Intravenous High-dose Anakinra Drops Venous Thrombosis and Myocardial Infarction in Severe and Critical COVID-19 Patients: A Propensity Score Matched Study

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Abstract

Introduction: In our study, we aimed to evaluate the effect of high dose intravenous anakinra treatment on development of thrombotic events in severe and critical COVID-19 patients. **Material and methods**: This retrospective observational study was conducted at a tertiary referral center in Aksaray, Turkey. The study population consisted of two groups as follows; the patients receiving high dose intravenous anakinra (anakinra group) added to background therapy and the patients treated with standard of care (SoC) as historical control group. Age, gender, mcHIS scores, and comorbidities such as DM, HT, and CHD of the patients were determined as the variables to be matched. **Results:** We included 114 patients in SoC and 139 patients in Anakinra group into the study. Development of any thromboembolic event (5% vs 12.3%, p=0.038; OR:4.3) and PTE (2.9% vs 9.6%, p=0.023; OR:5.1) were lower in Anakinra group than SoC. No patient experienced CVA and/or clinically evident DVT both in two arms. After 1:1 PS matching, 88 patients in SoC and 88 patients in Anakinra group were matched and included into the analysis. In survival analysis, development of any thromboembolic event, PTE, and MI were higher in SoC compared to Anakinra. Survival rate was also lower in patients with SoC arm than Anakinra in patients who had any thromboembolic event as well as MI. **Conclusion**: In our study, development of thrombosis were associated with hyperinflammation in patients with severe and critical COVID-19. Intravenous high-dose anakinra treatment decreases both venous and arterial events in patients with COVID-19.

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Intravenous High-dose Anakinra in COVID-19

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Introduction: In our study, we aimed to evaluate the effect of high dose intravenous anakinra treatment on development of thrombotic events in severe and critical COVID-19 patients.

Material and methods : This retrospective observational study was conducted at a tertiary referral center in Aksaray, Turkey. The study population consisted of two groups as follows; the patients receiving high dose intravenous anakinra (anakinra group) added to background therapy and the patients treated with standard of care (SoC) as historical control group. Age, gender, mcHIS scores, and comorbidities such as DM, HT, and CHD of the patients were determined as the variables to be matched.

Results: We included 114 patients in SoC and 139 patients in Anakinra group into the study. Development of any thromboembolic event (5% vs 12.3%, p=0.038; OR:4.3) and PTE (2.9% vs 9.6%, p=0.023; OR:5.1) were lower in Anakinra group than SoC. No patient experienced CVA and/or clinically evident DVT both in two arms.

After 1:1 PS matching, 88 patients in SoC and 88 patients in Anakinra group were matched and included into the analysis. In survival analysis, development of any thromboembolic event, PTE, and MI were higher in SoC compared to Anakinra. Survival rate was also lower in patients with SoC arm than Anakinra in patients who had any thromboembolic event as well as MI.

Conclusion : In our study, development of thrombosis were associated with hyperinflammation in patients with severe and critical COVID-19. Intravenous high-dose anakinra treatment decreases both venous and arterial events in patients with COVID-19.

Key words: Anakinra, COVID-19, thrombosis, inflammasome, hyperinflammation

Introduction

Coronavirus-19 (COVID-19) is an emerging infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and affects many organs mainly upper and lower respiratory tracts. Disease severity of COVID-19 is ranged from asymptomatic and/or mild symptoms to potential life-threatining disease including acute respiratory distress syndrome (ARDS), multi-organ failure and even death. Several risk factors such as male gender, advanced age, some comorbidities including diabetes mellitus (DM), hypertension (HT) and coronary heart disease (CHD), and immunosuppressive treatment were described for the development of poor prognosis as well as severe course in COVID-19 (Verity et al., 2020).

Hyperinflammation (cytokine storm) is one of the main features of severe disease in COVID-19 and also closely associated with poor outcome including ARDS, need of oxygen therapy and higher mortality (Tufan, Avanoğlu Güler, & Matucci-Cerinic, 2020). Several immunomodulatory treatments such as corticosteroids,

baricitinib, anakinra, and tocilizumab were found to be effective in COVID-19 patients with signs of hyperinflammation (Kyriazopoulou et al., 2021) (Marconi et al., 2021) (Horby et al., 2021) ("Tocilizumab in patients admitted to hospital with COVID-19 (RECOVERY): a randomised, controlled, open-label, platform trial," 2021).

In addition to cytokine storm, some patients suffer from thrombotic events including myocardial infarction (MI), cerebrovascular accident (CVA), and venous thromboembolism (VTE) such as deep vein thrombosis (DVT) and pulmonary thromboembolism (PTE) during the course of COVID-19 (Asakura & Ogawa, 2021). Thereby, prophylactic use of anticoagulant and/or antiaggregant therapies were applied especially in hospitalized COVID-19 patients in daily practise (Middeldorp et al., 2020). Although some studies has shown reduced mortality with profilactic use of anticoagulant therapy reduces mortality (Tang et al., 2020) (Albani et al., 2020) and also development of thromboembolic events (Lachant et al., 2020), there are conflicting results with benefit of anticoagulant therapy in terms of mortality and/or thrombosis (Sadeghipour et al., 2021). Moreover, it is not known whether immunomodulatory therapy reduces thromboembolic events in patients with severe COVID-19.

In our study, we aimed to evaluate the effect of high dose intravenous anakinra treatment on development of thrombotic events in severe and critical COVID-19 patients.

Material and Methods

Patients and data:

This retrospective observational study was conducted at a tertiary referral center in Aksaray, Turkey. Diagnosis of COVID-19 was performed by typical computer tomography (CT) findings in addition to clinical signs and symptoms and confirmed with positive polymerase chain reaction (PCR).

The study population consisted of two groups as follows; the patients receiving high dose intravenous anakinra (anakinra group) added to background therapy between 01.09.2021 and 01.02.2022 and the patients treated with standard of care (SoC) as historical control group who were hospitalized between 01.07.2021 and 01.09.2021. COVID-19 disease severity was evaluated according to the National Institute of Health (NIH) severity scale and only severe and critically ill patients who followed-up in the ward were included into the study ("COVID-19 Treatment Guidelines Panel. Coronavirus Disease 2019 (COVID-19) Treatment Guidelines.," 2022).

Individual written patient consent and local ethic committee approval was obtained for this study (date/number: 24.02.2022, 2022/04-09).

Laboratory evaluation

Laboratory values such as hemogram, liver enzymes, troponin levels, C-reactive protein (CRP) (mg/dL), ferritin (pg/mL), d-dimer (pg/mL), lactate dehydrogenase (LDH) (U/L), procalcitonin (pg/dL) at the admission and consecutive days (procalcitonin was every other day but others were once in a day); the peak levels of CRP, ferritin, d-dimer and LDH levels were recorded. Inflammatory state of the patients was evaluated and derived based on COVID hyperinflammatory syndrome score (cHIS) and it was calculated according to combination of neutrophil and lymphocyte counts at the admission and the peak levels of CRP, ferritin, D-dimer and LDH during to the follow-up (Webb et al., 2020). The item of fever was removed due to its lower frequency ($<$%10) in both arms. Therefore, the maximum score of the new version of cHIS score was 5 points (modified cHIS [mcHIS] score) were calculated in both groups (Bektaş et al., 2023a).

Treatment protocol and outcome

All patients received background corticosteroid therapy with 80 mg/day methylprednisolone (or its equivalent) and enoxaparin 0.4 mg/day at the admission and continued consecutive days (SoC). Anakinra was added to the background treatment in patients who did not respond to initial treatment at least two days or concomitantly with steroids in patients with higher risk and/or critical illness at admission and continued until discharge or death. Average starting dose of anakinra was 400 mg/day intravenously and increased

gradually to maximum 1600 mg/day if necessary (10 mg/kg/day). Anakinra dose adjustment was performed by the same experienced physician in COVID-19 (MB) according to daily clinical (respiratory symptoms, degree of oxygen supply, presence of fever) and laboratory findings.

Diagnosis of PTE was confirmed by thorax CT-angiography in patients with prominent d-dimer increase despite decrease in acute phase reactants (APR) such as CRP and ferritin and/or increase in need of oxygen therapy and respiratory distress despite the decrease in levels of APRs. Diagnosis of MI was made according to the Thygesen et al. study (Thygesen et al., 2018). Severe infection was defined as development of opportunistic infection, need of intravenous antibiotics, sepsis, or requirement of intensive care unit (ICU) admission or development of death due to secondary infection.

Statistical analysis

In our study, 22.0 version (IBM, Armonk, NY, USA) of the SPSS (Statistical Package for the Social Sciences) program was used for statistical analysis of data. Descriptive statistics, discrete and continuous numerical variables were expressed as mean, ± standard deviation or median (minimum-maximum). Categorical variables were expressed as number of cases (%). Cross table statistics were used to compare categorical variables (Chi-Square, Fisher' exact test). Normally distributed parametric data were compared with Student's t-test and non-parametric data that did not meet normal distribution were compared with Mann Whitney U and Kruskal Wallis tests. Correlation analysis was performed by Pearson or Spearman method according to normality distribution. Kaplan-Meier and log-rank methods were used for survival analysis. Multivariate analysis was performed by using logistic regression. Sensitivity and specificity calculation were performed by Receiver operating characteristic (ROC) analysis. $p<0.05$ value was considered statistically significant.

Propensity score matching

The first step in Propensity Score Matching (PSM) is to identify the covariates from which to calculate propensity scores (PS). Age, gender, mcHIS scores, and comorbidities such as DM, HT, and CHD of the patients were determined as the variables to be matched. The PS matching was done as 1:1 with the nearest neighbor method. Caliper value was 0.2. When matching, we performed this analysis by assigning values according to the averages of the parameters with missing data. PSM was performed with SPSS package program 28.0.1 using R package program and an auxiliary plugin (PS matching 3.0 SPE). Dot-plot of standardized mean differences for all covariates before and after PS matching was shown in supplementary figure 1. Jitter plots for trend scores and line-plot of standardized differences were described in supplemental figure 2 and 3, respectively.

Results

Analysis Before PS Matching

We included 114 patients in SoC and 139 patients in Anakinra group into the study. Baseline clinical and laboratory features of the patients were described in table 1. Frequency of male gender (51.8% vs 39.5%, p=0.05; Odds ratio [OR]: 3.8), chronic renal failure (CRF) (20% vs 5.3%, p=0.001; OR: 11.9), critical illness (61.2% vs 40.4%, p=0.001, OR:10.9) were higher in Anakinra group than SoC. Additionally, median (IQR) duration of hospitalization (11 [12] vs 9 [7.3] days; p=0.03), mcHIS scores ($p<0.001$), baseline NLR (p=0.002) and d-dimer levels (p=0.04), peak levels of CRP (p=0.012), ferritin ($p<0.001$), d-dimer (p=0.002), LDH ($p<0.001$) levels were higher in Anakinra receiving patients than SoC.

Development of any thromboembolic event (5% vs 12.3%, p=0.038; OR:4.3) and PTE (2.9% vs 9.6%, p=0.023; OR:5.1) were lower in Anakinra group than SoC. No patient experienced CVA and/or clinically evident DVT both in two arms. Although severe infection, pneumothorax and MI were not different between two arms (p=0.1, p=0.1, and p=0.2, respectively); ICU admission (39.6% vs 22%, p=0.003; OR:9) and mortality (36.7% vs 27%, p=0.026; OR:) were higher in Anakinra group compared to SoC before PS matching analysis (table 1).

Patients experienced any thromboembolic event had longer duration of hospitalization (p=0.03), higher

vaccination counts (p=0.028), more frequent CHD (p=0.001; OR:11.8), critical disease (p=0.001; OR:10.6), higher mcHIS scores (p<0.001), lower NLR (p=0.002) and higher baseline d-dimer levels (p=0.04), higher peak levels of CRP (p=0.012), ferritin (p<0.001), d-dimer (p=0.002), and LDH (p<0.001). Development of thrombosis was also higher in patients had mortality (62% vs 28%, p=0.001; OR:10.4) in univariate analysis (table 2). Patients developed PTE had longer duration of hospitalization (p=0.03), higher vaccination counts (p=0.03), critical disease (p=0.005; OR:7.8), higher mcHIS scores (p<0.001), and higher baseline d-dimer levels (p=0.04), higher peak levels of CRP (p=0.012), ferritin (p<0.001), d-dimer (p=0.002), and LDH (p<0.001). Development of PTE was also higher in patients had severe infection (p=0.028; OR:4.8), pneumothorax (p=0.046; OR:4), MI (p<0.001; OR:12.6), and SoC (p=0.023; OR: 5.1) in univariate analysis (table 3). In multivariate analysis, peak d-dimer levels (p<0.001, OR:1.1, 95% Confidence interval [CI]: 1.05-1.16), critical illness (p=0.044, OR:9.5, 95% CI: 1.06-85.5), and SoC (compared to Anakinra) (p=0.002, OR:11.2, 95% CI: 2.47-51.1) were associated with development of any thromboembolic event (supplementary table).

Analysis After PS Matching

After 1:1 PS matching, 88 patients in SoC and 88 patients in Anakinra group were matched and included into the analysis. Baseline clinical and laboratory features of the patients were described in table 1. After adjustment of potential confounders age, gender, presence of comorbidities (DM, HT, CHD, CRF, chronic lung disease, and malignancy), disease severity, vaccination history, mcHIS scores were not different between two groups (table 1). Only baseline d-dimer and peak levels of LDH were higher in Anakinra arm compared to SoC (p=0.05 and p<0.001). Severe infection (28.4% vs 16%, p=0.05; OR:3.9), development of any thromboembolic event (15.9% vs 3.4%, p=0.005; OR:7.9), PTE (12.5% vs 3.4%, p=0.026; OR:5), MI (6.8% vs 0, p=0.013; OR:6.2) were higher in SoC arm compared to Anakinra. ICU requirement and mortality did not differ between two arms (p=0.2 and p=0.4, respectively).

Patients experienced any thromboembolic event had more frequent CHD (p=0.04; OR:4.1), critical illness (p<0.001; OR:12.5), lower hemoglobin and baseline ferritin levels (p=0.03 and p=0.04, respectively), higher mcHIS scores (p=0.001), higher peak levels of CRP (p<0.001), d-dimer (p<0.001), LDH (p=0.038). Furthermore, severe infection (41% vs 20.3%, p=0.05; OR:3.9) and mortality (64.7% vs 27.7%, p=0.002; OR:9.8) were higher in patients had any thromboembolic event than those had not (table 2). Similarly PTE was higher in patients had critical illness (p=0.002; OR:9.5), lower hemoglobin and ferritin levels (p=0.02 and p=0.04, respectively), higher mcHIS score (p=0.002), peak levels of CRP (p<0.001), d-dimer (p<0.001), pneumothorax (p=0.03; OR:4.8), MI (p<0.001; OR:15), and mortality (p=0.03; OR:4.7) (table 3). PTE development was associated with peak levels of d-dimer levels (p=0.02, OR:1.08, 95% CI: 1.01-1.15) in multivariate analysis.

Development of MI was higher in patients had history of CHD and malignancy (p=0.007; OR:7.3 and p=0.02; OR:5.5, respectively), critical illness (p=0.02; OR:5.4), higher mcHIS scores (p=0.02), peak levels of CRP (p=0.043), d-dimer (p=0.03), LDH (p=0.004) (table 4). MI was also higher in SoC (P=0.016; OR:6.2) and patients had mortality (p<0.001; OR:13.7) in univariate analysis. MI development was associated with the history of CHD (p=0.038, OR:6.9, 95% CI:1.1-42.3) and PTE (p=0.008, OR:11.5, 95% CI:1.9-69.5) in multivariate analysis.

In survival analysis, development of any thromboembolic event, PTE, and MI were higher in SoC compared to Anakinra (Log-Rank; p=0.003 [figure 1], p=0.003 [supplementary figure 4], and p=0.007 [supplementary figure 5], respectively). Survival rate was also lower in patients with SoC arm than Anakinra in patients who had any thromboembolic event as well as MI (Log-Rank; p=0.03 [figure 2] and p<0.001 [figure 3], respectively). Survival rate of patients with and without PTE did not differ in patients with COVID-19 (supplementary figure 6).

ROC analysis revealed a cut-off value of d-dimer for the development of any thromboembolic event 16.75 (Area under curve [AUC]: 0.804, p<0.001 [95% CI: 0.710-0.898]) with 61.9% sensitivity and 84.8% specificity (likelihood ratio [LR]:4), for the development of PTE 14.97 (AUC: 0.867, p<0.001 [95% CI: 0.774-0.960])

with 86.7% sensitivity and 83.5% specificity (LR:5.3), for the development of MI 5.83 (AUC: 0.736, p<0.016 [95% CI: 0.585-0.887]) with 66.7% sensitivity and 66.7% specificity (LR:2) (Supplementary figure 7,8, and 9, respectively). Cut-off value of mcHIS score for the development of any thromboembolic event 3.5 (AUC: 0.726, p=0.001 [95% CI: 0.632-0.821]) with 71.4% sensitivity and 63.2% specificity (LR:1.94), for the development of PTE 3.5 (AUC: 0.740, p=0.002 [95% CI: 0.624-0.855]) with 73.3% sensitivity and 62.4% specificity (LR:1.95), for the development of MI 3.5 (AUC: 0.750, p=0.01 [95% CI: 0.630-0.870]) with 77.8% sensitivity and 61.7% specificity (LR:2) (Supplementary figure 10,11, and 12, respectively). Cut-off value of peak levels of CRP for the development of any thromboembolic event 171.2 mg/L (AUC: 0.780, p<0.001 [95% CI: 0.684-0.875]) with 76.5% sensitivity and 72.3% specificity (LR:2.8), for the development of PTE 201 mg/L (AUC: 0.800, p<0.001 [95% CI: 0.694-0.905]) with 71.4% sensitivity and 78.4% specificity (LR:3.3), for the development of MI 145.3 mg/L (AUC: 0.743, p=0.043 [95% CI: 0.629-0.857]) with 100% sensitivity and 54.7% specificity (LR:2.2) (Supplementary figure 13,14, and 15, respectively). Other results of ROC analysis were shown in table 5 and supplementary figure 16 and 17).

Discussion

It is well known that higher mortality rate and poor outcomes are mainly associated with development of cytokine storm in patients with COVID-19 (Gustine & Jones, 2021). Cytokine storm is a hyperinflammatory state that seen in several conditions such as hematological malignancies, infectious diseases and rheumatological conditions including adult-onset still disease (AOSD), systemic lupus erythematosus (Jarczak & Nierhaus, 2022). Development of cytokine storm depends on excessive production of several cytokines including interleukin-1 (IL-1), IL-6, tumor necrosis factor-alpha (TNF-α) and type 1 interferon (IFN) triggered by SARS-CoV-2 in COVID-19 (Chen et al., 2020). Recent studies revealed the importance of pulmonary macrophages' activation secondary to SARS-CoV-2 (23), which results in inflammasome activation in COVID-19 (Junqueira et al., 2022) (Sefik et al., 2022). Inflammasomes are essentials in the host defense against microorganisms including viruses that are present in various innate immune cells such as neutrophils, macrophages and dendritic cells. Activation of inflammasomes is leading to the cleavage of pro-IL-1β to produce active IL-1β (Vora, Lieberman, & Wu, 2021), and responsible for development of various immune-mediated diseases such as Familial Mediterranean Fever (FMF), gout, and AOSD (Gabay, Lamacchia, & Palmer, 2010). Furthermore, safety and efficacy of IL-1 blockade in these diaseases were established in these conditions (Dinarello, 2011).

Anakinra is an IL-1 receptor antagonist which is widely used in several rheumatological diseases such as FMF, AOSD, and gout (Marko et al., 2021) (Giacomelli et al., 2021) (Saag et al., 2021) and also several hyperinflammatory conditions such as cancer-related hemophagocytic syndrome, chimeric antigen receptor-modified (CAR) T cell associated cytokine storm, and macrophage activation syndrome (Bami et al., 2020) (Strati et al., 2020) (Grom, Horne, & De Benedetti, 2016). Safety and efficacy of Anakinra was also established in COVID-19-associated cytokine storm (Kyriazopoulou et al., 2021). Intravenous and high dose anakinra is an emerging therapeutic option both in rheumatology, other hyperinflammatory conditions, and COVID-19 (Nigrovic et al., 2011) (Mehta, Cron, Hartwell, Manson, & Tattersall, 2020) (Phadke, Rouster-Stevens, Giannopoulos, Chandrakasan, & Prahalad, 2021). Intravenous administration of anakinra ensures higher and fast maximum plasma concentration compared to subcutaneous form (Saunders, Kuijpers, Sloan, & Gertner, 2023). Daily dose adjustment of anakinra may allow early intervention of the cytokine storm according to daily clinical status, also withdrawing the drug in case of infection or other complications. Additionally, intravenous high-dose anakinra treatment reduced mortality in our previous study (Bektaş et al., 2023a).

Thromboembolic events are common in COVID-19 which is a remarkable finding from the beginning of pandemic (Asakura & Ogawa, 2021). In Middeldorp et al. study overall VTE frequency was 20% which was higher in patients in ICU (47%) than ward (3.3%). In the former study, ICU admission, increased d-dimer and NLR levels were associated with development of VTE which were similar with our results. In another observational study with 3334 patients, 16% of patients experienced a thrombotic event which 6.2% of them were VTE and 11.1% were arterial events (1.6% stroke and 8.9% MI) (Bilaloglu et al., 2020). The former study also revealed an association between development of thrombosis and prior history of CHD and

increased d-dimer levels which were consistent with our results. In the former study, thrombotic events were also higher in patients who had critical disease and/or deceased compared to those who had not. In a study with COVID-19-related deceased patients, although 9% of the patients had macroscopic thrombosis, most of the patients (87%) had microscopic evidence of thrombosis accompanying intense inflammation in autopsy specimens (Khismatullin et al., 2021). The authors also concluded a pathologic link between inflammation and thrombosis in the former study. In our study, higher mcHIS score and its components such as d-dimer and CRP levels in patients experienced thrombosis suggest that hyperinflammation is one of the key factors for the development of thrombotic events in patients with COVID-19. Moreover, the fact that higher values of peak levels of CRP, d-dimer, LDH, ferritin than those baseline levels emphasize the crucial role of hyperinflammation into the development of thrombotic events. This finding was also consistent with our previous results regarding the close association between peak levels of these laboratory tests and poor outcomes (Bektaş et al., 2023a).

In our study, lower frequency of PTE in anakinra group was a remarkable finding even though anakinra group had more severe disease before propensity score matching (Bektaş et al., 2023b). This finding persists after the PS matching procedure. As already known, endothelial dysfunction, thrombophilia and stasis are the main contributors into the development of venous thrombosis according to Virchow's triad. In COVID-19, endothelial dysfunction appears to be a more prominent factor for the development of thrombosis (Ahmed, Zimba, & Gasparyan, 2020). In our study, none of patients with PTE had clinical evident DVT which suggests COVID-19-related pulmonary thrombosis is an in-situ thrombosis rather than embolism which was claimed by Gabrielli et. al. study (Gabrielli, Lamendola, Esperide, Valletta, & Franceschi, 2020). In our study, all patients received background anticoagulant prophylaxis in two arms but could not prevent thrombotic events. This situation is recently called 'inflammothrombosis' which is similar to Behçet's disease (BD) associated venous thrombosis. While DVT and PT (in situ thrombosis, not embolism) may develop in BD separately, DVT is not expected to cause embolism due to its inflammatory nature (firmly attached to the vascular wall). Therefore, the definition of pulmonary thrombosis may be more accurate than pulmonary embolism in patients with COVID-19 similar to BD. Furthermore, while anticoagulant therapy does not prevent vascular thrombosis in BD patients, anti-inflammatory treatment improves the vascular outcomes such as recanalization and prevention of relapses (Bettiol et al., 2023). In the light of these data, pulmonary thrombosis in COVID-19 may be mainly associated with pulmonary inflammatory environment rather than stasis or other components of Virchow's triad and develops in situ thrombosis rather than embolism. Therefore, anti-inflammatory treatment may reduce thrombosis risk beyond the anticoagulant treatment in patients with severe COVID-19 which were shown in our study. However, it should be kept in mind that there is limited data showing the efficacy of anti-inflammatory therapy as an anticoagulant effect in patients with COVID-19.

Inflammation is an important contributor to the development of cardiovascular disease including acute coronary syndromes (ACS). During the pandemic arterial thrombotic events such as CVA and MI were increased in patients with COVID-19 (Stein, Mayman, Dhamoon, & Fifi, 2021) (Knight et al., 2022). The NLRP3 (NOD [nucleotide oligomerization domain]-, LRR [leucine-rich repeat]-, and PYD [pyrin domain]-containing protein 3) [NLRP3] inflammasome, an innate immune signaling complex, is the key mediator of IL-1 family cytokine production. Recent evidence has shown that NLRP3 inflammasome activation has a crucial role leading higher IL-1 production for the development of ACS (Afrasyab et al., 2016). Furthermore, colchicine, an inflammasome inhibitor was found to be effective for the prevention of MI in patients prior to ACS history (Tardif et al., 2019). Similarly, canakinumab, is an IL-1β monoclonal antibody that decreases composite cardiovascular events including MI, stroke, coronary revascularization, and cardiovascular death in the CANTOS study (Everett et al., 2020). In our study, decreased incidence of MI with Anakinra was consistent with previous studies. Additionally, higher mcHIS score in patients had MI compared with had not emphasized the crucial role of hyperinflammation into the development of arterial events.

This study has some strengths and limitations. Retrospective design of the study was the main limitation although controlled design of the study adjusting potential confounders by PS matching was important to prevent bias. We could not perform doppler USG screening in patients who had PTE since it did not cause

a change in treatment and critical situation of the patients. Diagnosis of MI could not be confirmed with cardiac catheterization. Having missing data is also a limitation of the study. On the other hand, the fact that the study is conducted in a single center enables homogeneity in terms of patient population and treatment decisions that are made by a single physician.

Conclusions

Thromboembolic events were seen despite the anticoagulant prophylaxis in our study. Development of thrombosis were associated with hyperinflammation in patients with severe and critical COVID-19. Intravenous high-dose anakinra treatment decreases both venous and arterial events in patients with COVID-19.

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Table 1 : Baseline clinical and laboratory features and outcomes of the patients before and after Propensity-score (PS) Matching

	Before PS Matching	Before PS Matching	Before PS Matching	Af
Variables	**Anakinra (n=139)**	**SoC (n=114)**	**p value (OR)**	**An**
Age, years, median (IQR)	71 (25)	65.5 (23)	0.09	70
Gender, male, n (%)	72 (51.8)	45 (39.5)	0.05 (3.8)	40
Duration of hospitalisation (days), median (IQR)	11 (12)	9 (7.3)	0.03	10
Comorbidities, n (%)				
Diabetes mellitus	36/137 (26.3)	39 (34.2)	0.17	27
Hypertension	79/135 (58.5)	64 (56)	0.7	49
Coronary heart disease	24/135 (17.8)	24 (21)	0.5	19
Chronic renal failure	28 (20)	6 (5.3)	0.001 (11.9)	15
Chronic obstructive lung disease	22/136 (16.2)	19 (16.7)	0.9	14
Malignancy	16/138 (11.6)	8 (7)	0.2	6 (
Vaccination history	44/86 (51.2)	26/63 (41.3)	0.2	31
Disease severity, n (%)				
NIH score 3 (severe)	54 (38.8)	68 (59.6)	0.001 (10.9)	36
NIH score 4 (critical)	85 (61.2)	46 (40.4)		52
Vaccination history, median (IQR)	2 (1)	2 (0)	0.9	3 (
mcHIS score, median (IQR)	3 (1)	3 (3)	<0.001	3 (
Laboratory results				
Neutrophil to lymphocyte ratio, median (IQR)	6.8 (8)	4.4 (4.44)	0.002	6.9
Hemoglobin (g/L), mean±SD	13.2±2.2	13.2±2	0.6	13
Creatinine (mg/dL), median (IQR)	0.9 (0.47)	0.83 (0.52)	0.5	0.8
Prokalsitonin (pg/dL), median (IQR)	0.2 (0.46)	0.16 (0.31)	0.7	0.1
C-reactive protein (mg/L), median (IQR)				
1	116 (113)	100.3 (100.3)	0.4	11
2	148 (120)	126 (88)	0.012	14
3	11.4 (64)	13.1 (91)	0.5	10
Ferritin (pg/mL), median (IQR)				
1	393 (592)	322 (423)	0.076	33
2	714 (969)	378 (660)	<0.001	63
3	392 (590)	268 (480)	0.007	37
D-dimer (pg/mL), median (IQR)				
1	1.2 (1.1)	0.85 (1.05)	0.04	1.2
2	4.1 (12.2)	2.25 (5)	0.002	2.7
3	1.4 (4.1)	1.14 (2.14)	0.15	1.3
Lactate dehydrogenase (U/L), median (IQR)				
1	404 (220)	414 (229)	0.7	39

	Before PS Matching	Before PS Matching	Before PS Matching	Af
2	559 (266)	408 (237)	<0.001	57
3	357 (231)	334 (170)	0.03	36
Outcomes, n (%)				
Severe infection	19/128 (14.8)	26 (22.8)	0.1	13
Pneumothorax	3/134 (2.2)	0	0.1	2/
Development of any thrombotic event	7 (5)	14 (12.3)	0.038 (4.3)	3 (
Pulmonary thromboembolism	4 (2.9)	11 (9.6)	0.023 (5.1)	3 (
Myocardial infarction	3 (2.2)	6 (5.3)	0.2	0
ICU requirement	55 (39.6)	25 (22)	0.003 (9)	33
Mortality	51 (36.7)	27 (23.7)	0.026 (5)	30

PS: Propensity score, SoC: Standard of care, OR: Odds ratio, IQR: Interquartile range, ICU: Intensive care unit, 1: Baseline levels, 2: Peak levels, 3: Last levels

Table 2: Univariate analysis of the patients had any thromboembolic event before and after Propensity-score (PS) Matching

	Patients with thrombosis before PSM	Patients with thrombosis before
Variables	**Yes (n=21)**	**No (n=232)**
Age, years, median (IQR)	71 (22)	68 (25)
Gender, male, n (%)	13 (62)	104(45)
Duration of hospitalisation (days), median (IQR)	11 (10)	9.5 (10)
Comorbidities, n (%)		
Diabetes mellitus	7 (33.3)	68/230 (29.6)
Hypertension	13 (62)	130/228 (57)
Coronary heart disease	10 (47.6)	38/228 (16.7)
Chronic renal failure	4 (19)	30 (13)
Chronic obstructive lung disease	4 (19)	37/229 (16.2)
Malignancy	3 (14.3)	21/231 (9)
Vaccination history	5/13 (38.5)	65/136 (48)
Disease severity, n (%)		
NIH score 3 (severe)	3 (14.3)	119 (51.3)
NIH score 4 (critical)	18 (85.7)	113 (48.7)
Vaccination counts, median (IQR)	3 (1.5)	2 (1)
mcHIS score, median (IQR)	4 (2)	3 (2)
Laboratory results		
Neutrophil to lymphocyte ratio, median (IQR)	5.6 (10.5)	5.6 (5.8)
Hemoglobin (g/L), mean±SD	12.6±1.7	13.3±2.2
Creatinine (mg/dL), median (IQR)	0.94 (0.73)	0.84 (0.48)
Prokalsitonin (pg/dL), median (IQR)	0.2 (0.7)	0.2 (0.43)
C-reactive protein (mg/L), median (IQR)		
1	118 (123)	108 (107)
2	212.5 (121)	137.5 (95)
3	87.4 (144)	11.5 (64)
Ferritin (pg/mL), median (IQR)		
1	204.5 (603)	371 (545)
2	714 (735)	546 (867)
3	551.4 (695)	331.5 (483)
D-dimer (pg/mL), median (IQR)		

	Patients with thrombosis before PSM	Patients with thrombosis before
1	1.44 (2)	1.15 (1.1)
2	21 (28)	2.7 (7.3)
3	5.6 (32.7)	1.2 (2.3)
Lactate dehydrogenase (U/L), median (IQR)		
1	418 (268)	409 (215)
2	655 (487)	476 (271)
3	482 (518)	348 (169)
Outcomes, n (%)		
Severe infection	7 (33.3)	38/221 (17.2)
Pneumothorax	1 (4.8)	2/227 (0.9)
Treatment		
Anakinra	7 (5)	132 (95)
SoC	14 (12.3)	100 (87.7)
ICU requirement	10 (47.6)	70 (30.2)
Mortality	13 (62)	65 (28)

PSM: Propensity score-matching, SoC: Standard of care, OR: Odds ratio, IQR: Interquartile range, ICU: Intensive care unit, 1: Baseline levels, 2: Peak levels, 3: Last levels

Table 3 : Univariate analysis of the patients had pulmonary thromboembolism before and after Propensity-score (PS) Matching

	Patients with pulmonary thromboembolism before PSM	Patients with
Variables	**Yes (n=15)**	**No (n=238**
Age, years, median (IQR)	71 (23)	68.5 (25)
Gender, male, n (%)	8 (53.3)	109 (45.8)
Duration of hospitalisation (days), median (IQR)	10 (5)	10 (10)
Comorbidities, n (%)		
Diabetes mellitus	3 (20)	72/236 (30.5)
Hypertension	8 (53.3)	135/234 (57.7
Coronary heart disease	5 (33.3)	43/234 (18.4)
Chronic renal failure	3 (20)	31 (13)
Chronic obstructive lung disease	3 (20)	38/235 (16.2)
Malignancy	2 (13.3)	22/237 (9.3)
Vaccination history	4/10 (40)	66/139 (47.5)
Disease severity, n (%)		
NIH score 3 (severe)	2 (13.3)	120 (50.4)
NIH score 4 (critical)	13 (86.7)	118 (49.6)
Vaccination history, median (IQR)	2.5 (1.75)	2 (1)
mcHIS score, median (IQR)	4 (2)	3 (2)
Laboratory results		
Neutrophil to lymphocyte ratio, median (IQR)	7.6 (12.2)	5.6 (5.7)
Hemoglobin (g/L), mean±SD	12.6±1.9	13.3±2.1
Creatinine (mg/dL), median (IQR)	0.94 (0.7)	0.84 (0.5)
Prokalsitonin (pg/dL), median (IQR)	0.13 (0.5)	0.2 (0.43)
C-reactive protein (mg/L), median (IQR)		
1	110 (110)	108 (105)
2	212.5 (122)	138.6 (96)
3	87.4 (158)	11.6 (68)

	Patients with pulmonary thromboembolism before PSM	Patients with
Ferritin (pg/mL), median (IQR)		
1	203 (413)	379 (543.5)
2	693.6 (739)	552 (863)
3	551.4 (614)	335.5 (487)
D-dimer (pg/mL), median (IQR)		
1	1.1 (4.8)	1.17 (1.1)
2	31.8 (18.2)	2.7 (7.4)
3	22.3 (31.5)	1.2 (2.3)
Lactate dehydrogenase (U/L), median (IQR)		
1	428.5 (207)	409 (219)
2	633 (426)	477 (282)
3	482 (495)	348 (171)
Outcomes, n (%)		
Severe infection	6 (40)	39/227 (17.2)
Pneumothorax	1 (6.7)	2/233 (0.9)
Myocardial infarction	3 (20)	6 (2.5)
Treatment		
Anakinra	4 (3)	135 (97)
SoC	11 (9.6)	103 (90.4)
ICU requirement	5 (33.3)	75 (31.5)
Mortality	8 (53.3)	70 (29.4)

PSM: Propensity score-matching, SoC: Standard of care, OR: Odds ratio, IQR: Interquartile range, ICU: Intensive care unit, 1: Baseline levels, 2: Peak levels, 3: Last levels

Table 4: Univariate analysis of the patients had myocardial infarction after Propensity-score (PS) Matching

	Patients with MI after PSM	Patients with MI after PSM	Patients w
Variables	**Yes (n=6)**	**No (n=170)**	**p value (**
Age, years, median (IQR)	77.5 (33)	69 (26)	0.1
Gender, male, n (%)	5 (83.3)	76 (44.7)	0.06
Duration of hospitalisation (days), median (IQR)	9 (14.5)	10 (9.3)	0.9
Comorbidities, n (%)			
Diabetes mellitus	2 (33.3)	54 (31.8)	0.9
Hypertension	3 (50)	99/168 (59)	0.7
Coronary heart disease	4 (66.7)	34/168 (20.2)	0.007 (7.3)
Chronic renal failure	1 (16.7)	23 (13.5)	0.8
Chronic obstructive lung disease	2 (33.3)	26/168 (15.5)	0.2
Malignancy	2 (33.3)	12 (7)	0.02 (5.5)
Vaccination history	1/4 (25)	48/102 (47)	0.4
Disease severity, n (%)			
NIH score 3 (severe)	0	82 (48.2)	0.02 (5.4)
NIH score 4 (critical)	6 (100)	88 (51.8)	
Vaccination history, median (IQR)		2 (1)	0.5
mcHIS score, median (IQR)	4.5 (1.25)	3 (2)	0.02
Laboratory results			
Neutrophil to lymphocyte ratio, median (IQR)	4 (6)	5.9 (6.8)	0.6
Hemoglobin (g/L), mean±SD	12.8±1.3	13.3±2.2	0.5
Creatinine (mg/dL), median (IQR)	1 (0.66)	0.87 (0.54)	0.5

	Patients with MI after PSM	Patients with MI after PSM	Patients w
Prokalsitonin (pg/dL), median (IQR)	NA	0.18 (0.44)	NA
C-reactive protein (mg/L), median (IQR)			
1	129 (164)	107 (117)	0.6
2	144.8 (74)	138.6 (110)	0.043
3	207.6 (80)	11.5 (80)	0.003
Ferritin (pg/mL), median (IQR)			
1	NA	331 (545)	NA
2	1001 (761)	542 (848)	0.096
3	1001 (687)	333 (483)	0.009
D-dimer (pg/mL), median (IQR)			
1	NA	1.2 (1.1)	NA
2	27.3 (32.1)	2.7 (8.6)	0.03
3	27.3 (33)	1.2 (2.8)	0.005
Lactate dehydrogenase (U/L), median (IQR)			
1	390 (97)	399 (206)	0.8
2	998 (759)	488 (278)	0.004
3	582.5 (684)	355 (172)	0.016
Outcomes, n (%)			
Severe infection	3 (50)	35/164 (21.3)	0.1
Pneumothorax	0	2/168 (1.2)	0.8
Treatment			
Anakinra	0	88 (100)	0.013 (6.2
SoC	6 (6.8)	82 (93.2)	
ICU requirement	4 (66.7)	53 (31.2)	0.07
Mortality	6 (100)	49 (28.8)	<0.001 (1

PSM: Propensity score-matching, SoC: Standard of care, OR: Odds ratio, IQR: Interquartile range, ICU: Intensive care unit, 1: Baseline levels, 2: Peak levels, 3: Last levels

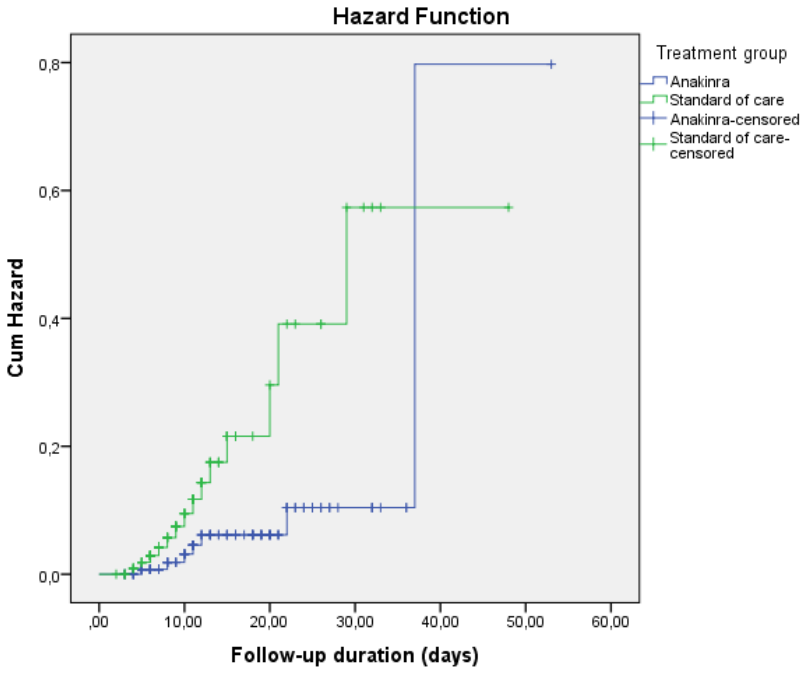
Table 5 : ROC analysis of laboratory features of the patients for development of thromboembolic events in patients with COVID-19

Variables	Cut-off value	Area under curve	p value (95% CI)	Sensitivity	Specificity	Likelihood ratio
mcHIS score						
Any thrombosis	3.5	0.726	0.001 (0.632-0.821)	71.4	63.2	1.94
PTE	3.5	0.740	0.002 (0.624-0.855)	73.3	62.4	1.95
MI	3.5	0.750	0.01 (0.630-0.870)	77.8	61.7	2
D-dimer (pg/mL)*						
Any thrombosis	16.75	0.804	<0.001 (0.710-0.898)	61.9	84.8	4
PTE	14.97	0.867	<0.001 (0.774-0.960)	86.7	83.5	5.3
MI	5.83	0.736	0.016 (0.585-0.887)	66.7	66.7	2

Variables	Cut-off value	Area under curve	p value (95% CI)	Sensitivity	Specificity	Likelihood ratio
C-reactive protein (mg/L)*						
Any thrombosis	171.2	0.780	<0.001 (0.684-0.875)	76.5	72.3	2.8
PTE	201	0.800	<0.001 (0.694-0.905)	71.4	78.4	3.3
MI	145.3	0.743	0.043 (0.629-0.857)	100	54.7	2.2
Lactate dehydrogenase (U/L)*						
Any thrombosis	496	0.676	0.008 (0.551-0.801)	66.7	53.4	1.4
PTE	NS	NS	NS	NS	NS	NS
MI	649.5	0.802	0.002 (0.675-0.928)	77.8	75.5	3.2
Ferritin (pg/mL)*						
Any thrombosis	NS	NS	NS	NS	NS	NS
PTE	NS	NS	NS	NS	NS	NS
MI	NS	NS	NS	NS	NS	NS
Neutrophil-lymphocyte ratio						
Any thrombosis	NS	NS	NS	NS	NS	NS
PTE	NS	NS	NS	NS	NS	NS
MI	NS	NS	NS	NS	NS	NS

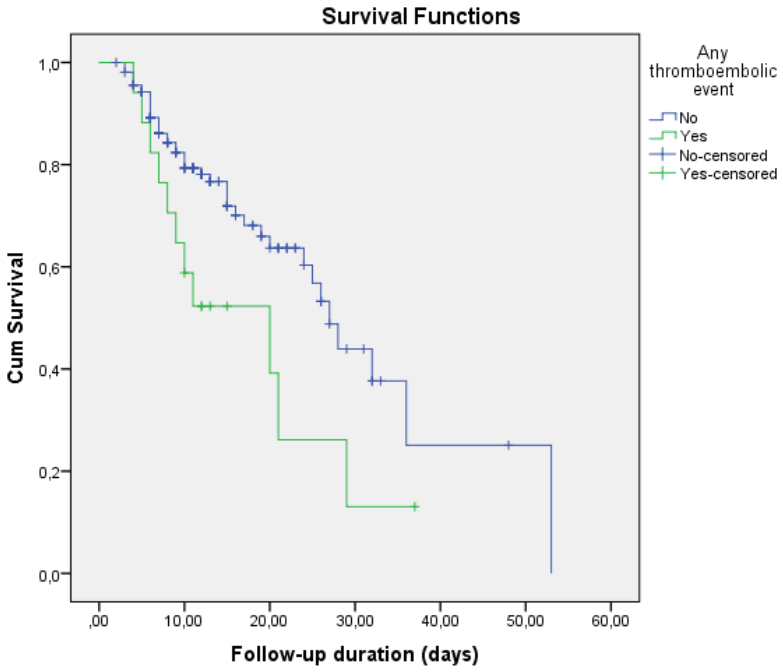
*Peak levels of value, PTE: Pulmonary thromboembolism, MI: Myocardial infarction, CI: Confidence interval

Figure 1 : Development of any thromboembolic event in patients with COVID-19 according to the treatment groups (Kaplan-Meier survival analysis)



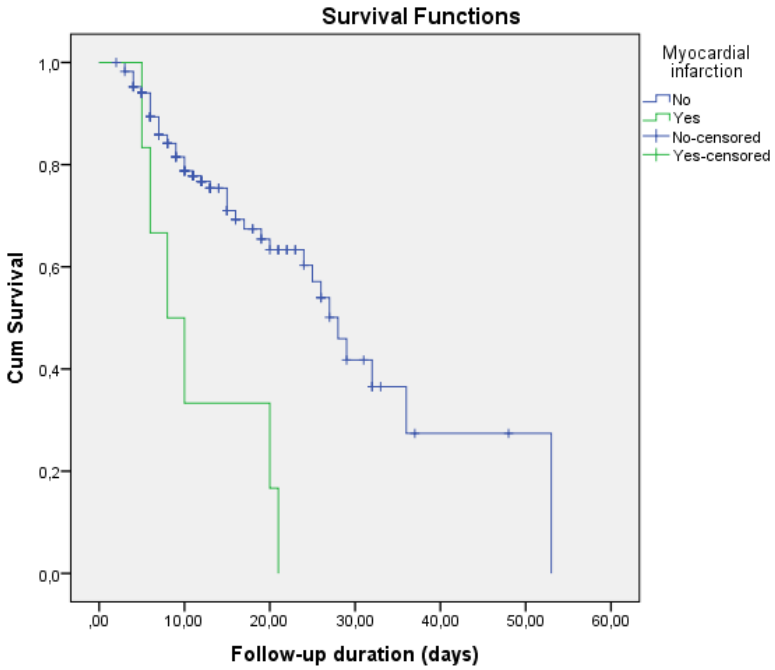
Log-Rank; p=0.003

Figure 2 : Survival rate of patients with COVID-19 according to presence of any thromboembolic event (Kaplan-Meier survival analysis)



Log-Rank; p=0.03

Figure 3 : Survival rate of patients with COVID-19 according to presence of myocardial infarction (Kaplan-Meier survival analysis)



Log-Rank; p<0.001