Amikishiyev Shirkhan (Orcid ID: 0000-0002-8355-0176)
Deniz Rabia (Orcid ID: 0000-0003-4537-894X)

Full-Length Article

Running Head: Criteria for Hyperinflammation of COVID-19

Title: Criteria for hyperinflammation developing in coronavirus disease-19: analysis of two cohorts from different periods of the pandemic

Authors:

Shirkhan Amikishiyev MD,[1] M. Guven Gunver PhD,[2] Murat Bektas MD,[1] Sarvan Aghamuradov MD,[1] Burak Ince MD,[1] Nevzat Koca MD,[1] Ege Sinan Torun MD,[1] Numune Aliyeva MD,[1] Selma Sari MD,[1] Cigdem Cetin MD,[1] Banu C. Yalcin-Dulundu MD,[1] Rabia Deniz MD,[3] Fatih Kemik MD,[3] Besim Fazil Agargun MD,[3] Ubeyde Ayse Gulseren MD,[3] Beliz Besisik MD,[3] Onur Alkan MD,[3] Ceren Bagriacik MD,[3] Yavuz B. Tor MD,[3] Naci Senkal MD,[3] Yunus Catma MD,[3] Gorkem Durak MD,[4] Sevim Mese MD,[5] Ali Agacfidan PhD,[5] Murat Kose MD,[3] Mustafa Erelel MD,[6] A. Atahan Cagatay MD,[7] Serap Simsek-Yavuz MD,[7] Sevgi Kalayoglu-Besisik MD,[8] Figen Esen MD,[9] Ahmet Gül MD[1]

[1] Department of Internal Medicine, Division of Rheumatology,

[2] Department of Medical Statistics,

[3] Department of Internal Medicine,

[4] Department of Radiology,

[5] Department of Medical Microbiology, Division of Virology and Fundamental Immunology,

[6] Department of Chest Diseases,

[7] Department of Infectious Diseases and Clinical Microbiology,

[8] Department of Internal Medicine, Division of Hematology,

[9] Department of Anesthesiology and Reanimation, Intensive Care Unit,

Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/art.42417

Corresponding author:

Professor Ahmet Gül

Department of Internal Medicine, Division of Rheumatology, Istanbul Faculty of Medicine, Istanbul University, 34093 Fatih, Istanbul, Turkey

E-mail: agul@istanbul.edu.tr

Word count: 4164

Keywords: COVID-19, hyperinflammation, cytokine storm, macrophage activation syndrome, criteria, anakinra, tocilizumab.

Funding: This study was partially funded by The Scientific and Technological Research Council of Turkey.

Abstract

Background Hyperinflammation (HI) developing in 2nd week of COVID-19 contributes to the worse outcome. Because of relatively milder laboratory findings, available criteria for classification of hemophagocytic lymphohistiocytosis or macrophage activation syndrome could not be helpful.

Methods Discovery cohort included symptomatic COVID-19 patients from Turkey, followed at hospital during the initial wave. Replication cohort consisted of hospitalized patients from a later period; all required oxygen support and received glucocorticoids. Diagnosis of HI was made by an expert panel and the majority received tocilizumab or anakinra. Daily clinical and laboratory data were recorded, and data of treatment start day were compared with the 5-6th day data of other patients. Values maximizing the sensitivity and specificity of each parameter were calculated to determine criteria items.

Results 685 patients were analyzed in discovery and 156 in replication cohorts; of whom 150 and 61 received treatment for HI, respectively. Mortality rate was higher in HI patients of discovery cohort (23.3%) compared to the rate of other patients (3.7%), and it was much lower in replication cohort for both groups. The 12-item criteria were developed to define HI of COVID-19 (HIC), and score of 35 provided 85.3% sensitivity, 81.7% specificity. The same criteria gave 90.0% sensitivity for HIC in replication cohort, but lower specificity values were observed, due to the inclusion of milder cases of HIC responding only glucocorticoids.

Conclusions The new criteria are expected to define patients with HIC better with reasonable sensitivity and specificity and enable us to start treatment as early as possible.

INTRODUCTION

Coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus infection, runs a severe course in a subset of patients, mainly due to acute respiratory distress syndrome (ARDS) resulting from diffuse alveolar damage. In addition to the direct cytopathic effects of the viral infection, hyperinflammation (HI) with features of cytokine storm developing in the second week of COVID-19 contributes to this worse outcome (1).

The umbrella term of cytokine storm has been used to define hyperinflammatory states leading to multiorgan dysfunction or failure in association with different causes including familial hemophagocytic lymphohistiocytosis (fHLH), infections, malignancies, autoimmune or autoinflammatory disorder-associated macrophage activation syndrome (MAS), or chimeric antigenic receptor T (CAR-T) cell associated cytokine release syndrome (2). Despite the recognition of the overlapping features of COVID-19-associated hyperinflammation (HIC) with cytokine storms at the beginning of the pandemic (3), the observed concentrations of proinflammatory cytokines and other inflammatory parameters have been reported as relatively lower compared to the levels developing in other conditions (4). Hence the available criteria for the classification of HLH and systemic juvenile arthritis-associated MAS were not helpful for this new condition (5-7), and novel sets of criteria were proposed aiming to characterize the COVID-19 associated hyperinflammatory syndrome leading to mechanical ventilation or death (Supplementary Table 1) (8-10).

Since anti-inflammatory treatments with glucocorticoids or anti-cytokine agents are effective in severe COVID-19, early recognition of HIC with its peculiar findings has become critical (11-14). We herein aimed to characterize the features of HIC further in a cohort of patients from the initial period of the pandemic. Using a large database enabling us to assess dynamic changes of the selected laboratory parameters, we developed a set of criteria defining this

hyperinflammatory state in the first week and then validated the results in an independent cohort of patients, followed at a different period of the pandemic.

PATIENTS AND METHODS

Study Design and Participants

Two cohorts of patients from Turkey were analyzed as the discovery and replication sets for the characterization of HIC. The discovery cohort was comprised of the hospitalized COVID-19 patients diagnosed by real-time polymerase chain reaction method (PCR) and/or clinical plus thorax computed tomography (CT) findings, and only the categories with high (CO-RADS 4), very high level of suspicion (CO-RADS 5) for the pulmonary involvement of COVID-19, and the PCR-proven cases (CO-RADS 6) were used to support the diagnosis (15). This cohort was followed between March and September 2020. In this early phase of the pandemic, the majority of symptomatic COVID-19 patients were followed at the hospital for close monitoring, irrespective of their respiratory status, and clinical and laboratory findings were collected and recorded daily.

The replication cohort also consisted of hospitalized COVID-19 patients diagnosed by PCR and/or clinical plus CT findings, and they were followed between September-December 2020. The algorithm for hospitalization was changed in the second period, and all patients required oxygen support and received glucocorticoids based on the findings of the Recovery trial (11).

We established an expert panel, consisting of a rheumatologist (AG), a hematologist (SB), and an intensive care specialist (FE) with the leadership of AG, based on their long-standing experience in the management of adult patients with HLH, MAS of all causes, and cytokine release syndrome. The panel followed the clinical findings and laboratory changes daily for the recognition of HIC, by taking all available criteria for the HLH and MAS into account along with their clinical judgment, to be able to select those patients requiring tocilizumab or anakinra

treatment. Because of the several unknowns and absence of a specific set of criteria, they classified the patients as “exact”, when they feel confident about HIC, or “borderline”, when they think that the patient possibly had HIC, but they could not rule out the contribution of other potential causes of inflammation, such as secondary infections based on the serum procalcitonin levels or clinical features.

Most of the patients diagnosed with HIC received tocilizumab or anakinra treatment. The data of both HIC groups were compared with the data of the remaining patients with a relatively milder course.

We collected data about demographic features of the patients as recorded in the hospital database. This database does not obtain information about the ethnicity of the patients, and patients’ sex were recorded as described by themselves. We searched the database for previously reported clinical and laboratory findings associated with severe COVID-19, three proposed criteria for COVID-19 associated hyperinflammatory syndrome as well as the criteria for the classification of HLH and juvenile idiopathic arthritis-associated MAS (5-10, 16) (Supplementary Table 1). Among the clinical and laboratory parameters used in the previous HLH and MAS criteria, increased triglyceride, and decreased fibrinogen levels as well as organomegaly findings indicating systemic inflammatory response were rarely observed in HIC. Therefore, we decided to collect daily follow-up data for the parameters of fever, complete blood count including the neutrophil, lymphocyte, and platelet count, serum concentrations of ferritin, D-dimer, C-reactive protein (CRP), lactate dehydrogenase (LDH), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) to be able to observe the dynamic changes in the discovery cohort. We also included the cycle threshold (Ct) values of the PCR results at the admission as well as serum procalcitonin values to the data set to be able to monitor accompanying bacterial infections in the differential diagnosis. These

parameters were extracted from the electronic medical record database of our tertiary referral center with the permission of our institution's committee dedicated to COVID-19 studies.

Statistical analysis

Daily changes of the selected parameters were evaluated within the first two weeks of the hospitalization in the HIC and non-HIC groups. The mean values of each laboratory parameter were calculated for each day.

The laboratory values on the onset day of tocilizumab or anakinra treatment in the HIC group and the corresponding values on the 5^{th} or 6^{th} day of hospitalization for the non-HIC group were used for the comparative analyses.

For the patients with missing values within the first 2 weeks, the values of the previous day, or if not available the values of the 2 days before, or if not available the values of the next day following drug administration were recorded for the analyses.

The receiver operating characteristics (ROC) were evaluated for the day of comparison with the lower and upper limits (10-90%) of the selected parameters, and the values maximizing the sensitivity and specificity of each parameter were calculated by the Youden Index to determine the predicted cut-off values for the preliminary criteria.

Achieving a threshold of at least 80% sensitivity and 80% specificity was aimed to be able to diagnose the patients with HIC as early as possible. The predicted cut-off value of each criterion was used for the initial data set. Adjustments were made for optimization of the results, and the area under the curve (AUC) values were calculated with the final cut-off values. Then, the set of preliminary criteria was applied to the replication cohort for the sensitivity and specificity analyses. Microsoft Excel and IBM SPSS statistics version 21.0 (IBM Corp., Armonk, N.Y., USA) were used for the statistical analysis.

Ethical Approval and Funding

The study protocol was approved by the Ethics Committee of Istanbul Faculty of Medicine and Ministry of Healthy COVID-19 registry and partially funded by The Scientific and Technological Research Council of Turkey's COVID-19 Platform.

RESULTS

Study cohorts

A total of 685 hospitalized COVID-19 patients (399 male, 286 female) were analyzed in the discovery cohort; of whom 150 (111 male, 39 female) were classified as patients with HIC and 141 received tocilizumab or anakinra treatment with the decision of the expert panel. The same panel retrospectively re-grouped 85 patients as those with exact-HIC and 65 of them as the borderline-HIC groups (Table 1). Their data were compared with the data of the remaining 535 patients with a milder course. The replication cohort consisted of 156 (106 male, 50 female) patients. The demographic and clinical features of both cohorts are given in Table 1. There was no significant difference between the discovery and replication cohorts regarding the age ($p=0.28$ for all groups, and $p=0.38$ for patients with HIC), sex ($p=0.055$ for all groups, and $p=0.69$ for patients with HIC), and co-morbidities.

The baseline Ct values of the PCR-confirmed patients were investigated for the prediction of nasopharyngeal viral loads on admission; and no statistically significant difference was found between the mean Ct values of patients with HIC (26.9, range 18-33 for exact-HIC; 27.0, range 21-35 for borderline-HIC) and the remaining patients (27.9, range 17-48).

About one-third of those patients diagnosed with HIC in the discovery cohort required intensive care unit follow-up, and the mortality rate was much higher compared to the remaining patients (all patients with HIC 23.3% *vs* other patients 3.7%) (Table 2). No

significant difference in the disease course was observed between the patients with HIC after re-grouping them as the exact-HIC and borderline-HIC.

The majority of patients diagnosed with HIC received tocilizumab (Table 2). The borderline-HIC patients with findings of HI, but also carrying the potential risk of secondary infection or sepsis, received anakinra more frequently because of its shorter half-life and relatively better safety profile. Twenty-two patients received anakinra or tocilizumab after the resolution of secondary infection findings, and two patients with HIC received anakinra despite the increased procalcitonin levels. Nine out of 65 patients with borderline findings did not receive anti-cytokine treatment because of a higher risk of secondary infections (Table 2). None of the patients received glucocorticoids before the anti-cytokine treatments during the Discovery cohort period; only 8 patients received glucocorticoids after starting the anti-cytokines, and 80 patients received glucocorticoids during the intensive care unit follow-up for the management of ARDS.

All of the 156 patients of the replication cohort were hospitalized because of COVID-19 pneumonia requiring oxygen support, and they all received glucocorticoids, usually dexamethasone 6-8 mg/day or methyl-prednisolone 40 mg/day (Table 1), but some refractory patients received 80-500 mg methyl-prednisolone at the later stages of hospitalization. Those patients not responding well to the glucocorticoids within 3 days were considered candidates for additional tocilizumab or anakinra treatments (Table 3). The mortality rates were much lower in the overall replication group, and the addition of tocilizumab or anakinra treatment to those not responding well to the glucocorticoids resulted in comparable mortality rates with those with a relatively milder course and receiving only glucocorticoids (Table 3).

Anti-cytokine treatments were started around the fifth day of hospitalization [average 4.6 days (range, 1-10) in the exact-HIC and 5.1 days (range, 1-10) of the borderline-HIC groups] in the discovery cohort, and on average 2.5 days (range, 1-12) in the replication cohort.

Selection of the criteria parameters

Mean laboratory values at the time of tocilizumab or anakinra onset of the patients with HIC and the corresponding values of the 5th or 6th days of the remaining patients with 10% and 90% ranges are given in Supplementary Table 2. The potential cut-off values maximizing the sensitivity and specificity of each criterion were determined, and AUC values were calculated if the values were equal to or greater (or less for lymphocyte, monocyte, and procalcitonin) than these initial cut-off values.

The items with at least >70% AUC values were aimed to be included in the criteria set. The WBC count and hemoglobin provided the lowest and serum ferritin levels provided the highest results. Because of the lower AUC results, we preferred not to include hemoglobin, WBC, neutrophil, platelet, fibrinogen, and troponin levels; and instead of neutrophil count, we selected neutrophil/lymphocyte ratio as a separate criterion. Despite the relatively high AUC value of 0.78 for IL-6, we preferred to use CRP as the parameter of acute phase response since it was easily available in all clinical centers.

We combined the increase of ALT or AST levels as a single criterion because of the lower AUC values of ALT; and we selected 90% upper limits as the cut-off values for the ALT or AST, and LDH parameters.

Because of the critical role of ferritin and D-dimer values, we also assessed their dynamic changes, by comparing their daily values to their baseline values in each patient (Figure 1). To increase the weight of these two parameters in the criteria set, we then added the >2.5 times increase of serum ferritin values and >2.0 times increase of D-dimer values within the first week of hospitalization as separate criteria items.

For the determination of procalcitonin cut-off values, we analyzed the patients with HIC according to the presence or absence of secondary infections (Supplementary Figure 1). Group

1 included 22 patients who had elevated procalcitonin levels, and they received anti-cytokine treatments after normalization of procalcitonin levels with antibiotic treatment. Group 2 included 9 patients, who developed secondary infections after starting anti-cytokine treatments. Group 3 included 117 patients with no findings of accompanying bacterial infections. Only 2 patients with HIC had increased procalcitonin levels when they started to receive anakinra along with antibiotic treatment for secondary infection, and their values were not given in Supplementary Figure 1.

According to this subgroup analysis, the 90% range of the potential cut-off value for procalcitonin was selected to be able to include those patients with a slightly increased levels due to COVID-19 itself without a secondary infection.

The optimized cut-off values for the selected 12 items are given in Table 4. The HIC scores were calculated by giving 1 point for each positive item assessed on Days 5-7. The sum of points given for each item meeting the definition was divided by 12 and then multiplied by 100. For those patients with missing values on the day of assessment, those with at least 9 items (without fever, ALT/AST, or LDH values) were also included in the analysis; and their scores were divided into the number of items assessed and then multiplied by 100.

The results of the discovery set revealed that starting from the total score of 35, the preliminary set of criteria achieved the targeted sensitivity and specificity scores, around day 5 in the discovery set; and this score was accepted as the limit for the early diagnosis of HIC. The ROC curve of the preliminary criteria for the score of 35 and sensitivity and specificity values in the discovery cohort are given in Figure 2.

Replication of the findings in the second cohort

The same criteria were applied to the replication cohort, and the score of 35 provided a better sensitivity (90%) in this independent group of patients who received tocilizumab or anakinra

with the diagnosis of HIC (Supplementary Table 3), which developed despite the glucocorticoid treatment. However, due to different patient characteristics, lower specificity values (47.9%) were obtained in those who were followed by glucocorticoids only, and 80.2% specificity was achieved with the score of 50. Between Day 6 and 10, an improvement in the specificity scores was observed (Supplementary Table 4), supporting the differentiation of the remaining patient group with a good response to glucocorticoids.

Comparative Analysis of the HIC Criteria with Other Criteria for HI of COVID-19

We applied the criteria proposed by Manson et al. (10) and Webb et al. (9) for the classification of HI in COVID-19 to our dataset on the same days we assessed our preliminary criteria. The number of patients classified as those with HI according to these criteria is given in Supplementary Table 5; and the sensitivity, specificity, and accuracy values for the Manson et al. criteria, Webb et al. criteria (for the scores of ≥2 and ≥3), and the new set of criteria (for the scores of 30 to 50) are given in Supplementary Table 6. Both previous criteria aimed to define the parameters associated with worse outcomes of the disease leading to mechanical ventilation and death; and they did not primarily aim to use the criteria for the early decision of starting treatment for HI and not considered the treatment as a factor affecting the results. Manson et al.'s criteria set was categorical and less sensitive than HIC. Its relatively higher specificity value (89.7%) was much closer to the value of HIC criteria score of 45 (90.8%), but due to better sensitivity values, the accuracy of the HIC criteria was superior to Manson et al. criteria (86.9% versus 83.7%).

Webb et al.'s criteria set was too sensitive for the score of ≥2 but less specific than the current set of criteria. Webb et al.'s sensitivity value (95.3%) was similar to the value of HIC criteria for the score of 30 (94.0%). However, better specificity and accuracy values were obtained with the current HIC criteria for this score. Likewise, the sensitivity value of Webb et al.'s

score of ≥3 was the same with the sensitivity value of HIC criteria for the score of 45, but the HIC criteria provided better specificity and accuracy values for this score (Supplementary Table 6).

Both of the previous criteria were found to be less specific in the Replication cohort similar to the new HIC set of criteria due to changing characteristics of the patient population. In the comparison of accuracy values, the current HIC criteria set's values were found to be better than the values of other criteria's relevant scores in the Replication cohort as well (Supplementary Table 6).

Discussion

This study investigated two cohorts of COVID-19 patients followed in two different periods of the pandemic with changing algorithms of hospitalization and management. Based on the clinical diagnosis of HIC by an expert panel and evaluation of daily changes of the selected laboratory parameters, a new set of 12-item criteria were developed to define HIC early, and the score of 35 provided 85.3% sensitivity and 81.7% specificity to identify those patients with much higher mortality despite targeted anti-inflammatory treatments. The same set of criteria gave 90.0% sensitivity for the diagnosis of HIC in an independent replication cohort, which included the patients, who required oxygen support and received glucocorticoids. On the other hand, specificity scores of the criteria were lower (47.9%) in the replication cohort largely due to using a different comparison group, which included milder cases of HIC who responded well to the glucocorticoids. This set was quite successful for the recognition of those patients with hyperinflammatory findings despite glucocorticoids, and timely intervention with anti-cytokine agents resulted in a similar mortality rate to the patients with a relatively milder course.

The mechanisms of hyperinflammatory response leading to respiratory and vascular complications have not been fully explored (1, 17). Although earlier studies suggested the role of higher viral load at baseline in the development of worse outcomes, the baseline Ct values of the PCR-positive patients in this study were comparable in both groups of patients with HIC and the remaining patients with no hyperinflammatory response, which suggests the critical role of uncontrolled viral replication, but not the initial viral load, during the early days of the disease (18). Later studies support this finding by documenting the importance of delayed-type I interferon (IFN) response either due to rare inborn errors or the presence of autoantibodies targeting IFN-α or IFN-ε in the development of severe disease (19, 20).

The contribution of HI to the development of severe COVID-19 with fatal outcomes has been confirmed by studies revealing the reduced mortality with anti-inflammatory treatments, including dexamethasone and more targeted agents such as tocilizumab, anakinra or Jak inhibitors (11, 12, 21, 22). Therefore, early recognition of HI has become a very important issue for a timely intervention to reduce the mortality of severe COVID-19 patients.

During the early days of the pandemic, similarities between the cytokine storms or MAS and HIC were recognized (3), but its less-defined characteristics have long been debated because of the relatively lower concentrations of inflammatory markers in HIC compared to the MAS associated with other disorders. Recent studies documented the importance of monocyte/macrophage activation by the Fcγ-receptor-mediated infection of monocytes and pulmonary macrophages following the development of anti-SARS-CoV-2 antibodies and opsonization of the virus by these antibodies (23), which then results in activation of inflammasome and pyroptosis leading to COVID-19 pathology (24). Increased ferritin levels support the role of gasdermin D-related pyroptosis (25), which contributes to the dissemination of inflammasome activation to the neighboring uninfected monocytes/macrophages, but not to endothelial cells and respiratory epithelial cells (26). Demonstration of autoantibodies against

IL-1 receptor antagonist in patients with severe COVID-19 and multisystem inflammatory disease in children (MIS-C) supports further IL-1-driven inflammasome mediated pathology (27). Therefore, available findings favor monocyte/macrophage-activation-driven hyperinflammatory response in COVID-19, but its relatively less prominent inflammatory features compared to the other types of MAS are possibly due to a more limited response resulting from activation of mainly pulmonary macrophages, at least in the early phase of the disease. Hypertriglyceridemia or hemophagocytosis, which may be associated with activation of hepatic and bone marrow macrophages, respectively, in systemic MAS were reported very rarely in COVID-19 during the later phases of the disease (28). In this regard, HIC could also be named as COVID-19-associated MAS (C-MAS) with its unique features. Systemic cytokine storms, such as the one seen in fHLH, which represents the most severe form, lead to a very strong inflammatory response resulting in multiple organ failure and a very high risk of mortality. On the other hand, more organ-specific or limited hyperinflammatory responses, such as the "HIC", which starts to develop primarily in the lungs can be considered at the milder end of the systemic inflammatory response spectrum, but it is still associated with increased mortality resulting from diffuse alveolar damage, respiratory failure, and thrombotic vascular complications (1). Therefore, a better definition of HIC with its unique characteristics would be crucial for preventing increased mortality (29-31).

We therefore aimed to develop a set of criteria to be able to diagnose HIC as early as possible, and the analysis of the discovery cohort provided us an opportunity to follow the natural disease course in early symptomatic days. In addition to fever and exclusion of secondary bacterial infections with low procalcitonin levels, presence of lymphopenia, increased neutrophil/lymphocyte ratio, monocytopenia, increased ferritin and D-dimer values and their ratio of increase compared to the baseline values within the first week, and increased LDH and ALT/AST enzymes constituted the preliminary set of criteria to define the HIC. This set of

criteria reached its target sensitivity and specificity scores, starting with the score of 35, and higher scores increased its specificity. Follow-up of daily changes in ferritin values are considered to be very informative for the recognition of ongoing macrophage activation within the first week. Higher sensitivity scores in the replication cohort also supported the importance of the early diagnosis of HIC, since anti-inflammatory treatments were started relatively later in this cohort due to changed management protocols. Timely interventions with glucocorticoids or treatments targeting IL-6/IL-6R, IL-1/IL-1R, or Janus kinases have been expected to help control the excessive inflammatory response and limit tissue damage as well as contribute to the recovery of immune dysfunction associated with lymphopenia and lymphocyte exhaustion (1).

During the initial period of the pandemic, higher mortality rates of 40% or higher were reported for patients with a severe disease course, such as 41.4% rate in patients requiring mechanical ventilation and 26.2% among those requiring oxygen support in the Recovery Platform trial; and dexamethasone treatment for 10 days resulted in significantly lower rates of 29.3% and 23.3%, in respective groups (11). The same platform's tocilizumab trial showed a decreased mortality rate from 32% to 28% compared to the standard of care, and 82% of the patients in both arms were using glucocorticoids. Much lower rates of mortality rates reported in this study (23.3% for the first and 9.8% for the second cohort) support the importance of early diagnosis and timely intervention. Relatively lower rates of mortality in the discovery cohort indicate the favorable effects of tocilizumab and anakinra; on the other hand, much lower mortality rates observed during the replication period, reveal the importance of combination treatment of glucocorticoids with more targeted drugs. It can be assumed that the addition of tocilizumab and anakinra resulted in better mortality rates in those patients with worse inflammatory and clinical findings not responding well to the glucocorticoid treatment, which were comparable with the rates observed in relatively milder cases.

This study was conducted in a single tertiary-referral center, which constitutes its major limitation. Different centers used variable approaches for the management of COVID-19 cases even in the same country, therefore external validation of the preliminary criteria would be very helpful. Using certain selected parameters, which were observed to be helpful for the definition of HIC in several studies, was also another limitation. After the advances in our understanding of the pathogenesis of HIC, novel laboratory parameters, which may assess the underlying pathology better, could be more successful criteria items in the early prediction of HIC. Also, during the pandemic, other potential causes of MAS associated mainly with pulmonary HI, such as those associated with other respiratory viruses or toxic substances such as vaping products could not be studied. Follow-up studies are expected to provide more information about the characteristics of HIC compared to similar conditions and the performance of the current criteria in other settings.

In conclusion, this new set of criteria is expected to help define those patients with features of HIC with reasonable sensitivity and specificity and enable us to start necessary treatments as early as possible. Its performance needs to be confirmed in different cohorts of COVID-19 patients and patients with HI due to the activation of pulmonary macrophages associated with other causes.

Contributions

All authors critically revised the manuscript and approved the final submitted version and take the responsibility for the completeness and accuracy of the data and analyses. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

All authors accessed and verified the underlying data.

AG, SA and MGG conceived and designed the study.

AG, SA, MGG, MK, ME, AAC, SSY, SKB, FE oversaw the implementation of the study.

SA, MB, SA, BI, NK, EGT, NA, SS, CC, BCYD, RD, FK, BFA, UAG, BB, OA, CB, YBT, NS, YC, GD, SM, AA, MK, ME, AAC, SSY, SKB, FE, AG collected the data.

AG, SA, MGG interpreted the data and drafted the manuscript.

MGG was the study statistician.

ME, AAC, SSY, SKB, and FE contributed to study conception and design.

All authors contributed to revising the manuscript critically for important intellectual content, and all authors approved the final version of the article to be published.

Funding: This Project was partially funded by The Scientific and Technological Research Council of Turkey COVID-19 Platform.

Competing interest

All authors confirm that they have no competing interest regarding this manuscript. ICMJE Conflict of Interest disclosure form was loaded by the first author, and other forms will be uploaded after peer review.

Disclosure: No additional disclosures for all authors. Preliminary form of this work was presented as an oral presentation at EULAR meeting in 2021.

Patient consent for publication Not applicable.

Ethical Approval and Funding

The study protocol was approved by the Ethics Committee of Istanbul Faculty of Medicine and Ministry of Healthy COVID-19 registry and partially funded by The Scientific and Technological Research Council of Turkey COVID-19 Platform.

Data Sharing: All data relevant to the study are included in the article or uploaded as supplementary information. Further data are available upon reasonable request.

References

1. Merad M, Blish CA, Sallusto F, Iwasaki A. The immunology and immunopathology of COVID-19. Science. 2022;375(6585):1122-7.
2. Fajgenbaum DC, June CH. Cytokine Storm. N Engl J Med. 2020;383(23):2255-73.
3. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ, et al. COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet. 2020;395(10229):1033-4.
4. Ombrello MJ, Schulert GS. COVID-19 and cytokine storm syndrome: are there lessons from macrophage activation syndrome? Transl Res. 2021;232:1-12.
5. Henter JI, Horne A, Arico M, Egeler RM, Filipovich AH, Imashuku S, et al. HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer. 2007;48(2):124-31.
6. La Rosee P, Horne A, Hines M, von Bahr Greenwood T, Machowicz R, Berliner N, et al. Recommendations for the management of hemophagocytic lymphohistiocytosis in adults. Blood. 2019;133(23):2465-77.
7. Ravelli A, Minoia F, Davi S, Horne A, Bovis F, Pistorio A, et al. 2016 Classification Criteria for Macrophage Activation Syndrome Complicating Systemic Juvenile Idiopathic Arthritis: A European League Against Rheumatism/American College of Rheumatology/Paediatric Rheumatology International Trials Organisation Collaborative Initiative. Ann Rheum Dis. 2016;75(3):481-9.
8. Caricchio R, Gallucci M, Dass C, Zhang X, Gallucci S, Fleece D, et al. Preliminary predictive criteria for COVID-19 cytokine storm. Ann Rheum Dis. 2021;80(1):88-95.
9. Webb BJ, Peltan ID, Jensen P, Hoda D, Hunter B, Silver A, et al. Clinical criteria for COVID-19-associated hyperinflammatory syndrome: a cohort study. Lancet Rheumatol. 2020;2(12):e754-e63.
10. Manson JJ, Crooks C, Naja M, Ledlie A, Goulden B, Liddle T, et al. COVID-19-associated hyperinflammation and escalation of patient care: a retrospective longitudinal cohort study. Lancet Rheumatol. 2020;2(10):e594-e602.
11. Group RC, Horby P, Lim WS, Emberson JR, Mafham M, Bell JL, et al. Dexamethasone in Hospitalized Patients with Covid-19. N Engl J Med. 2021;384(8):693-704.
12. Group RC. Tocilizumab in patients admitted to hospital with COVID-19 (RECOVERY): a randomised, controlled, open-label, platform trial. Lancet. 2021;397(10285):1637-45.
13. Angriman F, Ferreyro BL, Burry L, Fan E, Ferguson ND, Husain S, et al. Interleukin-6 receptor blockade in patients with COVID-19: placing clinical trials into context. Lancet Respir Med. 2021;9(6):655-64.
14. Mehta P, Cron RQ, Hartwell J, Manson JJ, Tattersall RS. Silencing the cytokine storm: the use of intravenous anakinra in haemophagocytic lymphohistiocytosis or macrophage activation syndrome. Lancet Rheumatol. 2020;2(6):e358-e67.
15. Prokop M, van Everdingen W, van Rees Vellinga T, Quarles van Ufford H, Stoger L, Beenen L, et al. CO-RADS: A Categorical CT Assessment Scheme for Patients Suspected of Having COVID-19-Definition and Evaluation. Radiology. 2020;296(2):E97-E104.
16. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020;395(10229):1054-62.
17. Paludan SR, Mogensen TH. Innate immunological pathways in COVID-19 pathogenesis. Sci Immunol. 2022;7(67):eabm5505.

18. Cheemarla NR, Watkins TA, Mihaylova VT, Wang B, Zhao D, Wang G, et al. Dynamic innate immune response determines susceptibility to SARS-CoV-2 infection and early replication kinetics. J Exp Med. 2021;218(8).
19. Zhang Q, Bastard P, Liu Z, Le Pen J, Moncada-Velez M, Chen J, et al. Inborn errors of type I IFN immunity in patients with life-threatening COVID-19. Science. 2020;370(6515).
20. Bastard P, Rosen LB, Zhang Q, Michailidis E, Hoffmann HH, Zhang Y, et al. Autoantibodies against type I IFNs in patients with life-threatening COVID-19. Science. 2020;370(6515).
21. Huet T, Beaussier H, Voisin O, Jouveshomme S, Dauriat G, Lazareth I, et al. Anakinra for severe forms of COVID-19: a cohort study. Lancet Rheumatol. 2020;2(7):e393-e400.
22. Marconi VC, Ramanan AV, de Bono S, Kartman CE, Krishnan V, Liao R, et al. Efficacy and safety of baricitinib for the treatment of hospitalised adults with COVID-19 (COV-BARRIER): a randomised, double-blind, parallel-group, placebo-controlled phase 3 trial. Lancet Respir Med. 2021;9(12):1407-18.
23. Junqueira C, Crespo A, Ranjbar S, de Lacerda LB, Lewandrowski M, Ingber J, et al. FcgammaR-mediated SARS-CoV-2 infection of monocytes activates inflammation. Nature. 2022;606(7914):576-84.
24. Sefik E, Qu R, Junqueira C, Kaffe E, Mirza H, Zhao J, et al. Inflammasome activation in infected macrophages drives COVID-19 pathology. Nature. 2022;606(7914):585-93.
25. Wang D, Yu S, Zhang Y, Huang L, Luo R, Tang Y, et al. Caspse-11-GSDMD pathway is required for serum ferritin secretion in sepsis. Clin Immunol. 2019;205:148-52.
26. Junqueira C, Crespo A, Ranjbar S, de Lacerda LB, Lewandrowski M, Ingber J, et al. FcgammaR-mediated SARS-CoV-2 infection of monocytes activates inflammation. Nature. 2022.
27. Pfeifer J, Thurner B, Kessel C, Fadle N, Kheiroddin P, Regitz E, et al. Autoantibodies against interleukin-1 receptor antagonist in multisystem inflammatory syndrome in children: a multicentre, retrospective, cohort study. Lancet Rheumatol. 2022;4(5):e329-e37.
28. Harris CK, Hung YP, Nielsen GP, Stone JR, Ferry JA. Bone Marrow and Peripheral Blood Findings in Patients Infected by SARS-CoV-2. Am J Clin Pathol. 2021;155(5):627-37.
29. McGonagle D, Ramanan AV, Bridgewood C. Immune cartography of macrophage activation syndrome in the COVID-19 era. Nat Rev Rheumatol. 2021;17(3):145-57.
30. McGonagle D, O'Donnell JS, Sharif K, Emery P, Bridgewood C. Immune mechanisms of pulmonary intravascular coagulopathy in COVID-19 pneumonia. Lancet Rheumatol. 2020;2(7):e437-e45.
31. Morris G, Bortolasci CC, Puri BK, Marx W, O'Neil A, Athan E, et al. The cytokine storms of COVID-19, H1N1 influenza, CRS and MAS compared. Can one sized treatment fit all? Cytokine. 2021;144:155593.

Figure legends

Figure 1. Daily dynamic changes of serum D-dimer and ferritin values in comparison to the baseline values in each patient. The mean values of the ratios for each day are given for HIC and the remaining patients are given on the left side, and for the exact and borderline HIC groups and the remaining patients on the right side.

Figure 2. The ROC curve obtained with the application of the criteria to the discovery cohort. The right side of the figure shows sensitivity and specificity results for different scores of the criteria.

Supplementary Figure 1. Mean serum procalcitonin levels of the patients with HIC. Group 1 (n=22), patients with elevated procalcitonin levels at baseline, who received anti-cytokine treatments after normalization of procalcitonin levels with antibiotic treatment; Group 2 (n=9), patients, who developed secondary infections after starting anti-cytokine treatment; Group 3 (n= 117) patients with no findings of accompanying bacterial infections.

Tables

Table 1. Characteristics of the patients in the discovery and replication cohorts

	Discovery set				Replication set
	All patients (n=685)	Exact-HIC (n=85)	Borderline HIC (n=65)	Remaining Patients (n=535)	(n=156)
Age, years	57.8 (18-98)	58.9 (31.0-94.0)	60.7 (24.0-89.0)	57.4 (18.0-98.0)	59.3 (23-92)
Sex					
Male	399 (58.2%)	66 (77.6%)	45 (69.2%)	288 (53.8%)	106 (67.9%)
Female	286 (41.8%)	19 (22.4%)	20 (30.8%)	247 (46.2%)	50 (32.1%)
SARS-CoV-2					
RT-PCR positivity	299 (43.7%)	49 (57.6%)	40 (61.5%)	210 (39.3%)	132 (84.6%)
Ct values (mean, range)	27.68 (17-48)	26.9 (18-33)	27.0 (21-35)	27.9 (17-48)	25.2 (14.0-38.8)
Comorbidities (%)					
Diabetes	176 (25.7%)	21 (24.7%)	20 (30.8%)	135 (25.2%)	51 (32.7%)
Hypertension	252 (36.8%)	33 (38.8%)	28 (43.1%)	191 (35.7%)	66 (42.3%)
Coronary artery disease	68 (10%)	11 (12.9%)	9 (13.8%)	48 (9.0%)	19 (12.2%)
Chronic pulmonary disease	73 (10.7%)	4 (4.7%)	9 (13.8%)	50 (9.3%)	9 (5.8%)
Congestive heart failure	32 (4.7%)	3 (3.5%)	3 (4.6%)	26 (4.9%)	3 (1.9%)
Chronic kidney disease	34 (5%)	4 (4.7%)	4 (6.2%)	26 (4.9%)	11 (7.1%)
Renal transplantation	18 (2.7%)	3 (3.5%)	0	15 (2.8%)	7 (4.5%)
Chronic liver disease	3 (0.5%)	0	0	3 (0.6%)	0
Cerebrovascular disease	18 (2.7%)	0	2 (3.1%)	16 (3.0%)	5 (3.2%)
Dementia	13 (1.9%)	1 (1.2%)	0	12 (2.2%)	1 (0.6%)
Peripheral artery disease	2 (0.3%)	1 (1.2%)	1 (1.5%)	0	2 (1.3%)
Systemic lupus erythematosus	4 (0.6%)	2 (2.4%)	1 (1.5%)	1 (0.2%)	1 (0.6%)
Rheumatoid arthritis	3 (0.5%)	1 (1.2%)	0	2 (0.4%)	2 (1.3%)
Familial Mediterranean fever	7 (1%)	1 (1.2%)	1 (1.5%)	5 (0.9%)	0
Dermatomyositis	1 (0.1%)	1 (1.2%)	0	0	0
Behçet's disease	3 (0.5%)	0	0	3 (0.6%)	0
Solid-organ malignancy	66 (9.7%)	7 (8.2%)	4 (6.2%)	55 (10.3%)	11 (7.1%)
Hematologic malignancy	25 (3.7%)	4 (4.7%)	5 (7.7%)	16 (3.0%)	4 (2.6%)
Treatment					
Tocilizumab *(n, %)*					
400mg	59 (8.7%)	30 (35.2%)	29 (44.6%)	0	20 (12.8%)

600mg	23 (3.3%)	16 (18.8%)	7 (10.7%)	0	5 (3.2%)
800mg	35 (5.1%)	27 (31.7%)	8 (12.5%)	0	12 (7.6%)
***Anakinra** (n, %)*	24 (3.5%)	12 (14.1%)	12 (18.4%)	0	24 (15.3%)
Cumulative dose (mean)	2600	2800	2400		2000
Cumulative dose (range)	800-5600	800-5600	800-5100		700-5000
Mean days	9.9 ± 5.2	11 ± 6.2	8.8 ± 4.0		7.2±2.4
Glucocorticoids* (n)	0	0	0	0	156
Cumulative dose (mean)					500
Cumulative dose (range)					120-2000
Mean days					7.2 ± 2.4

*Glucocorticoid dose is given as the equivalent dose of prednisolone. The numbers in the discovery cohort indicate the period before the start day of tocilizumab or anakinra.

Table 2. The number of patients followed up at the ward or intensive care unit (ICU) and the number of died patients as well as the number of patients who received tocilizumab or anakinra with the diagnosis of HIC (n=150) in the discovery set

	Exact-HIC (n=85)	**Borderline-HIC** (n=65)	**Remaining Patients** (n=535)
Course			
Ward	56 (65.9%)	44 (67.7%)	534 (99.8%)
ICU	29 (34.1%)	21 (32.3%)	1 (0.2%)
Died	20 (23.5%)	15 (23.1%)	20 (3.7%)
Anti-cytokine Treatments			
Tocilizumab	73 (85.9%)	44 (67.7%)	0
Anakinra	12 (14.1%)	12 (18.5%)	0
None	0	9 (13.8%)	535 (100%)

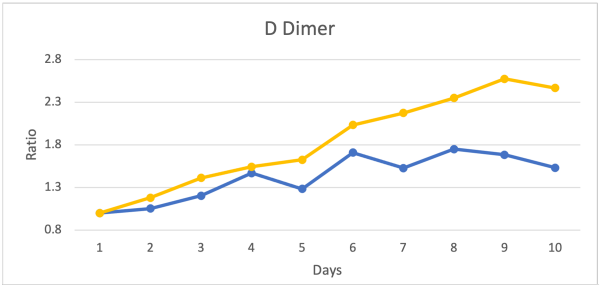
Table 3. Treatments and mortality rate of the patients in the replication set

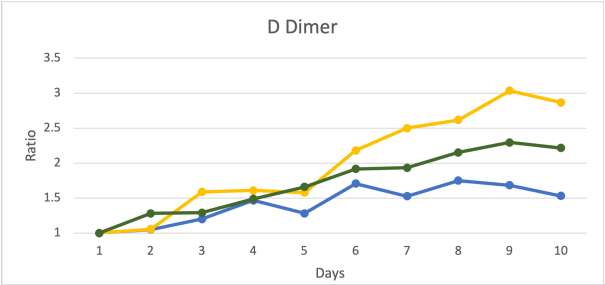
Treatments	**Tocilizumab (n=37)**	**Anakinra (n=24)**	**Glucocorticoids (n=95)**	**Total (n=156)**
Survived	34 (91.8%)	21 (87.5%)	85 (89.4%)	140 (89.7%)
Died	3 (8.2%)	3 (12.5%)	10 (10.6%)	16 (10.3%)

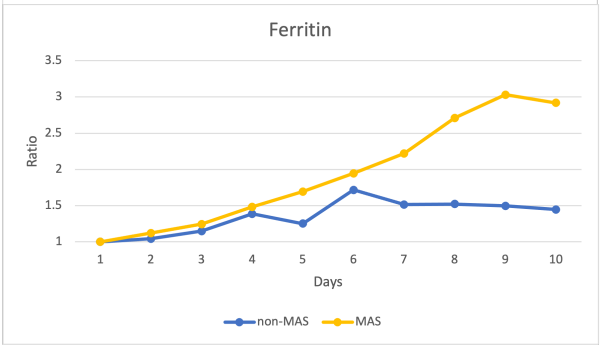
Table 4. Preliminary Set of Criteria for the Hyperinflammation of Coronavirus Disease-19

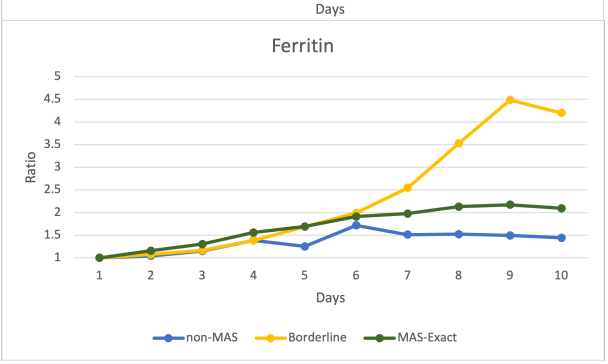
1. Fever (≥ 37.0 °C)
2. CRP concentration ≥ 40 mg/L
3. Lymphopenia ≤ 900 cell/mm^3
4. Neutrophil/lymphocyte ratio ≥ 5
5. Monocyte ≤ 390 cell/mm^3
6. Ferritin concentration ≥ 680 ng/mL
7. More than 2.5 times increase of ferritin concentration within 7 days of disease onset
8. D-dimer concentration ≥ 885 ng/ml
9. More than 2.0 times increase of D-dimer concentration within 7 days of disease onset
10. LDH concentration ≥ 360 U/L
11. ALT or AST concentration ≥ 70 U/L
12. Procalcitonin concentration ≤ 0.8 ng/ml

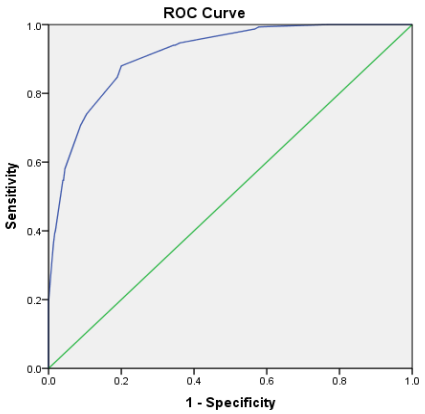
1 point for each positive item assessed on Days 5-7











Score	Sensitivity (%)	Specificity (%)
30	94·0	68·2
35	85·3	81·7
40	82·0	82·8
45	72·0	90·8
50	52·0	96·4
55	48·7	96·8
60	35·3	98·7