Association of early nasopharyngeal immune markers with COVID-19 clinical outcome: predictive value of CCL2/MCP-1

Beatriz Sierra*1a, Ana B. Pérez1a, Eglis Aguirre1, Claudia Bracho1, Odalys Valdés2, Narciso Jimenez3, Waldemar Baldoquin4, Guelsys Gonzalez2, Lilia M. Ortega5, Maria C. Montalvo6, Sonia Resik7, Delmis Alvarez8, Maria G. Guzmán9

1Cellular Immunology Laboratory, Virology Department, Center for Research, Diagnostic and Reference (CIDR), Pedro Kourí Institute of Tropical Medicine (IPK), Havana, Cuba.

2Respiratory Viruses Laboratory, Virology Department, Center for Research, Diagnostic and Reference, Pedro Kourí Institute of Tropical Medicine (IPK), Havana, Cuba.

3Medical Attention Branch, Pedro Kourí Institute of Tropical Medicine, (IPK), Havana, Cuba.

4Epidemiology Department Center for Research, Diagnostic and Reference, Pedro Kourí Institute of Tropical Medicine, (IPK), Havana, Cuba.

5Intensive Care Unit (Head), Medical Attention Branch, Pedro Kourí Institute of Tropical Medicine, (IPK), Havana, Cuba.

6Hepatitis Laboratory, Virology Department, Center for Research, Diagnostic and Reference, Pedro Kourí Institute of Tropical Medicine, (IPK), Havana, Cuba.

7Enterovirus Laboratory. Virology Department (Head), Center for Research, Diagnostic and Reference, Pedro Kourí Institute of Tropical Medicine, (IPK), Havana, Cuba.

8Department of Computing, Pedro Kourí Institute of Tropical Medicine, (IPK), Havana, Cuba.

© The Author(s) 2020. Published by Oxford University Press on behalf of Infectious Diseases Society of America. This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs licence (http://creativecommons.org/licenses/by-nc-nd/4.0/), which permits non-commercial reproduction and distribution of the work, in any medium, provided the original work is not altered or transformed in any way, and that the work is properly cited. For commercial re-use, please contact journals.permissions@oup.com
Center for Research, Diagnostic and Reference (Head), Pedro Kourí Institute of Tropical Medicine, (IPK), Havana, Cuba.

Footnote page

Funding statement

This work was supported by the Cuban Ministry of Public Health. Publishing charges of this article are supported by VLIR-UOS through Project CU2019SIN243A102 (SI2019-SEL012).

Potential conflict of interest.

All No reported conflicts of interest. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Previous presentation of information:

Results of this study have not been presented at any meeting.

Corresponding author:

Beatriz Sierra, Md, PhD. Immunology Laboratory, Virology Department

National Center for Research, Diagnostic and Reference

Pedro Kourí Institute of Tropical Medicine (IPK)

Autopista Novia del Mediodía , km 61/2, Habana, 11400, Cuba.

Phone:53-7-2553559 Email: siebet@ipk.sld.cu; sierrab@infomed.sld.cu

*B.S. and AB.P. contributed equally to this work
Abstract

Early recognition of severe forms of COVID-19 is essential for an opportune and effective intervention, reducing life risk complications. An altered inflammatory immune response seems to be associated to COVID-19’s pathogenesis and progression to severity. Here we demonstrated the utility of the early nasopharyngeal swab sample for detection of the early expression of immune markers and the potential value of CCL2/MCP-1 in predicting disease outcome.

Keywords: COVID-19, Cytokines, Chemokines, Prognostic biomarkers, Inflammation
Background

Coronavirus disease 2019 (COVID-19) is an emerging infectious disease highly pathogenic, threatening at present the global health. Cuba reported the first confirmed COVID-19 cases on March 11, 2020, and autochthonous transmission on April 7. First confirmed cases in Cuba were diagnosed and hospitalized at the Institute of Tropical Medicine Pedro Kourí (IPK), Cuban National Center of Reference for Infectious Diseases.

SARS CoV-2 virus, the etiological agent of COVID-19 produces both symptomatic and asymptomatic infections. As a result, patients can be asymptomatic, have a mild respiratory disease, acute respiratory distress syndrome (ARDS), or pneumonia of varying degrees of severity (1).

SARS-CoV-2 virus shows, similar to the observed by Middle Eastern respiratory syndrome (MERS) coronavirus infection, a high viral replication in the upper airway epithelial cells resulting in enhanced production of multiple cytokines and chemokines(2)(3). Some proinflammatory mediators, like TNF alpha, CCL2 and CCL3 could be directly responsible of vascular leakage and alveolar edema ultimately causing hypoxia, ARDS and potentially death, through the apoptosis of both lung epithelial and endothelial cells (4).

In view of this we considered the possible utility of early nasopharyngeal swabs samples to detect the presence of messenger RNA of proinflammatory and regulatory mediators already associated to COVID-19 disease, like TNFα, CCL2/MCP-1, CCL3/MIP-1α, and TGFβ and IL-10, respectively. We hypothesized that the activity of this set of cytokines/chemokines could trigger/suppress the severe outcome of infection, resulting in confirmed COVID-19 patients with asymptomatic infections or different clinical evolutions.

We found significant differences between the groups with favorable and unfavorable clinical evolution, suggesting the value of the early detection of TNFα, CCL2/MCP-1, CCL3/MIP-1
alpha mRNA in nasopharyngeal swab samples, and the predictive value of CCL2/MCP-1 for the COVID-19 clinical outcome.

**Methods**

**Clinical Samples**

Nasopharyngeal samples were collected separately with sterile polyester tipped swabs (Puritan Medical Products Co., LLC, Guilford, ME, USA) following the PAHO/WHO Laboratory Guidelines for Detection and Diagnosis of the Novel Coronavirus (2019-ncov). Both swabs of each patient (nasopharyngeal samples) were placed into the same tube with universal transport medium in one collection tube (Puritan UniTranz-RT transport System).

Sample manipulation was performed in a biosafety class II lab. Total RNA was isolated from nasopharyngeal swabs by means of RNeasy Mini QIAcube Kit, in QIAcube® instrument (Qiagen, Hilden, Germany), following the supplier’s instructions.

COVID-19 infection was confirmed by real-time reverse transcription-polymerase chain reaction (RT-PCR) assay for nasopharyngeal swab specimens, following WHO guidelines for qRT-PCR (5).

Isolated RNA from nasopharyngeal swab samples from 54 cases received at the Reference Laboratories of the Pedro Kourí Institute of Tropical Medicine as part of the COVID-19 surveillance in the period of March 11 to April 7, was studied. Thirty-eight samples were confirmed as COVID-19 positive. Patients were admitted at the Medical Attention Branch of the IPK. From these patients, 12 remained asymptomatic and 26 developed COVID-19 symptoms (fever, cough, myalgia, diarrhea rhinorrhea, sore throat, vomiting, asthenia, dyspnea and respiratory distress) (table 1).
Symptomatic cases were classified according to the number of symptoms, and the presence of symptoms previously associated to severity in Cuban patients (distress respiratory, dyspnea and asthenia). Patients with 1 or 2 symptoms, excluding those associated to severity, were classified as Mild Disease (eleven patients). Patients with 5 or more symptoms, including asthenia and dyspnea, were classified as Very Symptomatic (nine patients); Those patients with respiratory distress (respiratory frequency>30/min, O$_2$ Saturation index <93 %, PaO$_2$/FiO$_2$ Ratio <300) and intensive care requirement, but progressing to recovering phase, were classified as Severe (three patients), and those with respiratory distress and intensive care requirement, that unfortunately died, as Fatal (three patients). None of the asymptomatic or mild disease cases showed levels of C-reactive protein over 20 mg/l, levels of ferritin over 400 μg/L, or lymphopenia (lymphocyte count below 1.0× 10$^9$/l). In order to identify biomarkers which discriminate between a favorable and unfavorable clinical evolution, we joined asymptomatic subjects and patients with mild disease in the category of “favorable evolution”, and all those patients who developed a more severe picture (Very symptomatic, complicated and fatal cases) in the category “unfavorable evolution” (Table 1).

All swab samples from symptomatic patients were collected between the first and fifth day after onset of symptoms for COVID-19. Samples from 16 healthy individuals confirmed as negative for SARS-CoV-2 were used as controls. The information about age, sex, days from symptoms onset to sample collection and co-morbidities of the studied subjects are shown in table 1.

**Cytokines gene expression analysis**

The cDNA was synthesized from mRNA with poly(dT) primers and Superscript II reverse transcriptase (Life Technologies, Rockville, MD, USA). Quantitative real-time PCR (qPCR) was performed with Rotor-Gene Q 5plex HRM (Qiagen) in 36-wells Gene Discs,
using QuantiFast SYBR® Green RT-PCR Kit (Qiagen) following manufacturer instructions. PCR protocol is described in detail in supplementary materials.

Gene expression variations of TNFα, CCL2/MCP-1, CCL3/MIP-1 alpha, TGFβ and IL10 were evaluated in terms of fold induction with respect to the housekeeping gene hypoxanthine phosphoribosyltransferase-1 (HPRT-1) by both the 2-ΔΔCT method. Experiments were conducted in triplicate. Sequence of primers used in this work is described in supplementary data.

**Statistical analysis**

Distribution of data was tested using Kolmogorov-Smirnov test (data not normally distributed). Kruskal-Wallis non-parametric test was used to know if there were significant differences in the gene expression of each cytokine among the three groups of cases (controls, cases with favorable evolution and cases with unfavorable evolution). If there was statistical significance we proceed to the Dunn’s correction for multiple comparisons. For analyzing the differences in the gene expression of each cytokine according to the time from symptom onset to sample collection and the association of cytokine gene expression with symptoms we used the non-parametric Wilcoxon–Mann–Whitney U mean rank test. Graphics, showed as individual dots, and with data on a logarithmic scale, were made with the program GraphPad Prism 5 for Windows, Version 5.01, 2007. Data are displayed as median and range. Correlations between variables were evaluated with the Spearman correlation test. The values of the cytokines were log transformed to address skewed distribution before performing univariate and multivariate logistic regression analysis. Data analyses were considered as statistically significant when p < 0.05. Calculations were made with the Statistical Package for Social Sciences, SPSS 11.5 and R version 4.0.1 (2020-06-06), for Microsoft Windows 10.
**Patient Consent Statement**

Written informed consent was obtained from each individual upon enrolment in the study. This study was conducted according to the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects. The design of the work has been approved by the Institutional Ethical Review Committee of the Institute of Tropical Medicine “Pedro Kouri”, and the Cuban Ministry of Public Health.

**Results**

The objective of this study was to explore the early expression of TNF-alpha, CCL2/MCP-1, CCL3/MIP-1 alpha, IL-10, and TGFβ, previously associated with the uncontrolled cytokines response in the ARDS pathogenesis produced by human pathogenic coronaviruses, thereby taking advantage of the nasopharyngeal swab sample, mandatory for diagnosis of COVID-19 diagnosis in Cuban suspected cases.

To determine the possible association of early gene expression of immune mediators in nasopharyngeal swab sample with the clinical evolution, we studied patients with a mild or severe COVID-19 clinical picture, and asymptomatic subjects. SARS-CoV-2 negative individuals were included as controls.

Figure 1a shows the comparison of TNF alpha expression among negative controls, patients with favorable and unfavorable evolution. We did not find significant differences between the controls and patients with favorable evolution The group of unfavorable evolution showed a significantly higher TNF alpha expression in comparison to the patients with favorable evolution (p<0.001), and with negative controls (p<0.001).

A significantly higher expression however of CCL2 was observed in cases with unfavorable outcome compared to cases with favorable evolution (p<0.001) or to negative controls (p<0.001) (figure 1b). Similar results were obtained for CCL3: also here a significantly
higher expression in cases with unfavorable outcome was observed, compared to cases with favorable evolution (p<0.001) or to controls (p<0.001) (figure 1c).

The analysis of TGFβ expression among the three groups is shown in figure 1d. Individuals grouped as favorable outcome showed a significantly higher expression compared to controls (p<0.05). Patients with unfavorable outcome showed higher expression than controls (p<0.001), and cases with favorable outcome (p<0.01). In contrast, we did not find statistical relevant differences in IL-10 expression between individuals with favorable and unfavorable evolution or with negative controls (supplementary data).

We also analyzed if there were significant differences in the gene expression of each cytokine according to the time from symptom onset to sample collection. We found a significantly higher expression only for TGF beta in the period from 0 to 3 days, compared to the period from 4 to 5 days (p=0.005). We checked then which group of cases, according to clinical evolution, was supporting this difference in the in the period from 0 to 3 days, and we found significantly higher expression in cases with unfavorable evolution compare those with favorable evolution (p=0.033).

An association of TNFα with marked asthenia (p=0.0004), and with the dyspnea presence (p=0.006) in the unfavorable evolution group was found. Dyspnea has been reported to be more frequent in COVID-19 severe cases, and indeed, in some studies, it was included as a marker of severe disease (15, 16). A similar association with these clinical symptoms was observed for CCL2/MCP-1 and TGFβ (asthenia: p=0.003 and p=0.006 resp.; dyspnea: p=0.043 and p=0.006 resp.) while CCL3/MIP-1 alpha was shown to be associated only with asthenia (p=0.004).

The univariate logistic regression models showed that the coefficients of three of the five cytokines evaluated were statistically associated with the unfavorable outcome (CCL2/MCP-
1, TGFβ and TNFα). Nevertheless, only CCL2/MCP-1 was significant in the multivariate analysis- beta_(Estimate = 2.93, p= 0.024).

**Discussion**

Early recognition of severe forms of COVID-19 is crucial for an opportune and effective early intervention that reduces life risk complications.

A deregulated antiviral immune response, resulting in the release of large amounts of pro-inflammatory cytokines and subsequent uncontrolled local or systemic inflammation, has been recognized as causal for severity and lethality in pathogenic human coronavirus infections, including COVID-19 (3) (4).

As a consequence, several inflammatory markers, in particular cytokines, have been associated to severity and prognosis of COVID-19 (6). However, most of these were detected in peripheral blood, and after the fifth day of onset of symptoms, which is relatively late (7).

To our knowledge, this is the first study evaluating mRNA expression of cytokines/chemokines, already linked to COVID-19 pathogenesis, in swab samples from the upper airway at the early start of the symptoms (first 5 days). Although the sample size was rather limited (16 healthy controls, 23 patients with favorable evolution and 15 patients with unfavorable evolution), clearly statistical differences were observed.
Concurring with recent reports in Chinese COVID-19 patients, we found significantly higher expression of TNFα, CCL2/MCP-1 and CCL3/MIP-1α in COVID-19 cases with an unfavorable evolution compared to those with a favorable evolution (1) (8).

These markers have a high amplifying potential, quickly enhancing the inflammatory cytokine/chemokine responses in the upper airway, probably predicting later pathologic events in the lower airway associated to ARDS (9) (10) (11).

The higher levels of TGFβ in cases with severest outcome could be in apparent contradiction with the regulatory and anti-inflammatory role attributed to this cytokine (12). However, the TGFβ function seems to be highly dependent on location and context and could play a major role under inflammatory conditions (13), in fact, it has been also associated to hypoxia and lung damage (14). The analysis of IL-10 gene expression did not showed statistical relevant differences among the groups.

Cytokines are pleiotropic and interact as a network, in which the analysis of a single cytokine may not provide trustable information about serial immune events. Considering this, univariate logistic statistical analysis was conducted to detect differences in concentrations of each measured cytokines, and to detect the confounders in patients with favorable versus patients with unfavorable evolution. Also, in order to control the effect confounders like age, sex and the presence of comorbidities, a multivariate logistic regression analysis was used.

The univariate logistic regression model confirmed the association of TNFα, CCL2/MCP-1 and TGFβ with the COVID-19 outcome. However, after discard the “noise” introduced by confounder variables, with a multivariate logistic regression model, only CCL2/MCP-1 was significant which suggested a predictive value for this marker.

CCL2/MCP-1 regulates the migration and infiltration of monocytes, memory T lymphocytes, and natural killer (NK) cells, favoring inflammatory process in tissues, including lung (2).
Several recent reports showed MCP-1 as a biomarker predicting disease severity of COVID-19 in serum and plasma (1) (15) (16). We are reporting the predictive value of this chemokine in mucosal environment, where the infection is occurring, and very early after symptoms onset.

Moreover, the present study demonstrates the utility of nasopharyngeal swab samples collected within five days after the onset of symptoms for very early detection of immune markers. They may be a valuable and simple aid to improve the early identification of patients with risk for evolution towards a severe COVID-19 taking timely measures and treatments to preserve the life. Further research in a higher number of cases may validate CCL2/MCP-1 as early mucosal biomarker predicting disease severity of COVID-19.
Acknowledgments

We thank Professor Xaveer Van Ostade, Laboratory for Protein Science, Proteomics and Epigenetic Signaling (PPES), Department of Biomedical Sciences (BMW), Faculty of Pharmaceutical, Biomedical and Veterinary Sciences (FBD), University of Antwerp, for the revision of the manuscript.
References

1. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020; 395 (10223):497-506.

2. Chu CM, Poon LL, Cheng VC, et al. Initial viral load and the outcomes of SARS. CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne 2004; 171 (11):1349-52.

3. Channappanavar R, Perlman S. Pathogenic human coronavirus infections: causes and consequences of cytokine storm and immunopathology. Semin Immunopathol. 2017; 39 (5):529-39.

4. Mehta P, McAuley DF, Brown M, et al. COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet 2020; 395 (10229):1033-4.

5. World Health Organization. Laboratory testing strategy recommendations for COVID-19: interim guidance, 21 March 2020. World Health Organization 2020. Available at: https://apps.who.int/iris/handle/10665/331509. Accessed 24 May 2020.

6. Zeng F, Huang Y, Guo Y, et al. Association of inflammatory markers with the severity of COVID-19: A meta-analysis. Int J Infect Dis. 2020; 96:467-74.

7. Velavana TP, Meyer CG. Mild versus severe COVID-19: Laboratory markers. Int J Infect Dis 2020; 95:304-7.

8. Xiong Y, Liu Y, Cao L, et al. Transcriptomic characteristics of bronchoalveolar lavage fluid and peripheral blood mononuclear cells in COVID-19 patients. Emerg Microbes Infect 2020; 9 (1):761-70.

9. Deshmane SL, Kremlev S, Amini S, Sawaya BE. Monocyte chemoattractant protein-1 (MCP-1): an overview. J Interferon Cytokine Res 2009; 29 (6):313-26.
10. Zelova H, Hosek J. TNF-alpha signalling and inflammation: interactions between old acquaintances. Inflam Res 2013; 62 (7):641–51.

11. Glaser L, Coulter PJ, Shields M, et al. Airway Epithelial Derived Cytokines and Chemokines and Their Role in the Immune Response to Respiratory Syncytial Virus Infection. Pathogens 2019; 8 (3).

12. Sanjabi S, Zenewicz LA, Kamanaka M, Flavell RA. Anti- and Pro-inflammatory Roles of TGF-β, IL-10, and IL-22 in Immunity and Autoimmunity. Curr Opin Pharmacol 2009; 9 (4):447-53.

13. Pahlman LI, Jogi A, Gram M, Mori M, Egesten A. Hypoxia down-regulates expression of secretory leukocyte protease inhibitor in bronchial epithelial cells via TGF-beta1. BMC Pulm Med. 2015; 15:19.

14. Rosenbloom J, Macarak E, Piera-Velazquez S, Jimenez SA. Human Fibrotic Diseases: Current Challenges in Fibrosis Research. Methods Mol Biol 2017; 1627:1-23.

15. Yang Y, Shen C, Li J, Yuan J, et al. Plasma IP-10 and MCP-3 levels are highly associated with disease severity and predict the progression of COVID-19. J Allergy Clin Immunol. 2020; 146 (1):119-127.

16. Chen Y, Wang J, Liu C, et al. IP-10 and MCP-1 as Biomarkers Predicting Disease Severity of COVID-19 (6/2/2020). Available at SSRN: https://ssrn.com/abstract=3619756 or http://dx.doi.org/10.2139/ssrn.3619756.
Figure legends

Figure 1. Relative gene expression of immune mediators in nasopharyngeal swab samples from COVID-19 cases and controls. (A) Expression of TNFα transcripts in different study groups of subjects: Controls (Samples from 16 healthy individuals confirmed as negative for SARS-CoV-2); Favorable (23 subjects with favorable evolution: 12 asymptomatic subjects and 11 patients with mild disease) Unfavorable (15 patients with unfavorable evolution: 9 very symptomatic patients, and 6 that required intensive care, from these, 3 recovered and 3 deceased). (B) Expression of CCL2/MCP-1 transcripts in those three groups; (C) Expression of CCL3/MIP-1α transcripts in the three groups. (D) Expression of TGFβ transcripts in the three groups. mRNA was determined for real-time RT-PCR analysis and genes /housekeeping gene ratio was determined according to the delta/delta Ct method. Data are shown as dot-plots graphics, on log scale. Each dot plot shows statistic data about the gene expression determination. The central horizontal line in the dot marks the median of the samples, the hinges marks the ranges. Statistical significant differences in cytokine production among groups are indicated with horizontal lines over dots. A p < 0.05 was considered statistically significant, p > 0.05 was considered not statistically significant. (*p < 0.05, **p < 0.01, ***p < 0.001).
**Tables.**

Table 1: Characterization of the studied subjects.

| Clinical Classification | Healthy | Asymptomatic  | Mild disease | Very symptomatic | Severe | Fatal |
|-------------------------|---------|---------------|--------------|------------------|--------|-------|
|                         | 16      | 12            | 11           | 9                | 3      | 3     |

| Study’s Groups          | Controls | Favorable evolution | Unfavorable evolution |
|-------------------------|----------|---------------------|-----------------------|
|                         | n=16     | n=23                | n=15                  |

| Age         | n  | %  | n  | %  | n  | %  |
|-------------|----|----|----|----|----|----|
| 15-54       | 9  | 56.3 | 7  | 58.3 | 8  | 61.5 |
| 55-64       | 5  | 31.2 | 1  | 8.3  | 3  | 23.1 |
| > 65        | 2  | 12.5 | 4  | 33.3 | 2  | 15.4 |

| Sex | Female | 8 | 50 | 6 | 50 | 5 | 38.5 |


| Male | 8  | 50 | 6  | 50 | 8   | 61.5 |
|------|----|----|----|----|-----|------|
| **Comorbidities** |    |    |    |    |     |      |
| Hypertension     | -  | 1  | 3  | 2  | 2   | 1    |
| Cardiopathy      | -  | 0  | 0  | 1  | 1   | 1    |
| Diabetes mellitus| -  | 0  | 0  | 0  | 1   | 2    |
| Bronchial asthma | 2  | 1  | 1  | 1  | 1   | 1    |
| Obesity          | -  | 0  | 0  | 0  | 0   | 1    |
| Other (Autoimmunity, Epilepsy, Cancer, HIV) | 5  | 0  | 0  | 0  | 0   | 1    |
| **Signs / Symptoms** |    |    |    |    |     |      |
| Fever            | 0  | 0  | 7  | 53.8 | 9 | 100 | 3 | 100 | 3 | 60 |
| Cough            | 0  | 0  | 8  | 61.5 | 9 | 100 | 3 | 100 | 4 | 80 |
| Sore throat      | 0  | 0  | 3  | 23.1 | 2 | 22.2 | 3 | 100 | 1 | 20 |
| Rhinorrhea       | 0  | 0  | 3  | 23.1 | 1 | 11.1 | 0 | 0   | 0 | 0  |
| Myalgia          | 0  | 0  | 0  | 0   | 4 | 44.4 | 1 | 33.3 | 1 | 20 |
| Asthenia         | 0  | 0  | 0  | 0   | 5 | 55.5 | 1 | 33.3 | 4 | 80 |
| Symptom                        | Count | Count2 | Count3 | Count4 | Count5 | Count6 | Count7 | Count8 | Count9 | Count10 |
|--------------------------------|-------|--------|--------|--------|--------|--------|--------|--------|--------|---------|
| Dyspnea                        | 0     | 0      | 0      | 4      | 44.4   | 1      | 33.3   | 4      | 80     |
| Diarrhea                       | 0     | 0      | 1      | 7.6    | 3      | 33.3   | 0      | 0      | 1      | 20      |
| Vomiting                       | 0     | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0       |
| Respiratory distress*          | 0     | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0       |

*Respiratory distress*: Respiratory frequency > 30/min, O2 Saturation index < 93 %, PaO₂/FiO₂ Ratio < 300

Abbreviations: COVID-19, coronavirus disease 2019; ICU, intensive care unit; PaO₂/FiO₂, ratio of arterial oxygen partial pressure to fraction of inspired oxygen.
Figure 1

(a) TNF alpha gene expression
(b) MIP1 alpha gene expression
(c) MCP1 gene expression
(d) TGF beta gene expression

Groups: Control, Favorable, Unfavorable