



Low Lymphocytes and IFN-Neutralizing Autoantibodies as Biomarkers of COVID-19 Mortality

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To the Editors,

Type I interferons (IFN) are part of both intrinsic and innate anti-viral immunity. Their deficiency may result in the viral spread and unleash excessive inflammation in SARS-CoV-2 infected individuals, which may underlie poor clinical outcomes or even death. Autoantibodies (auto-Abs) neutralizing type I IFNs are present in around 4% of uninfected individuals over 70 years old [1], at least in 15% of cases of critical coronavirus disease 2019 (COVID-19) pneumonia [2], and are found in around 20% of COVID-19 deaths across ages [1].

The relationship between type I IFNs neutralizing auto-Abs, which precede infection and may underlie critical disease, and clinical progression or death has not been yet explored, unlike the many other clinical variables that may also play a role. Some hematological markers such as low

lymphocyte count below 1000 cells/mm³ [3], C reactive protein (C-RP) over 100 mg/L, ferritin over 500 ng/mL, and D-dimer > 1000 µg/L have been repeatedly identified as risk predictors of severity and mortality in COVID-19 patients.

We performed a retrospective review in a death-enriched cohort at the Infanta Leonor University Hospital in Madrid, Spain, of 178 COVID-19 patients (47 survivals and 131 deaths) with the availability of remaining plasma or serum samples from the first and second waves of COVID-19, which occurred between March and September 2020 in Spain. We analyzed the potential impact of the presence of neutralizing auto-Abs against type I IFNs and other clinical and biological parameters including lymphocytes, C-RP, ferritin, and D-dimer within the first 10 days of symptoms, in terms of survival defined as time, in days, between hospitalization and death. Individuals alive at 90 days of first symptoms were included in the study as censored data.

Clinical data were collected retrospectively from electronic medical records and entered into an anonymous Research Electronic Data Capture (REDCap) database. Biological blood samples were processed for the determination of anti-IFN autoantibodies (IFN-α2, IFN-β, and IFN-ω). Univariate survival curves were modeled using the Kaplan–Meier method and a log-rank test was performed to assess univariate differences in survival time according to established cutoffs in analytic parameters according to published data. Death risk was estimated by the Cox proportional-hazard and Aalen’s additive model. Age and sex were included as covariables. The continuous native form of the different variables was used to develop these analyses. The survival Cox proportional-hazard model’s goodness of fit was evaluated by the Harrell’s concordance index (C-index). The study protocol was approved by the local ethics committee, code 008–2. Informed consent was obtained orally when clinically possible. In the remaining cases, the informed consent waiver was authorized by the ethics committee.

Our cohort comprised 178 COVID-19 patients with critical pneumonia who required hospital admission, of

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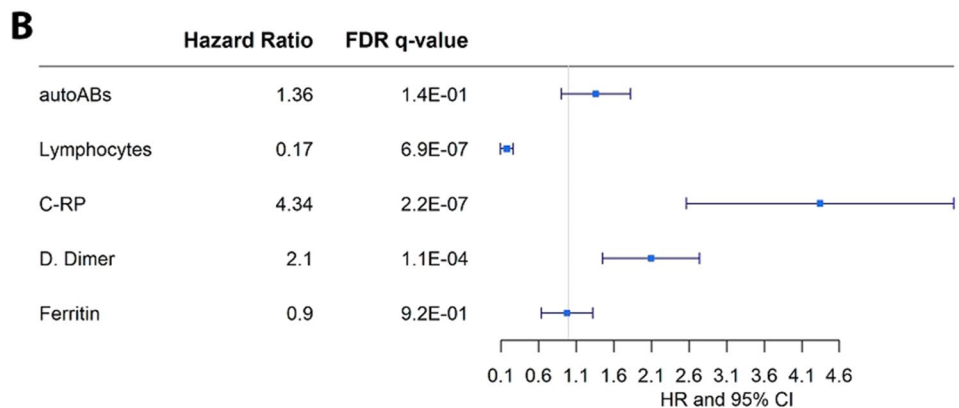
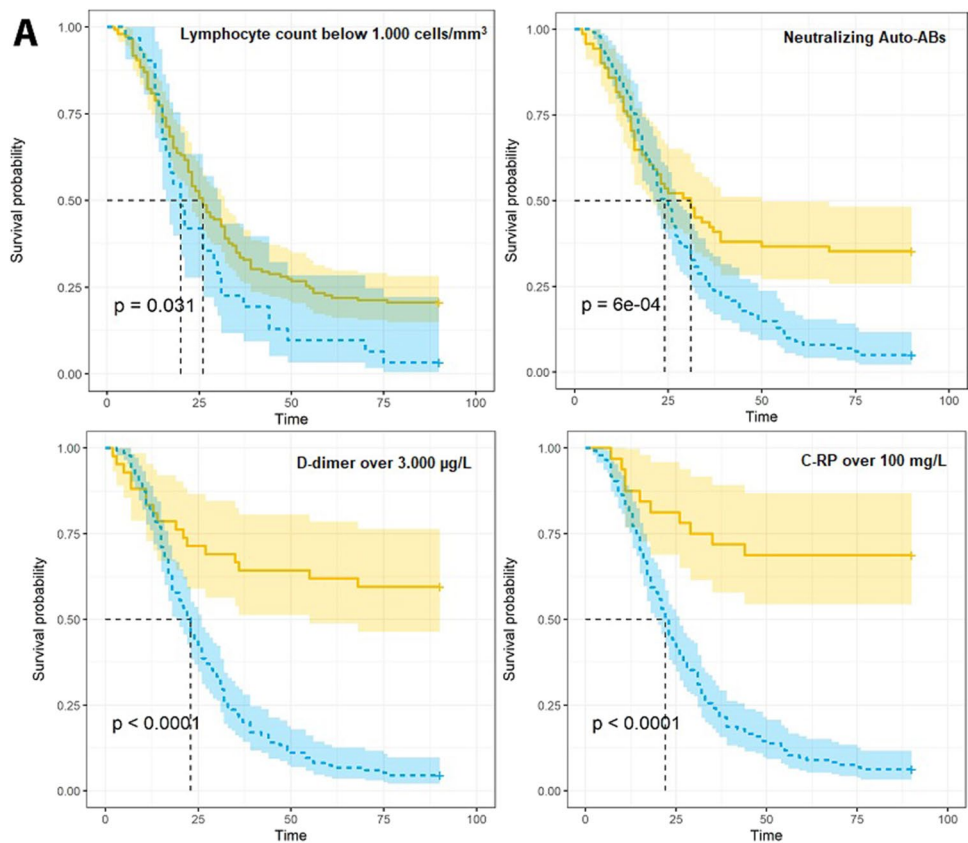
whom 115 (64.6%) were men, 72% patients were over 70 years old, and 85.6% were Spaniards. The main characteristics of this population and clinical parameters are presented in the supplementary material (Supplementary Table 1).

Neutralizing auto-Abs against IFN type I were present in 17.4% (31/178) of our population and in 19.2% (26/135) of patients who died. These results are in consonance with those recently published by the international consortium COVIDHGE (COVID Human Genetic Effort) [1, 2]. In our cohort, neutralizing auto-Abs accounted for IFN- α 2 in 27 patients, IFN- ω in 26 patients, and IFN- β in one patient. In 17 patients, auto-Abs against IFN- α 2 and IFN- ω were found at the same time (Supplementary Table 2).

Even though mortality among patients with neutralizing autoantibodies to IFN was 95.6% (30/31) [4] compared with 79.5% in no carriers, we must be cautious to draw conclusions since we used a cohort enriched in patients who died. Thus, we propose an analysis of survival instead.

Kaplan–Meier univariate analysis showed that the presence of neutralizing auto-Abs led to an increased risk of death during hospitalization ($p = 6e - 04$). Same outcome is shown for lymphocyte count below 1.000 cells/mm³ ($p = 0.0031$), C-RP over 100 mg/L ($p = 0.0001$), and D-dimer over 3.000 μ g/L ($p = 0.0001$) (Fig. 1A). However, in multivariate analysis (Cox or Aalen’s regression), after adjusting by age sex, C-RP, D-dimer, and lymphocyte count, statistical significance, found using the univariate Kaplan–Meier

Fig. 1 **A** Kaplan–Meier survival analysis. Uncorrected univariable analysis on individual survival. The presence of neutralizing autoantibodies against IFN, lymphocyte count below 1.000 cells/mm³, C-RP over 100 mg/L, and D-dimer over 3.000 μ g/L reduces the probability of survival. **B** Cox-hazard model forest plot. Hazard ratios and their 95% confidence intervals, along with the q -values are shown



approach, was lost for the effect of the presence of neutralizing auto-Abs (Fig. 1B, Supplementary Table 3). A statistical power analysis showed that, if we assume a hazard ratio of 1.4, we would need at least 172 individuals with neutralizing auto-Abs to reach statistical significance.

Nevertheless, Cox semi-parametric proportional hazard model (Fig. 1A) showed a strong effect of lymphocyte count, C-RP, and D-dimer over the risk of mortality. A lymphocyte count above 1000 cells/mm³ reduces the risk of mortality by 82.2%. A C-PR and D-dimer over 100 mg/L and 3000 µg/L respectively, increases the risk of mortality 334% and 109% correspondingly. Aalen's additive model, however, did not find such a strong effect of C-RP and D-dimer under a truly non-parametric model (Supplementary Table 3). This finding can be explained because coefficients of Aalen's model are related to risk differences, but coefficients of the Cox model are related to risk ratios.

No differences were found in terms of comorbidities, symptoms before admission, oxygen needs, treatment needs, or intensive care unit (ICU) admission, between both groups with and without neutralizing auto-Abs, by using chi-square tests with *p* values computed by Monte Carlo simulations. Thus, since we have a homogenous cohort, we did not include these covariables in the model. Nevertheless, it is worth mentioning that clinical management of disease varied largely depending on the period in which they were admitted to the hospital, given the overload of the public healthcare system and limited knowledge of the disease in the first weeks of the pandemic, which might have induced a bias for this type of analysis.

In our study, we corroborate the strong effect of the low lymphocyte counts on the mortality risk within a SARS-CoV-2 infection. In this sense, we posit that low lymphocytes counts appear as the most robust marker for unfavorable prognosis or even death, as described in other cohorts [3]. Furthermore, we detected an association between having neutralizing auto-Abs and an increased risk of mortality through a univariate model. However, when adjusting for other risk factors using multivariable hazard models, this association is lost; a trend remains but without statistical significance. Thus, we consider that it will be extremely interesting to confirm these findings with a larger sample to have enough statistical potency to demonstrate this association.

This study has two major limitations that should be considered, regarding its relatively small sample size and its unbalanced design, and therefore we present the work as a pilot exploratory analysis. Most likely, a lack of statistical power prevented us from clearly showing an association between having neutralizing auto-Abs to type I IFN and unfavorable prognosis.

These results invite to pool data with other groups for reaching higher statistical power, such as with a French

COVID-19 cohort [5] which recently published clinical data regarding IFN neutralizing auto-Abs, to elucidate with a larger population if the presence of neutralizing auto-Abs definitely impacts COVID-19 progression in clinical terms. Meanwhile, low lymphocyte counts may be used as a robust biomarker of COVID-19 unfavorable progression and high mortality risk.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10875-022-01241-5>.

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Author Contribution Authors have participated in (a) conception and design; (b) analysis; (c) interpretation of the data; (d) drafting the article; (e) revising it critically for important intellectual content; and (f) approval of the final version:

Jesús Troya: a, c, d, e, f

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Data availability Clinical data files are stored at Infanta Leonor University Hospital. It may be shared if needed.

Declarations

Ethics Approval The study was approved by the Committee for Ethical Research of the Infanta Leonor University Hospital, code 008–20.

Consent to Participate Informed consent was obtained orally when clinically possible. In the remaining cases, the informed consent waiver was authorized by the ethics committee.

Consent for Publication This consent was obtained with consent to participate.

Conflict of Interest The authors declare no competing interests.

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