Perforin gene variant A91V in young patients with severe COVID-19

Since early 2020 SARS-CoV-2 infectious disease (COVID-19) has been responsible for more than 300.000 deaths across the globe.[1] Advanced age and previous comorbidities are clearly related to the development of severe forms of the disease (sCOVID) and an increased mortality risk.[2,3] However young healthy subjects with sCOVID are also admitted to intensive care units (ICU) and die, suggesting that individual variations and/or genetic predisposing factors might play a role in modifying the clinical course and severity of the disease.[4]

sCOVID-19 is characterized by fever, bilateral pneumonia, lymphopenia, hyperferritinemia, elevated acute phase reagents and cytokine storm, altogether conforming a hyperinflammation scenario similar to that in secondary hemophagocytic lymphochystiocytosis (sHLH), also known as macrophage activation syndrome.[5] In adults, sHLH is mostly triggered by viral infections and approximately 50% of patients experience pulmonary disease.[6] In contrast, familial HLH (fHLH) is genetically determined by mutations in genes coding for proteins related to lymphocyte cytotoxicity such as perforin (*PRF1* gene). Studies in juvenile idiopathic arthritis or systemic lupus erithematosus patients show that up to 40% of individuals suffering sHLH carry heterozygous mutations in fHLH genes. In a fatal influenza A (H1N1) series, 36% of patients carried one or several mutations in fHLH-related genes.[7,8] These findings suggest an important, not yet totally recognized overlap between primary and secondary forms of HLH.

It has been previously observed that the highly prevalent, fHLH-associated c.272C>T variant (p.A91V; rs35947132) in the *PRF1* gene impairs the processing to the active form of perforin protein.[9] Published reports associate this variant with immune diseases but it has not been validated as pathological in larger cohorts.[10] The A91V *PRF1* gene translates into a protein with reduced stability and abnormal trafficking which associates with a significant decrease of NK-cell cytotoxicity.[11,12] Previous studies reported higher prevalence of the A91V variant in

Table 1. Description of the main clinical and laboratory characteristics of patients positive for the c.272C>T (p.A91V; rs35947132) change in the perforin *PRF1* gene.

	Patient 1	Patient 2
Demographic characteristics		
Age (years)	45	46
Sex	Female	Male
Initial findings		
Medical history	Hypertriglyceridemia	Grade 1 obesity
Symptoms at disease onset	Confusion, tachypnea and dyspnea	Dry cough and fever
Imaging features	Multi-lobar/bilateral patchy consolidations (100% involvement of both lungs). Worst radiologic findings of the series.	Multi-lobar/bilateral patchy consolidations (75% involvement of both lungs)
Treatment before admission to ICU	No previous treatment	Azithromycin 500 mg/24h, Lopinavir/Ritonavir 400/100 mg/12h, hydroxychloroquine 200 mg/12h
Days from disease onset to death	19	14
Findings at ICU admission		
Days from disease onset to ICU admission	0	6
Disease severity	Severe	Severe
Laboratory findings at ICU admission		
Albumin (g/deciliter) [3.5 - 5.0]	3.6	3.1
Alanine aminotransferase (U/liter) [5 - 45]	25	49
Aspartate aminotransferase (U/liter) [5 - 33	29	81
Creatinine (mg/deciliter) [0.70 - 1.20]	0.78	1.01
Lactate dehydrogenase (U/liter) [135 - 225]	663	757
Triglycerides (mg/deciliter)	483	Not tested
Creatinine Kinase U/liter [34 - 171]	110	297
Troponin T (ng/liter) [< 14]	21.3	8.9
Procalcitonin (ng/liter) [≤ 0.50]	1.15	0,52
Prothrombin time (sec)	12.5	12.8
D-dimer (ng/mililiter) [0 - 500]	1020	672
Serum ferritin ng/mililiter [30 - 400]	1107	3032
Fibrinogen mg/deciliter [200 - 560]	870	765
C-reactive protein mg/deciliter [0.10 - 0.50]	22.93	23.96
Hemoglobin (g/deciliter)	10.9	12.1
White-cell count (x10^3 per mm^3) [4.0 - 11.3]	16	4.9
Lymphocytes (x1^3 per mm^3) [1.2 - 4.0]	1.2	1.1
Platelets (x10^3 per mm^3) [140 - 450]	380.000	175.000
Neutrophils (x10^3 per mm^3) [1.8 - 7.4]	13.5	3.4
c.272C>T (p.A91V; rs35947132) variant	Heterozygosis	Heterozygosis

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	Patient 1	Patient 2
Evolution of laboratory parameters	Initial lymphopenia resolved (from 0.5 to 2.7 per mm^3), hemoglobin decreased from 9.9 to 7.9 g/dL, requiring transfusion of 2 erythrocytes concentrates. D-dimer stabilized at 2240 ng/mL. CRP values increased up to 46.7 mg/dL and fell to 4,5 mg/dl after antibiotics.	Sustained lymphopenia (0.5 to 1.1 per mm^3), hemoglobin decreased, platelets recovered to normal levels. D-Dimers abruptly rose up to 8706 ng/ml. CRP increased up to 35.5 mg/dL. LDH stabilized. Creatinine increased to 2.81 mg/dl and Troponin T to 20 ng/L.
Ventilation management	Mechanical ventilation was initiated soon after admission. Tracheostomy was performed on day 11. After 3 days without improvement, extracorporeal membrane oxygenation was implemented.	Oxygen therapy with face mask with reservoir bag was initiated at admission. Due to progressive deterioration, mechanical ventilation was indicated on day 1.
Treatment received	Hydroxychloroquine 200 mg/12h, Azithromycin 500 mg/24h, Lopinavir/Ritonavir 400/100 mg/12h, Interferon β 250 mcg/48h, Methylprednisolone 250 mg/12h and antibiotics.	Hydroxychloroquine 200 mg/12h, Azithromycin 500 mg/24h, Lopinavir/Ritonavir 400/100 mg/12h, Methylprednisolone 50 mg/12h, Tocilizumab 20 mg/mL, and antibiotics.
HScore for HLH (without bone marrow aspirate and organomegaly evaluation)[1]	188 points	175 points

HLH: hemophagocytic lymphochystiocytosis; CPR: c-reactive protein; ICU: intensive care unit; LDH: lactate dehydrogenase.

HLH patients.[13,14] It is reasonable to think that perforin bearing the A91V change could be related to suboptimal activation and effector capacities of CD8 and/or natural killer (NK) cells. In the context of a viral infection, the correct function of these cells is required to contain the viral replication, clear the virus and overcome the infection. Ineffective killing of SARS-CoV-2 infected cells might lead to a sustained activation of lymphocytes and macrophages contributing to the cytokine storm and hyperinflammation that characterizes sCOVID-19.

Based on the above premises, we hypothesized that the fHLH-associated A91V *PRF1* variant is prevalent in patients suffering severe forms of COVID-19. We therefore tested for the A91V *PRF1* variant in all sCOVID-19 patients in the ICU of our hospital on a random day (March 27). Exon 2 of the *PRF1* gene coding region was amplified using PCR. PCR products were purified and sequenced as previously reported.[15] Elderly and patients with comorbidities were excluded. Twenty-two previously healthy patients between the age of 24-52 years were identified: 17 of 22 males; 14 of 22 Latin- American, 7of 22 Spanish and 1 of 22 Polish.

Among the studied patients, 2 of 22 showed A91V *PRF1* in heterozygosis (allele frequency of 0.045). According to the Genome Aggregation Database (*gnomAD gnomad.broadinstitute.org*), the calculated A91V *PRF1* variant frequency in European plus Latino population is 0.031. Considering that no A91V-positive patients were detected among the Latin-American patients in intive care, the allele frequency found in our Europeans COVID-19 cohort was 0.125, almost 3-times higher than that described for Europeans in gnomAD (0.046).

After 6 weeks, 17of 20 A91V-negative patients had been discharged, 2 of 20 continued hospitalization with significant clinical improvement without ventilator requirement and 1 of 20 had died. Remarkably, both A91V-positive patients died. In these patients we calculated the value of the HScore, a previously validated score which includes the most important variables independently associated with sHLH and helps to form an accurate diagnosis of HLH. HScore values higher than 169 are considered positive for HLH, with a sensitivity of 93% and specificity of 86%.[16] Both patients showed a high HScore for HLH (188 and 175),[5] a shorter time from the disease onset to ICU admission (0 and 6 *vs.* 9.36 days on average) and more severe initial radiological findings (Table 1). Clinically, our A91V-positive patients had high fever associated with the respiratory symptoms. The HLH-related laboratory parameters triglycerides, fibrinogen, ferritin and aspartate aminotransferase were markedly elevated in both subjects, even while receiving immunosuppressive therapy. Unfortunately, because of the pandemic situation and rapid death of both patients, functional studies with cell samples could not be performed.

In conclusion, in our young sCOVID-19 patient cohort, A91V PRF1 was prevalent. A defective A91V *PRF1* may translate into suboptimal lymphocyte cytotoxicity and ineffective SARS-CoV-2 clearance, favoring the progress to sCOVID-19 with an HLH-like clinical phenotype and high mortality. Our observation merits further investigations to assess the specific influence of this variant in COVID-19 clinical course. International collaborative efforts are needed to elucidate the role of genetics in COVID-19.

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