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Cytokine prediction of mortality in COVID19 patients

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ABSTRACT

Coronavirus disease 2019 (COVID19) is a life-threatening infection with uncertain progression and outcome. Assessing the severity of the disease for worsening patients is of importance in making decisions related to supportive mechanical ventilation and aggressive treatments. This was a prospective, non-randomized study that included hospitalized patients diagnosed with COVID19. Pro-inflammatory cytokines were assessed during hospitalization, and we calculated a prediction paradigm for 30-day mortality based on the serum levels of interleukin1β (IL1β), interleukin6 (IL6), interleukin8 (IL8), and tumor necrosis factor alpha (TNFα) measured by next-generation ELISA.

Data of 71 COVID19 patients, mean age 62 years, SD13.8, 50 males, 21 females, were analyzed. Twelve (16.9%) patients died within 7–39 days of their first COVID19 positive nasopharyngeal test. Levels of IL6 and TNFα were significantly higher in patients that did not survive. IL6 predicted mortality at the cut-off value of 163.4 pg/ml, with a sensitivity of 91.7% and specificity of 57.6%.

Our findings demonstrate that IL6 expression is significant for the prediction of 30-day mortality in hospitalized COVID19 patients and, therefore, may assist in treatment decisions.

1. Introduction

Cytokine-mediated inflammation, also described as a cytokine storm, plays an important role in severe cases of COVID19 and is reported as a major cause of death. The implicated mechanism for COVID19 cytokine-mediated inflammation relates to infection of the alveolar epithelial cells through the ACE2 receptor. The resulting acute inflammatory response activates macrophages as well as B and T lymphocytes that release pro-inflammatory cytokines directly promoting the ongoing inflammatory process. Under the stimulation of these inflammatory factors, a large number of inflammatory exudates and erythrocytes enter the alveoli, resulting in dyspnea, respiratory failure and death [1–5].

Accurate knowledge regarding clinical worsening that results in death is crucial to choose appropriate interventions aimed to reduce mortality. To date, information related to the cytokine profile of COVID19 infected patients is scarce, and data is currently being collected.

Based on our findings, we propose recommendations regarding cytokine assessment that can determine with reasonable accuracy the possibility of death for COVID19 patients. Using these data and a method to assess the degree of certainty, we suggest a cytokine paradigm that could help treatment decisions.

2. Methods

2.1. Patients

Demographic and clinical data of patients diagnosed with COVID19 pneumonia whose peripheral blood cytokines were tested were collected. Serum samples from age-matched healthy subjects served as cytokine-assay controls.

2.2. Cytokine assay

The cytokine level of interleukin1β (IL1β), interleukin6 (IL6), interleukin8 (IL8), and tumor necrosis factor alpha (TNFα) were measured by next-generation ELISA (Simple PlexTM Ella microfluidic platform, Protein Simple, CA, USA). Briefly, 25 μl diluted serum samples were added to a microfluidic cartridge, separated into triplicates, and coated with biomarker-specific capture monoclonal antibodies. Detection of the antibodies and streptavidin-DyLight650 conjugates, as well as all washing steps, were automatically performed. Raw data were analyzed using the SimplePlex Explorer software.

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2.3. Statistics

All measured variables and derived parameters were tabulated by descriptive statistics; for categorical variables – sample size, absolute and relative frequency by status (Dead or Alive), and for continuous variables – sample size, arithmetic mean, standard deviation, percentiles (25%, median, 75%), minimum and maximum for means of variables by status (Dead or Alive), were calculated. The two-sample T-test for independent samples was applied for testing the statistical significance of the difference in the cytokines level between status (Dead or Alive). Chi-square test was applied for testing the statistical significance of the difference in mortality rate between the subjects who had a high level of cytokines (≥median) and subjects who had a low level of cytokines (< median).

Survival analysis using Kaplan-Meier survival function curve was applied for testing the statistical significance of the difference in survival between the subjects who had a high level of cytokines (≥median) and the subjects who had a low level of cytokines (< median). All probabilities were calculated relatively to group status (Dead or Alive). The log-rank test was used for comparison between groups. Cox model was applied for comparative analysis of Kaplan-Meier curves with adjustment for age and gender as covariates. Adjusted hazard ratios (HR) and 95% confidence intervals were estimated via the Cox regression model. All tests were two-tailed, and a p-value of 5% or less was considered statistically significant. Data was analyzed using the SAS® version 9.4 (SAS Institute, Cary North Carolina).

3. Results

Seventy-one COVID19 patients, mean age 62.0 years, SD = 13.8, 50 males, 21 females were included in the analysis. Twelve (16.9%) patients died within 7–39 days of their first COVID19 positive nasopharyngeal test, median 25 days, 3–42 days of the first day of hospitalization, median 17 days, and 0–23 days of serum cytokine assay, median 8 days. Serum samples of 11 healthy subjects, mean age 48.9 years, SD = 8.4, 7 males, 4 females served as controls for the cytokine-assay. COVID19 patients had very high cytokine levels compared to healthy subjects confirming the disease-related cytokine storm. The pro-inflammatory cytokine levels of patients by status (Dead or Alive) are presented in Table 1. All cytokines were higher in COVID19 patients as compared to healthy subjects. Levels of IL6 and TNFα were significantly higher in patients that did not survive.

Survival analysis calculating the probability of death given a cytokine level above the 50th percentile, showed a sensitivity of 91.7% and a specificity of 57.6% for IL6, suggesting that patients with IL6 level above the 50th percentile, e.g., at the cut-off value above 163.4 pg/ml, had a probability of 42.4% to remain alive and 91.7% probability of dying, p = 0.0018. Similarly, given a cytokine level above the 50th percentile for TNFα, e.g., a cut-off level above 33.91 pg/ml, showed a sensitivity of 75.0% and specificity of 54.2%, suggesting that patients with TNFα level above the 50% percentile had a probability of 45.8% to remain alive and a probability of 75% to die, p = 0.0648.

Kaplan-Meier curves with adjustment for time of event (Dead or Alive) were performed for all cytokines but here we present only those with significant trend with mortality data. Accordingly, IL6 levels above the 50th and 75th percentiles are shown in Fig. 1a, and b, demonstrating that 15% and 25% of patients with high cytokine levels on day 5, respectively, will die. In comparison, 95% and 85% of patients with low cytokine levels on day 15, respectively, will survive (log-rank test p = 0.1134 and p = 0.0323, respectively). Due to the relatively small number of patients that died in each group, 7 patients in the early (0–14) mortality group, and 5 patients in the late (15–30) mortality group, we could not assess significant differences between the cytokine levels and time until death.

Cox model for overall survival demonstrated that the likelihood estimates of death were associated with older age (HR, 1.082, 95% CI, 1.004–1.165, p = 0.0385), suggesting that for each yearly increase in age the probability of death increases by 8.2%, but not with male gender (HR, 0.905, CI, 0.184–4.449, p = 0.905), Table 2. An increase in IL6 level by 100 units resulted in a 20% increase in death probability, and an increase in TNFα level by 10 units resulted in a 22% increase in death probability.

4. Discussion

In the current study, we found that IL6 can predict survival in COVID19 severe patients. In the literature, IL6 was reported as a highly indicative inflammatory marker to predict mortality and hospitalization in the oldest patients and patients with multiple chronic diseases [6,7]. Moreover, using IL6 levels in the first 24 h following trauma predicted immunological complications and mortality in trauma patients [8]. In severe viral infections such as Ebola virus, Dengue virus, and highly pathogenic avian influenza viruses (H5N1, H1H1), high cytokine levels correlated with morbidity and mortality [9–11]. In Corona virus infections, cytokine storm has been described in patients with SARS-CoV in 2002 and MERS-CoV in 2012 [12,13], and was associated with disease severity. In the recent COVID19 pandemic, the first studies that reported the association of cytokines and COVID19 infection found that the levels of IL6 and IL10 were associated with the severity of COVID19 pneumonia [14], and that TNFα and IL8 levels were potential targets for immunotherapy of COVID19 infection as their levels were high in patients with severe disease [2,15]. Similarly, we also found high cytokine levels in COVID19 patients and specifically IL6 and TNFα levels differentiated between patients that died and those that remained alive.

Moreover, we calculated an IL6-based paradigm that predicted mortality with high sensitivity and specificity and defined a cut-off level for mortality prediction within 30 days of the cytokine assay. We further demonstrated that an increase of 100 units in the IL6 level would result in a 20% increase in death probability. However, levels of other pro-inflammatory TNFα, IL1β, and IL8, though elevated, did not contribute to mortality prediction. Herold et al. [16], reported that level of

Table 1
Cytokine levels in COVID19 patients by status.

*Cytokine pg/ml	Survival														
	Alive, N = 59							Dead, N = 12							
	Mean	SD	Min	25%	Median	75%	Max	Mean	SD	Min	25%	Median	75%	Max	p
IL1β	0.67	1.38	0.05	0.17	0.31	0.67	10.15	0.64	0.77	0.25	0.27	0.41	0.58	3.01	0.9491
IL6	117.24	229.48	0.44	17.81	39.65	99.77	1206.61	605.69	710.99	43.33	87.36	163.40	1222.01	1829.97	< 0.0001
IL8	51.62	59.27	5.49	18.06	29.68	56.52	290.51	102.04	117.90	26.63	33.16	52.44	119.69	435.32	0.1739
TNFα	22.88	12.15	5.46	14.74	19.09	28.10	62.80	36.00	17.33	10.65	22.36	33.91	50.13	66.06	0.0023

* Cytokine levels in healthy controls (mean + SD, median) were as follows: IL1β (0.10 ± 0.15, 0.03); IL6 (1.80+ ± 0.88, 1.61); IL8 (13.09 ± 5.89, 10.04); TNFα (9.92 ± 2.04; 9.65).

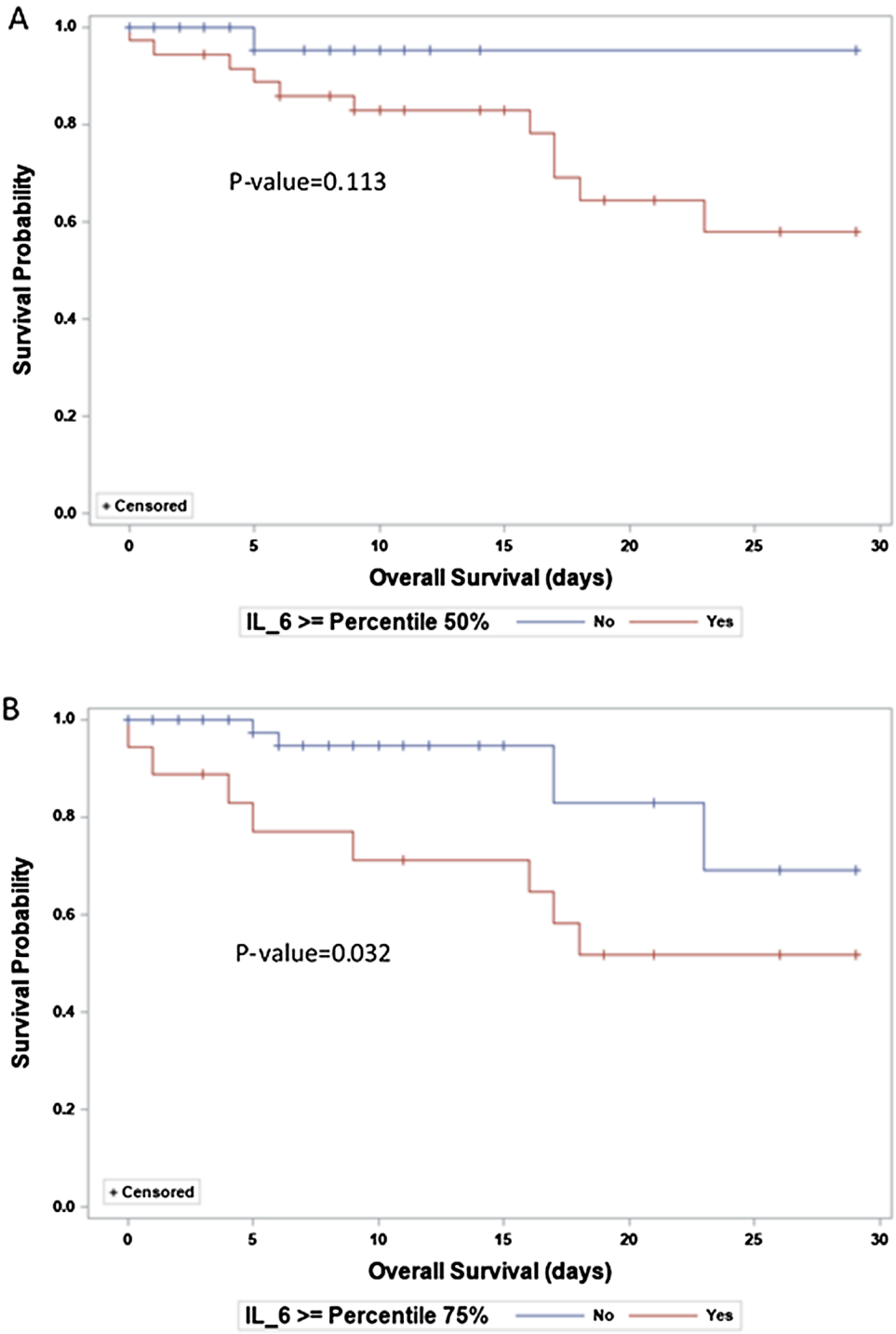

Fig. 1. IL6-based survival curves in COVID19 patients. Kaplan–Meier estimates for the cumulative mortality with adjustment for time of event (Dead or Alive) for IL6 levels above the 50th (Panel A) and 75th (Panel B) percentiles.

Table 2
Maximum likelihood estimates for mortality prediction.

Parameter	Hazard Ratio	95% confidence interval	P value
Age, years	1.082	1.004–1.165	0.0385
Male gender	0.905	0.184–4.449	0.9023
IL6	1.002	1.001–1.003	0.0040
TNFα	1.022	0.955–1.057	0.2121

IL6 could predict respiratory failure in hospitalized symptomatic COVID19 patients requiring mechanical ventilation, suggesting that this cytokine is important to assess deterioration.

We could not detect significant differences in the cytokine levels for the risk for early and late mortality due to the relatively small numbers of patients in each group.

As IL6 drives the acute inflammatory response and is thus responsible for immune dysregulation during acute viral inflammation, its role in predicting survival during COVID19 infection is central. Therefore, elevated IL6 levels during COVID19 infection may alert

clinicians to consider more aggressive treatment interventions.

CRediT authorship contribution statement

Mathilda Mandel: Conceptualization, Investigation, Writing - original draft. **Gil Harari:** Formal analysis, Writing - review & editing. **Michael Gurevich:** Investigation, Writing - review & editing. **Anat Achiron:** Conceptualization, Writing - original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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