because LDH was missing in 76 patients.

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