

Article

Measurement of serum paraoxonase-1 arylesterase activity as a screening test for the diagnosis of COVID-19

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Abstract: The development of inexpensive, fast and reliable screening tests for COVID-19 is, as yet, an unmet need. The present study was aimed at evaluating the usefulness of serum arylesterase activity of paraoxonase-1 (PON1) measurement as a screening test in patients with different severity levels of COVID-19 infection. We included 615 COVID-19 positive patients who were classified as asymptomatic, mildly symptomatic, severely symptomatic, or fatally symptomatic. Results were compared with 50 healthy volunteers, 330 patients with cancer and 343 with morbid obesity. Results showed PON1 activity greatly decreased in COVID-19 compared to healthy volunteers; receiver operating characteristics plot showed a high diagnostic accuracy. The degree of COVID-19 severity did not influence PON1 levels. Our results indicated that PON1 determination was efficient for disease diagnosis but not for prognosis. Further, patients with obesity or cancer presented alterations similar to those of COVID-19 patients. As such, elevated levels of PON1 indicate the absence of COVID-19, but low levels may be present in various other chronic diseases. The assay is fast and inexpensive. We suggest that PON1 measurement could be used as an initial, high cut-off point screening method, while lower values should be confirmed with the more expensive nucleic acid amplification test.

Keywords: biomarkers; COVID-19; paraoxonase-1; SARS-CoV-2.

1. Introduction

Coronavirus disease 2019 (COVID-19) continues to spread worldwide at dangerous levels, with high morbidity and mortality rates. Despite the relative success of vaccination campaigns in high-income countries, the majority of populations in large areas of the world do not have access to vaccines, or to effective measures to prevent virus transmission. Further, variants such as omicron (PANGO identifier B.1.1.529) clearly demonstrated that new variants can appear which are more infective, and more adept at escaping the action of existing vaccines. The current consensus in the scientific community is that COVID-19 will not be eradicated easily, and that populations would need to accommodate for the infection in the future. In this context, developing cheap, fast and reliable screening tests remains a peremptory need.

Although antigen tests have demonstrated their efficacy in confirming infection in symptomatic patients, the gold standard method for the diagnosis of COVID-19 remains

the detection of the viral messenger RNA by nucleic acid amplification tests (NAATs) [1]. However, these techniques are not without drawbacks. NAATs are expensive, and require time and specialized laboratories that tend to be saturated or even overwhelmed during times of outbreak. Also, many countries cannot afford the cost of performing NAATs on several thousands of people. Conversely, antigen tests are unreliable in asymptomatic patients. We recently reported preliminary results suggesting that the measurement of paraoxonase-1 (PON1) arylesterase activity can be a good marker of COVID-19 with excellent sensitivity and specificity [2]. However, our study was performed in a limited number of hospitalized patients and, therefore, we could not propose it as a blood test for application in the general population. In the present study we aimed to evaluate the usefulness of serum arylesterase activity of PON1 measurement as a screening test in a larger series of outpatients and hospitalized patients, with different levels of severity of COVID-19.

2. Materials and Methods

2.1. Study design and data collection

This is a retrospective cohort study in sera from 615 patients with SARS-CoV2 infection, confirmed by NAAT or antigen test. They were recruited between April 2020 and September 2021 in *Hospital Universitari de Sant Joan*. Ours is a reference hospital with 352 beds located in Reus, a medium-sized city (100,000 inhabitants) located in Catalonia, on the northeast coast of Spain. Our Hospital offers health coverage to the surrounding counties, comprising a population of about 250,000 mainly rural inhabitants, but including primary care centers and residences for the elderly. According to our care protocols, and in accordance with the recommendations of the health authorities [3], a NAAT or antigen test was performed on all patients who attended our hospital as outpatients seeking hospitalization, or receiving attention in the Emergency Department. NAAT testing for COVID-19 was regardless of the reason for entry into the hospital and whether or not they had symptoms suggestive of COVID-19. The only exclusion criteria were to be younger than 18 years, and having missing clinical data. Patients were classified according to the recommendations of the National Institute of Health [4], as follows: Asymptomatic infected patients were those who had a positive test with no symptoms of COVID-19; mildly symptomatic were those patients with positive test and COVID-19-compatible symptoms who did not require hospitalization (oxygen saturation, SpO₂, >94% respiratory rate <22 breaths/min and no dyspnea); severely symptomatic were those patients with positive test and evidence of lower respiratory disease during clinical assessment (or imaging and who had an SpO₂ <94% on room air at sea level and a respiratory rate >22 breaths/min); fatally symptomatic were those patients with positive test who required high-flow oxygen therapy or mechanical ventilation (invasive or non-invasive) or extracorporeal membrane oxygenation. For statistical purposes, patients who died within 30 days post-diagnosis were considered within the fatally symptomatic group. We also recorded the days elapsed between obtaining a sample for PON1 determination and the beginning of the disease as determined by the date-of-onset of symptoms or first-positive test. For comparisons, we used sera from 50 healthy volunteers, 238 patients with breast cancer, 32 with lung cancer, 28 with head and neck cancer, 32 with rectal cancer and 343 patients with morbid obesity (body mass index ≥ 40 kg/m²). Healthy volunteers were selected from an epidemiological study conducted in our area, and who had no clinical or biochemical evidence of renal insufficiency, liver disease, neoplasia, or neurological disorders. Patients with cancer and morbid obesity were recruited from clinical control subjects prior to receiving treatment with radiation therapy or bariatric surgery. Samples from all these subjects were collected before January 2020, and detailed descriptions of these populations have been reported previously [5-9].

Serum samples from all participants were stored in our Biobank at -80 °C until the time of analyses. This study was approved by the *Comitè d'Ètica i Investigació en Medicaments* (Institutional Review Board) of *Institut d'Investigació Sanitària Pere Virgili* (Resolu-

tions CEIM 014/2017, 040/2018 amended on 16 April 2020, INFLAMET/15-04/4proj7 and OBESPAD/14-07-31proj3).

2.2. Laboratory procedures

PON1 is an esterase and lactonase that hydrolyzes multiple substrates, and various assays have been described using some of these substrates. For the current study, we chose the determination of its arylesterase activity by measuring the hydrolysis of phenylacetate, because this substrate is not toxic, it is cheap and easy to obtain, and the assay is fast and is not affected by the enzyme's genetic polymorphisms [10]. The activity was measured at 280 nm, in a 9 mM Tris-HCl buffer, pH 8.0, and supplemented with 0.9 mM CaCl₂, as previously reported [11]. The detection limit of the assay was 2.3 ± 1.1 U/L (mean \pm SD, n = 3). Intra-assay coefficient of variation was 8% (n = 35). Inter-assay coefficient of variation was 9% (n = 12).

2.3. Statistical analyses

All statistical calculations and graphic representations were performed with the Statistical Package for Social Sciences (SPSS 24.0, Chicago, IL, USA) and GraphPad Prism 6.01 (GraphPad Software, San Diego, CA, USA). Quantitative data are presented as medians and 95% Confidence Intervals and assessed for differences with the Mann-Whitney *U* test. The diagnostic accuracy of PON1 measurement was assessed by Receiver Operating Characteristics (ROC) curves and confusion matrices [12]. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated as previously reported [13].

3. Results

3.1. Characteristics of the COVID-19 positive patients in the study

Raw data associated with this article are shown as Supplementary Materials. Asymptomatic patients were similar in age to those with mild symptomatic infection and younger than those with severe or fatal disease. Males composed the majority in all groups, except in the mild COVID-19. The most frequent symptoms were dyspnea, pneumonia, cough and fever, and occurred most often in patients with severe and fatal disease. Vaccination rates on entry into hospital varied between 17% and 30% (Table 1).

Table 1. Demographic and clinical characteristics of studied patients

| Characteristic | Healthy subjects (n=50) | Asymptomatic COVID-19 (n=113) | Mild COVID-19 (n=284) | Severe COVID-19 (n=143) | Fatal COVID-19 (n=75) |
|-----------------|----------------------------|-------------------------------------|-----------------------------|-------------------------------|-----------------------------|
| Age (years) | 76 (67-80) | 50 (33-70) | 45 (32-56) | 73 (57-84) | 72 (53-86) |
| Sex | | | | | |
| Men (%) | 38 (76) | 70 (61.9) | 79 (27.7) | 83 (58.0) | 44 (61.1) |
| Women (%) | 12 (24) | 43 (38.1) | 205 (72.2) | 60 (42.0) | 28 (38.9) |
| Symptoms | | | | | |
| Pneumonia (%) | - | - | 12 (4.2) | 37 (25.9) | 17 (22.7) |
| Cough (%) | - | - | 91 (32.0) | 79 (55.2) | 40 (53.3) |
| Fever (%) | - | - | 115 (40.5) | 85 (59.4) | 39 (52.0) |
| Chills (%) | - | - | 5 (1.8) | 2 (1.4) | 5 (6.7) |
| Dyspnea (%) | - | - | 47 (16.5) | 73 (51.0) | 54 (72.0) |
| Vomiting (%) | - | - | 15 (5.3) | 9 (6.3) | 5 (6.7) |
| Diarrhea (%) | - | - | 36 (12.7) | 20 (14.0) | 11 (14.7) |
| Anosmia (%) | - | - | 49 (17.2) | 8 (5.6) | 1 (1.3) |
| Ageusia (%) | - | - | 40 (14.1) | 10 (7.0) | 2 (2.7) |
| Odynophagia (%) | - | - | 24 (8.5) | 7 (4.9) | 2 (2.7) |
| Headache (%) | - | - | 82 (28.9) | 11 (7.7) | 9 (12.0) |

| | | | | | |
|-------------------------|---|-----------|-----------|-----------|-----------|
| Anorexia/hyporexia (%) | - | - | 8 (2.8) | 8 (5.6) | 3 (4.0) |
| Myalgia (%) | - | - | 39 (13.7) | 15 (10.5) | 5 (6.7) |
| Arthralgia (%) | - | - | 31 (10.9) | 12 (8.4) | 4 (5.3) |
| Respiratory failure (%) | - | - | 5 (1.8) | 6 (4.2) | 12 (16.0) |
| Hospital stay (days) | - | - | - | 9 (6-14) | 13 (7-32) |
| Vaccine* | | | | | |
| Vaccinated (%) | - | 32 (28.3) | 50 (17.6) | 43 (30.1) | 20 (26.7) |
| 1st dose (%) | - | 11 (9.7) | 11 (3.9) | 16 (11.2) | 9 (12) |
| 2nd dose (%) | - | 20 (17.7) | 39 (13.7) | 27 (18.9) | 11 (14.7) |
| 3rd dose (%) | - | 1 (0.9) | 0 (0) | 0 (0) | 0 (0) |
| Deceased (%) | - | - | - | - | 31 (41.3) |

* Samples from the healthy subjects were collected before the COVID-19 pandemic. Results are shown as medians and 95% Confidence Intervals (in parenthesis) or as number of cases and percentages.

Serum PON1 arylesterase activity was 120.3 (59.5 – 176.1) U/L in COVID-19 positive patients and 213.3 (141.8 – 436.3) U/L in the healthy subjects ($P < 0.001$). The area under the curve (AUC) of the ROC plot was 0.951 (0.919 – 0.979) when comparing all COVID-19 positive patients with the healthy volunteers (Figure 1A). Similar results were obtained when COVID-19 positive patients were segregated according to the severity of their disease (Figure 1 B to E). The best combination of sensitivity and specificity was obtained at PON1 = 161 U/L. We investigated the possibility of maximizing the sensitivity with the aim of using PON1 measurement as a screening test and found that at PON1 = 205 U/L, sensitivity was 100%, specificity was 56.0%, PPV was 96.6% and NPV was 100.0% (Table 2). We also wished to know the usefulness of PON1 measurement as a prognostic test but failed to observe differences in relation to the severity of the disease. Indeed, we found similar serum PON1 activities when comparing asymptomatic and mild versus severe and fatal disease the AUC of the ROC curve was low, and the test misclassified a high percentage of patients (Figure 2). Finally, we wished to know whether there were any difference in serum PON1 values in relation to the days elapsing between the commencement of the infection and the measurement of PON1. No differences were observed regardless of the severity of the disease (Figure 3). We found no significant differences in PON1 activity between vaccinated and unvaccinated patients [115.3 (61.2 – 166.6; n = 146) U/L and 119.2 (58.2 – 178.2; n = 470) U/L, respectively].

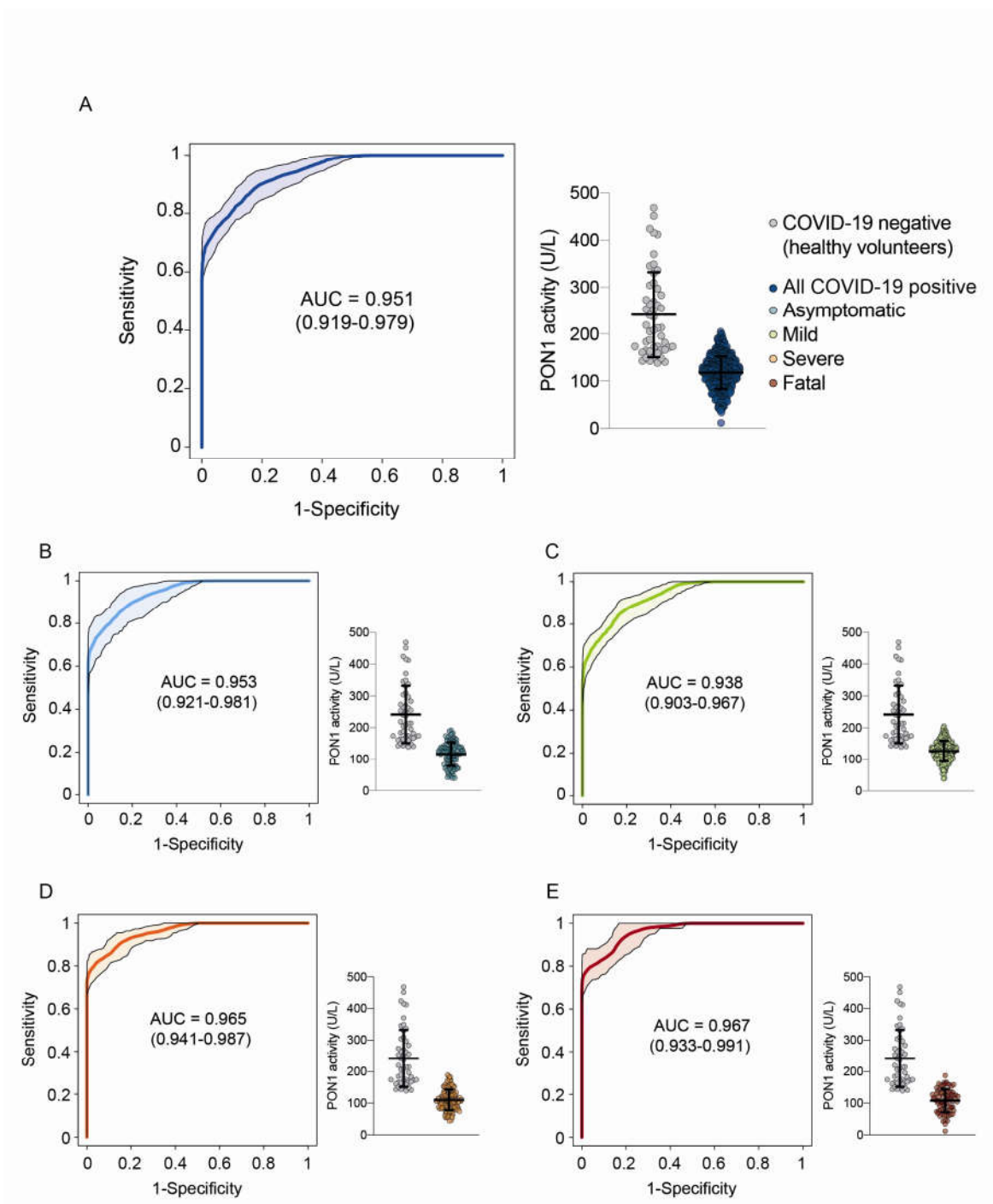


Figure 1. Serum paraoxonase-1 (PON1) activities and Receiver Operating Characteristics (ROC) curves comparing COVID-19 positive patients and the healthy controls; (A): All patients combined; (B): Asymptomatic patients; (C): Mild disease; (D): Severe disease; (E): Fatal disease. AUC: Area under the curve.

Table 2. Diagnostic accuracy of paraoxonase-1 (PON1) measurement at two cut-off levels in the differentiation of patients with COVID-19 from non-COVID-19

| PON1 activity (U/L) | Diagnosis | | | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) |
|---------------------|--------------|-----------|------------|------------------------|---------------------|---------------------|------------------------|
| | Negative | Positive | Total | | | | |
| 161 | Negative | 41 | 61 | 90.1 (87.4-92.3) | 82.0 (68.1-91.0) | 98.4 (96.9-99.2) | 40.2 (30.8-50.4) |
| | Positive | 9 | 554 | | | | |
| | Total | 50 | 615 | 665 | | | |
| 205 | Negative | 28 | 0 | 100.0 (100.0-100.0) | 56.0 (41.4-69.8) | 96.6 (94.7-97.8) | 100.0 (100.0-100.0) |
| | Positive | 22 | 615 | | | | |
| | Total | 50 | 615 | 665 | | | |

Results are shown at 161 U/L, which is the cut-off with the best combination of sensitivity and specificity, and at 205 U/L, which is the cut-off with 100% sensitivity. The numbers represent the number of cases or the percentages and 95% Confidence Intervals (in parenthesis). NPV: Negative predictive value. PPV: Positive predictive value. NAAT: Nucleic acid amplification test.

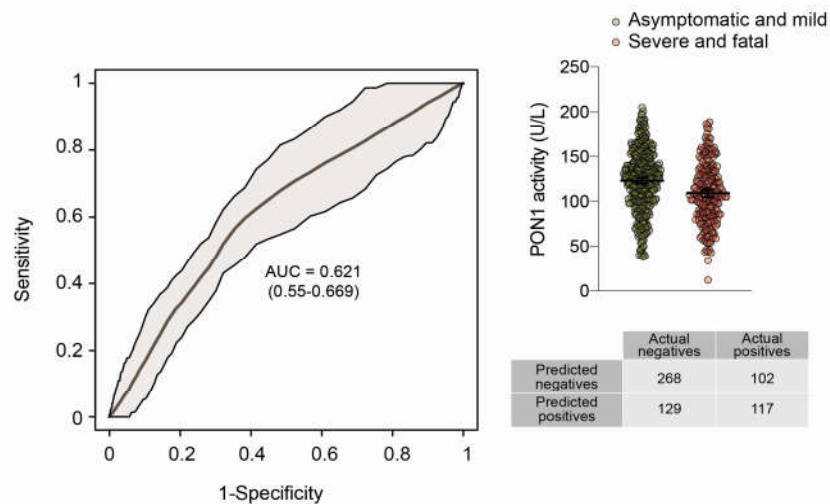
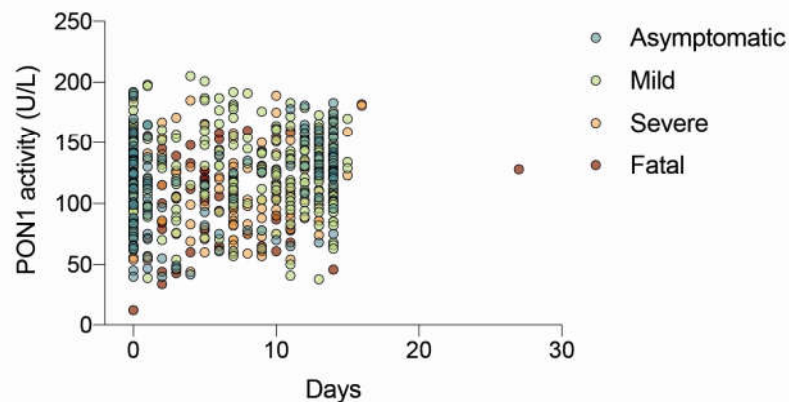
**Figure 2.** Serum paraoxonase-1 (PON1) and Receiver Operating Characteristics (ROC) curves comparing asymptomatic and mild *versus* severe and fatal COVID-19 positive patients. AUC: Area under the curve.

Figure 3. Serum paraoxonase-1 (PON1) activities in COVID-19 patients segregated according to the severity of symptoms and the days post-onset of the infection.

3.3. Paraoxonase-1 activity showed a similar trend toward decrease in COVID-19, cancer and morbid obesity patients

PON1 activity was similar in COVID-19 positive patients and in patients with cancer or morbid obesity, when the latter group was considered together or when considered separately according to disease. The ROC curve showed that PON1 measurement was unable to segregate COVID-19 positive patients from subjects with the other diseases in the study group (Figure 4).

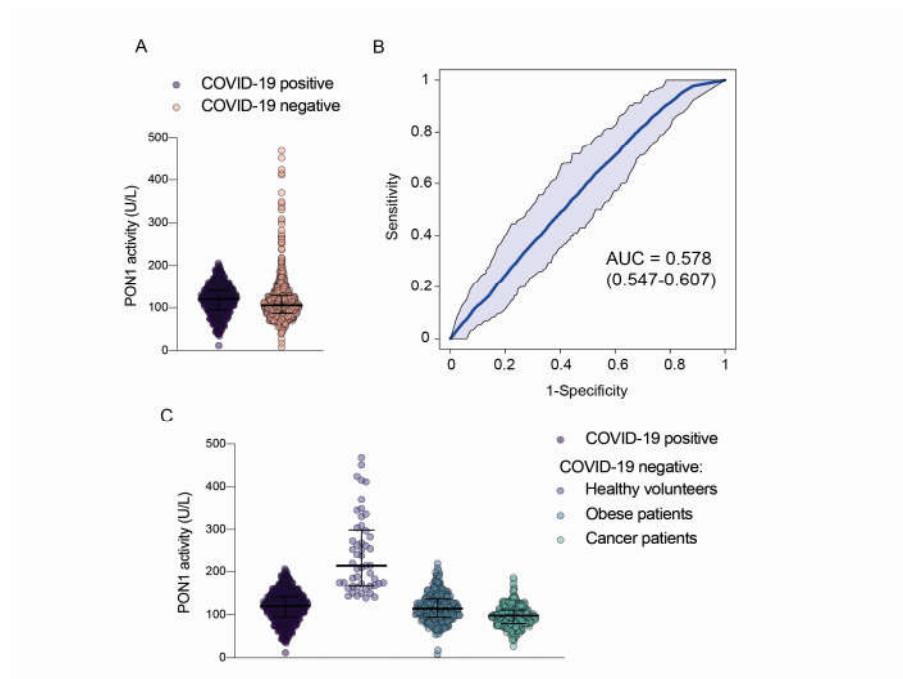


Figure 4. Serum paraoxonase-1 (PON1) activities in COVID-19 positive patients and all COVID-19 negative patients (obesity + cancer) and healthy volunteers combined; (A): Receiver Operating Characteristics (ROC) curves comparing COVID-19 positive patients and COVID-19 negative patients; (B): Serum paraoxonase-1 (PON1) activities in COVID-19 positive and COVID-19 negative patients separated according to their disease; (C): AUC: Area under the curve.

4. Discussion

PON1 is an enzyme synthesized mainly by the liver, and carried into the circulation bound to high-density lipoproteins. It can be internalized in peripheral cells and, thus, its protein expression is practically ubiquitous in almost all tissues. The enzyme is a lipoperoxide hydrolase which degrades lipoperoxides in lipoproteins and cells, and participates in the subject's innate immune system and defense against oxidative stress [10,14]. Decreased serum PON1 activities have been reported in several diseases that have an infectious or inflammatory component including bacterial infections [15-17], and non-communicable diseases such as chronic liver diseases, cardiovascular diseases and cancer [10,14]. To elicit its physiological action, the active center of the enzyme needs to bind covalently to the substrate molecules; the outcome being that each enzyme molecule that binds to a lipoperoxide is inactivated and can no longer carry out its function. The result is a decreased enzyme activity [10].

The present study, in a large series of patients, confirms our preliminary data indicating that serum PON1 activity is strongly decreased in COVID-19 infection [2] and demonstrates that this is an alteration that appears early in the course of the disease. Also, it is of note that the change is independent of whether or not the disease is severe. There are very few data on PON1 alterations in COVID-19, and the role that this enzyme may play in the course of the disease. Proteomic studies have observed increased PON1 protein expression in high-density lipoproteins of COVID-19 patients [18-20]. Indeed, our preliminary study observed increased serum PON1 concentrations and decreased activities. This apparently contradictory result has also been observed in other infectious and non-communicable diseases [10,14]. The explanation is probably related to an attempt by the organism to increase synthesis of the enzyme to counteract the decrease in enzyme activity.

The results from the present study suggest that the widespread determination of serum PON1 arylesterase activity may be a useful parameter for the community-based diagnosis of COVID-19. The activity of this enzyme was greatly decreased in our patients, to levels approximately half of that of the healthy population, and regardless of whether or not the patient has concurrent COVID-19 symptoms, or even regardless of the severity of the symptoms. Perhaps this determination could be especially useful in low-income countries where millions of people have not yet been vaccinated, and the State cannot afford massive campaigns to detect positive patients using NAAT technology. Determination of PON1 arylesterase activity in serum is extremely simple and inexpensive. It only requires a spectrophotometer capable of reading at 280 nm. If desired, the determination can be done in a plastic tube or plate at home, and it can be easily automated for specialist centers. The cost of reagents ought not to exceed a few cents. For example, the price of a 500 mL bottle of phenylacetate substrate is around 40-50 euros. We use 25 μ L per sample in our assay, and one plate of 96 determinations costs around 10 euros. However, an important drawback is that observing low levels of this enzyme is not specific to COVID-19 infection, since similar levels of activity can be found in other chronic diseases such as morbid obesity or cancer. This limitation implies that the patient's clinical history must be taken into account before interpreting the results. We suggest that the determination of PON1 be used as an initial screening method with a high cut-off point (205 U/L, for example, as our results highlight), and subsequent confirmation of the lower values using NAAT. This could save millions of dollars.

One criticism that can be made of our study is that, currently, the epidemiological status of the COVID-19 infection has improved thanks to the predominance of the omicron strain where the symptoms are considerably milder. As such, the test we are proposing is no longer necessary. However, the omicron strain is much more transmissible than the previous ones and the number of infected individuals is considerably higher. Hence, the numbers of severe cases remain high, in absolute terms. In addition, although vaccines considerably restrict the numbers of severe cases, it is also true that vaccination rates are far from adequate in large areas of the world. As such, we believe that the determination of PON1 arylesterase activity can still be very useful. Other limitations of our study are that it was carried out in a single center, in a specific geographical area, with a specific lifestyle and in which the Caucasian ethnic group is the most abundant. Nevertheless, we believe that our data can stimulate further research, including more extensive national and international studies.

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Informed Consent Statement: Written informed consent was waived to avoid contamination and spread of infection through paper

Data Availability Statement: The data presented in this study are available in the article and supplementary material.

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References

- Bostan, M.; Ataman, M.; Bostan, I.S.; Bleotu, C. Targets and assay types for COVID-19 diagnosis. *J. Immunoassay Immunochem.* **2020**, *41*, 946-959. doi:10.1080/15321819.2020.1862866.
- Rodríguez-Tomás, E.; Iftimie, S.; Castañé, H.; Baiges-Gaya, G.; Hernández-Aguilera, A.; González-Viñas, M.; Castro, A.; Camps, J.; Joven, J. Clinical performance of paraoxonase-1-related variables and novel markers of inflammation in coronavirus disease-19. A machine learning approach. *Antioxidants (Basel)* **2021**, *10*, 991. doi: 10.3390/antiox10060991.
- Gobierno de España. Ministerio de Sanidad. Recomendaciones de seguridad del paciente y profesionales en procedimientos intervencionistas en la fase de transición de la pandemia COVID-19. Available at: https://www.sanidad.gob.es/gl/profesionales/saludPublica/ccayes/alertasActual/nCov/documentos/COVID19_Procedimientos_intervencionistas.pdf. Accessed 20 May 2022.
- NIH. COVID-19 treatment guidelines. Clinical spectrum of SARS-CoV-2 infection. Available at: <https://www.covid19treatmentguidelines.nih.gov/overview/clinical-spectrum/>. Accessed 20 May 2022.
- Fort-Gallifa, I.; García-Heredia, A.; Hernández-Aguilera, A.; Simó, J.M.; Sepúlveda, J.; Martín-Paredero, V.; Camps, J.; Joven, J. Biochemical indices of oxidative stress and inflammation in the evaluation of peripheral artery disease. *Free Radic. Biol. Med.* **2016**, *97*, 568-576. doi: 10.1016/j.freeradbiomed.2016.07.011.
- Arenas, M.; García-Heredia, A.; Cabré, N.; Luciano-Mateo, F.; Hernández-Aguilera, A.; Sabater, S.; Bonet, M.; Gascón, M.; Fernández-Arroyo, S.; Fort-Gallifa, I.; Camps, J.; Joven, J. Effect of radiotherapy on activity and concentration of serum paraoxonase-1 in breast cancer patients. *PLoS One* **2017**, *12*, e0188633. doi: 10.1371/journal.pone.0188633.
- Rodríguez-Tomás, E.; Murcia, M.; Arenas, M.; Arguís, M.; Gil, M.; Amigó, N.; Correig, X.; Torres, L.; Sabater, S.; Baiges-Gaya, G.; Cabré, N.; Luciano-Mateo, F.; Hernández-Aguilera, A.; Fort-Gallifa, I.; Camps, J.; Joven, J. Serum paraoxonase-1-related variables and lipoprotein profile in patients with lung or head and neck cancer: Effect of radiotherapy. *Antioxidants (Basel)* **2019**, *8*, 213. doi: 10.3390/antiox8070213.
- Cabré, N.; Luciano-Mateo, F.; Fernández-Arroyo, S.; Baiges-Gaya, G.; Hernández-Aguilera, A.; Fibla, M.; Fernández-Julà, R.; París, M.; Sabench, F.; Castillo, D.D.; Menéndez, J.A.; Camps, J.; Joven, J. Laparoscopic sleeve gastrectomy reverses non-alcoholic fatty liver disease modulating oxidative stress and inflammation. *Metabolism* **2019**, *99*, 81-89. doi: 10.1016/j.metabol.2019.07.002.
- Rodríguez-Tomás, E.; Arenas, M.; Gómez, J.; Acosta, J.; Trilla, J.; López, Y.; Árquez, M.; Torres, L.; Araguas, P.; Hernández-Aguilera, A.; Baiges-Gaya, G.; Castañé, H.; Camps, J.; Joven, J. Identification of potential metabolic biomarkers of rectal cancer and of the effect of neoadjuvant radiochemotherapy. *PLoS One* **2021**, *16*, e0250453. doi: 10.1371/journal.pone.0250453.
- Camps, J.; Marsillach, J.; Joven, J. The paraoxonases: Role in human diseases and methodological difficulties in measurement. *Crit. Rev. Clin. Lab. Sci.* **2009**, *46*, 83-106. doi: 10.1080/10408360802610878.
- Gaita, L.; Manzi, B.; Sacco, R.; Lintas, C.; Altieri, L.; Lombardi, F.; Pawlowski, T.L.; Redman, M.; Craig, D.W.; Huettelman, M.J.; Ober-Reynolds, S.; Brautigam, S.; Melmed, R.; Smith, C.J.; Marsillach, J.; Camps, J.; Curatolo, P.; Persico, A.M. Decreased serum arylesterase activity in autism spectrum disorders. *Psychiatry Res.* **2010**, *180*, 105-113. doi: 10.1016/j.psychres.2010.04.010.
- Zweig, M.H.; Campbell, G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. *Clin. Chem.* **1993**, *39*, 561-577.
- Trevelan R. Sensitivity, specificity, and predictive values: Foundations, pliabilities, and pitfalls in research and practice. *Front. Public Health* **2017**, *5*, 307. doi: 10.3389/fpubh.2017.00307.

14. Camps, J.; Castañé, H.; Rodríguez-Tomás, E.; Baiges-Gaya, G.; Hernández-Aguilera, A.; Arenas, M.; Iftimie, S.; Joven J. On the role of paraoxonase-1 and chemokine ligand 2 (C-C motif) in metabolic alterations linked to inflammation and disease. A 2021 Update. *Biomolecules* **2021**, *11*, 971. doi: 10.3390/biom11070971.
15. Camps, J.; Iftimie, S.; García-Heredia, A.; Castro, A.; Joven, J. Paraoxonases and infectious diseases. *Clin. Biochem.* **2017**, *50*, 804-811. doi: 10.1016/j.clinbiochem.2017.04.016.
16. Iftimie, S.; García-Heredia, A.; Pujol, I.; Ballester, F.; Fort-Gallifa, I.; Simó, J.M.; Joven, J.; Camps, J.; Castro, A. Preliminary study on serum paraoxonase-1 status and chemokine (C-C motif) ligand 2 in hospitalized elderly patients with catheter-associated asymptomatic bacteriuria. *Eur. J. Clin. Microbiol. Infect. Dis.* **2016**, *35*, 1417-1424. doi: 10.1007/s10096-016-2679-8.
17. Iftimie, S.; García-Heredia, A.; Pujol, I.; Ballester, F.; Fort-Gallifa, I.; Simó, J.M.; Joven, J.; Castro, A.; Camps, J. A preliminary study of paraoxonase-1 in infected patients with an indwelling central venous catheter. *Clin. Biochem.* **2016**, *49*, 449-457. doi: 10.1016/j.clinbiochem.2015.11.006.
18. Villar, M.; Urra, J.M.; Rodríguez-Del-Río, F.J.; Artigas-Jerónimo, S.; Jiménez-Collados, N.; Ferreras-Colino, E.; Contreras, M.; de Mera, I.G.F.; Estrada-Peña, A.; Gortázar, C.; de la Fuente, J. Characterization by quantitative serum proteomics of immune-related prognostic biomarkers for COVID-19 symptomatology. *Front. Immunol.* **2021**, *12*, 730710. doi: 10.3389/fimmu.2021.730710.
19. Geyer, P.E.; Arend, F.M.; Doll, S.; Louiset, M.L.; Virreira Winter, S.; Müller-Reif, J.B.; Torun, F.M.; Weigand, M.; Eichhorn, P.; Bruegel, M.; Strauss, M.T.; Holdt, L.M.; Mann, M.; Teupser, D. High-resolution serum proteome trajectories in COVID-19 reveal patient-specific seroconversion. *EMBO Mol. Med.* **2021**, *13*, e14167. doi: 10.15252/emmm.202114167.
20. Begue, F.; Tanaka, S.; Mouktadi, Z.; Rondeau, P.; Veeren, B.; Diotel, N.; Tran-Dinh, A.; Robert, T.; Vélia, E.; Mavingui, P.; La-grange-Xélot, M.; Montravers, P.; Couret, D.; Meilhac, O. Altered high-density lipoprotein composition and functions during severe COVID-19. *Sci. Rep.* **2021**, *11*, 2291. doi: 10.1038/s41598-021-81638-1.