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SARS-CoV-2 infection- induced growth factors play differential roles in COVID-19 pathogenesis

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A R T I C L E   I N F O

A B S T R A C T

Aims: Biologically active molecules cytokines and growth factors (GFs) are critical regulators of tissue injury/ repair and emerge as key players in COVID-19 pathophysiology. However, specific disease stage of GFs dysregulation and, whether these GFs have associations with thromboembolism and tissue injury/repair in COVID-19 remain vague.

Main methods: GF profiling in hospitalized moderate (non-ICU) and critically ill (ICU) COVID-19 patients was performed through legendPlex assay.

Key findings: Investigation revealed profound elevation of VEGF, PDGFs, EGF, TGF-α, FGF-basic, and erythropoietin (EPO) in moderate cases and decline or trend of decline with disease advancement. We found strong positive correlations of plasma VEGF, PDGFs, and EPO with endothelial dysfunction markers P-selectin and sCD40L. Interestingly, the HGF and G-CSF were upregulated at the moderate stage and remained elevated at the severe stage of COVID-19. Moreover, strong negative correlations of PDGFs ($r^2 = 0.238$, $P = 0.006$), EPO ($r^2 = 0.18$, $P = 0.01$) and EGF ($r^2 = 0.172$, $P = 0.02$) and positive correlation of angiopoietin-2 ($r^2 = 0.267$, $P = 0.003$) with D-dimer, a marker of thromboembolism, was observed. Further, plasma PDGFs ($r^2 = 0.199$, $P = 0.01$), EPO ($r^2 = 0.115$, $P = 0.02$), and EGF ($r^2 = 0.108$, $P = 0.07$) exhibited negative correlations with tissue injury marker, myoglobin.

Significance: Taken together, unlike cytokines, most of the assessed GFs were upregulated at the moderate stage of COVID-19. The induction of GFs likely occurs due to endothelial dysfunction and may counter the adverse effects of cytokine storms which is reflected by inverse correlations of PDGFs, EPO, and EGF with thromboembolism and tissue injury markers. The findings suggest that the assessed GFs play differential roles in the pathogenesis of COVID-19.

1. Introduction

Accumulating dataset suggests that growth factors (GFs) may play essential roles in the pathogenesis of COVID-19 by modulating the cardiovascular complications and tissue injury and repair [1]. GFs are biologically active intermediate molecules secreted by various cell types during health and diseased conditions [2]. They regulate several pathophysiological conditions including tissue injury and repair through binding to specific receptors on the cell *via* paracrine and/or autocrine

mechanisms [3,4]. SARS-CoV-2-induced cytokines and GFs mediate coagulation dysfunction and tissue injury, which contribute to the clinical severity of COVID-19 [5,6]. Changes in plasma cytokines and GFs level increases the risk of venous thromboembolism (VTE) [7,8], characterized by elevated D-dimer and, a major cause of tissue injury in the severe COVID-19. The tissue injury marker, myoglobin, and D-dimer levels have been used to predict the severity of COVID-19 patients. These biomarkers possess significant predictive potential in evaluating disease outcomes in COVID-19 patients [9,10]. The robust correlations

of dysregulated GFs with these biomarkers may help identify whether aberrant levels of GFs in COVID-19 are pathological or compensatory.

Angiopoietin-2 (angpt-2) gets activated in response to inflammation of the endothelial lining, a relevant indicator of endothelial damage and commonly observed in COVID-19 patients [11]. It has been linked to pulmonary dysfunction, particularly in critically ill COVID-19 patients admitted to the intensive care unit (ICU) [12]. Resistance to GFs, such as erythropoietin (EPO), was reported to cause anemia in patients with chronic kidney and lung diseases [13]. *In-vivo* studies suggest the protective role of EPO by limiting lung injury through the downregulation of NLRP3 inflammasome *via* activation of JAK2/STAT3 and inhibition of the NF-kB signaling pathways [14]. The vascular endothelial growth factor (VEGF), a pro-angiogenic growth factor, has a mitogenic and anti-apoptotic effect on endothelial cells [15]. Hypoxic condition induces VEGF expression that disrupts vascular permeability in lung tissues causing pulmonary inflammation and congestion [16,17]. Since a severe infection of SARS-CoV-2 often leads to endothelial dysfunction, acute respiratory distress syndrome (ARDS), and dyspnea with systemic hypoxia [18], VEGF may play a critical role in the COVID-19 pathogenesis.

Epidermal growth factor (EGF) regulates cell growth and differentiation by binding to its receptor EGFR, which is abundantly expressed on cells derived from the epithelial, mesenchymal, and neuronal origins [19]. EGFR-mediated signaling regulates cell rejuvenation, mucus overproduction, and pulmonary fibrosis in SARS-CoV-1 infection [20]. Similarly, TGF-α, a member of the EGF family, promotes the proliferation of type II alveolar epithelial cells and is associated with fibrosis [21]. Besides these factors, basic- fibroblast growth factor (bFGF) also plays strong mitogenic roles in smooth muscle cells, myofibroblasts, and fibroblasts and ultimately in pulmonary fibrosis [22].

Hepatocyte growth factor (HGF) is produced by stromal and mesenchymal cells and regulates epithelial cell proliferation, motility, morphogenesis, and angiogenesis in multiple organs [23,24]. Studies reveal that lung injury triggers HGF production, which promotes tissue repair *via* inhibiting apoptosis of lung epithelial and endothelial cells, and by counteracting several pro-apoptotic and pulmonary fibrosis factors such as TGF-β, IL-1β, IL-8, TNF-α, the bFGF, the insulin-like growth factor (IGF), and the platelet-derived growth factor (PDGF) [1,25,26].

Colony-stimulating factors (CSFs) are pro-inflammatory cytokines that regulate granulocyte and macrophage populations. These immune cell populations are the key players of the innate immune system to defend against viral, bacterial, and fungal infections [27]. The number of these cell populations remains stable in a healthy environment; however, the demand for these cells increases rapidly upon infections. The increased demand for immune cell production is tightly regulated by different CSFs, including granulocyte-CSF (G-CSF), macrophage-CSF (M-CSF), and granulocyte-macrophage CSF (GM-CSF). The CSFs are primarily synthesized by lymphocytes, but other cell populations like endothelial cells, fibroblasts, and epithelial cells can also produce CSFs under activating stimulus [28]. Considering these facts, it is clinically important to carefully evaluate the plasma levels of these GFs at different severity stages of SARS-CoV-2 infection. Moreover, assessing their potential association with thromboembolism and tissue injury and/or repair would help better understand their roles in COVID-19 pathogenesis.

Here, we report the upregulation of VEGF, EPO, PDGF isoforms, TGF-α, and bFGF in moderate non-ICU COVID-19 patients, and levels decline in the critically ill patients admitted to the ICU. The levels of G-CSF, but not GM-CSF, increase in moderate patients and remain elevated in severe patients. Similarly, the levels of HGF increase at the moderate stage and remain elevated in critically ill patients. Of these, only PDGF isoforms, EPO and EGF negatively correlate with D-dimer and positively correlate with tissue injury marker myoglobin. In contrast, the level of plasma angpt-2 positively correlates with the level of D-dimer. These observations indicate that the assessed GFs may play differential roles in the COVID-19 progression and severity.

2. Materials and methods

The hospitalized SARS-CoV-2 infected patients, confirmed by real-time-PCR (RT-PCR), were recruited from the ICU and general (non-ICU) COVID-19 ward of Rashid hospital Dubai during the early pandemic period (May–June 2020). A total of 30 (28 males: 2 females) patients were recruited, including 15 critically ill patients with pneumonia and/or acute respiratory distress syndrome (ARDS) requiring a ventilator and 15 patients without serious illness. After taking the written informed consent, blood samples were collected from patients when admitted to their respective wards. Patients with pre-existing medical conditions, including myocardial infarction, stroke, or DVT were excluded from the study. Patients treated with anticoagulants were also excluded from the study. A total of 10 healthy controls (8 males: 2 females) without any current medications and/or a recent history of any disease were recruited for comparison. The study was provided by the research ethics committees of the University of Sharjah and the Dubai Health Authority.

2.1. Blood sample processing

The anticoagulated blood was processed rapidly and centrifuged at 3000 rpm for 20 min. The platelet-poor plasma was transferred to a fresh tube and stored at -80 °C freezer until used for the experiments.

2.2. Growth factor assay

A flow cytometry-based multiplex assay was performed using a flow cytometry-based LegendPlex kit (BioLegend #740180) to assess the levels of 13 plasma GFs including angpt-2, FGF-basic, TGF-α, EGF, EPO, M-CSF, G-CSF, GM-CSF, SCF, HGF, PDGF-AA, PDGF-BB, and VEGF following manufacturer's instruction as reported previously [4]. Briefly, plasma samples from COVID-19 patients and healthy controls were diluted 1:1 and used to measure the levels of mentioned growth factors. Diluted samples were loaded onto a 96-well assay plate, and beads were added to the wells. The plate was sealed and incubated on an orbital shaker at 300 rpm for 2 h at room temperature. The plate was centrifuged, and the supernatant was aspirated. The beads were washed twice using wash buffer, and detection antibodies were added to each well, followed by incubation on a shaker for 1 h. The SA-PE solution was added to the plate wells, followed by incubation on a shaker. After 30 min of incubation, the supernatant was carefully aspirated, and beads were re-suspended in wash buffer. The beads were assessed under BD FACS Aria III using FACS Diva software, and data analysis was performed using Legendplex software (Biolegend, USA). All the samples were tested in duplicate, and the average was taken as a final reading.

2.3. Statistics

Data group differences were evaluated for significance using one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons. A linear regression analysis was performed to identify the potential correlation between different GFs, D-dimer and tissue injury markers (Graph Pad Prism Software Inc., San Diego, CA). Data are expressed as mean ± SEM. A P-value <0.05 was considered for statistical significance.

3. Results

3.1. Phenotypic and clinical presentation

The recruited patients and healthy controls were of Arab and Asian backgrounds. The age range of moderate patients was between 32 and 67 years (median age of 48 years) and between 38 and 69 years (median age of 57 years) for severe patients. The detailed clinical laboratory parameters and medications of these patients were reported recently

[6,29]. Briefly, there was an induction of inflammatory reaction in these patients which was attested by an elevated level of plasma c-reactive protein (CRP), a higher number of white blood cells (WBCs) including absolute lymphocyte counts (ALC), particularly in severe COVID-19 cases. The plasma creatinine levels were within the normal range; however, significantly increased levels of ferritin were observed in both patient groups. All the patients in the moderate group survived however, the fatality was reported in a total of five (33.3 %) of 15 severe patients. Irrespective of severity, most of the recruited patients were treated with Chloroquine or Hydroxychloroquine with or without anti-inflammatory and antiviral medication like Favipiravir, Kaletra (lopinavir/ritonavir),

Azithromycin, Tocilizumab, and Corticosteroids.

3.2. SARS-CoV-2 induces the release of different growth factors at the moderate stage of COVID-19

Injury and infection-induced GFs play critical roles in inflammation and tissue repair [26,30]. Therefore, we sought to identify when particularly GFs dysregulation occurs during the COVID-19 pathogenesis and whether GFs have a role in the modulation of thromboembolism and tissue injury in COVID-19. Investigation revealed that the levels of VEGF and EPO were profoundly upregulated in the moderate non-ICU
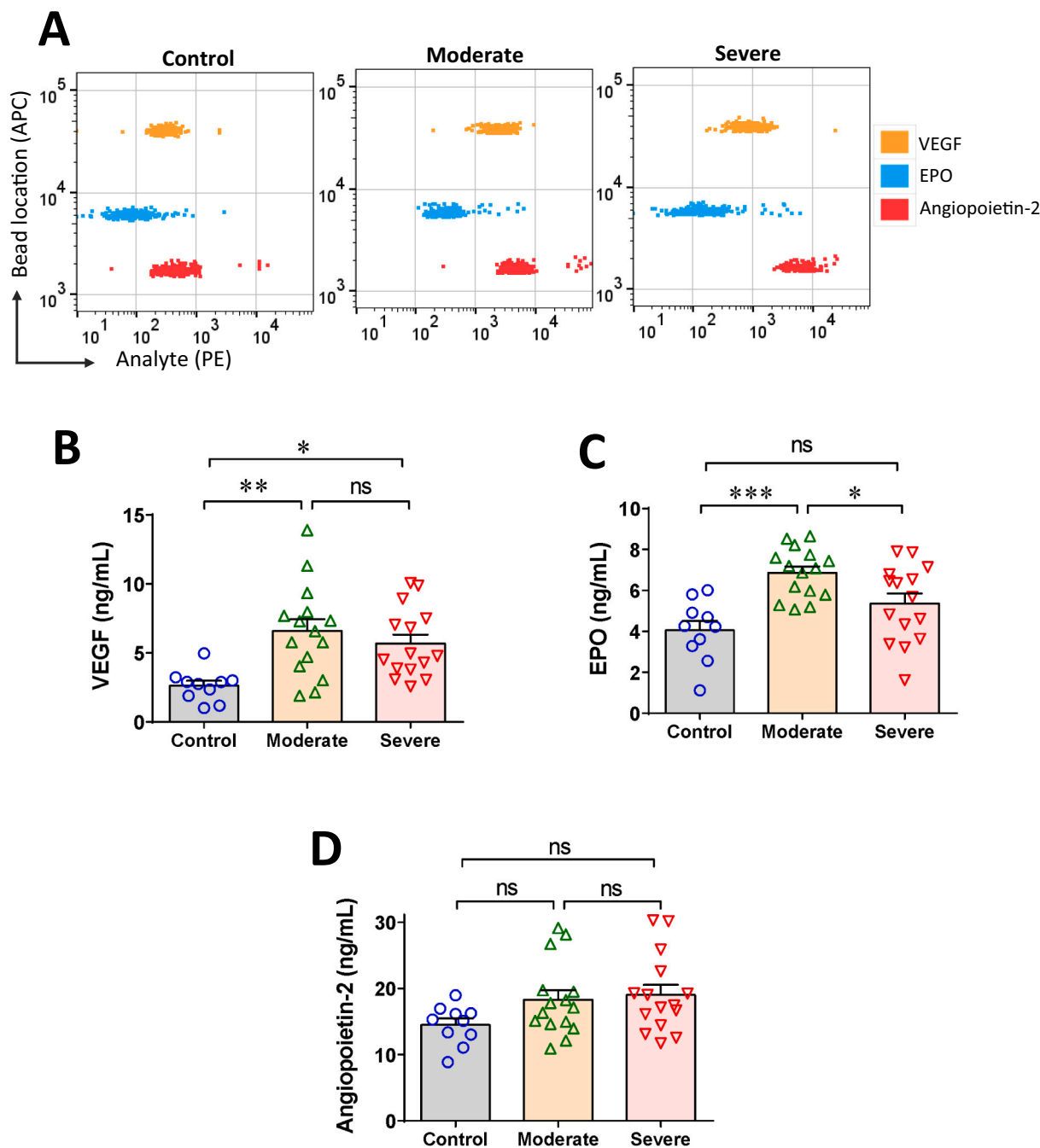

Fig. 1. Elevation of plasma VEGF and erythropoietin at the moderate stage of COVID-19. (A) Representative flow cytometry scatter dot plots show the levels of plasma vascular endothelial growth factor (VEGF), erythropoietin (EPO) and angiopoietin-2 in moderate and severe COVID-19 patients *vs.* healthy controls. Scatter dot plots show significantly elevated levels of (B) VEGF and (C) EPO in moderate patients and lower levels in severe *vs.* moderate COVID-19 patients. (D) The angiopoietin-2 levels were comparable in both moderate and severe cases. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

COVID-19 patients *vs.* healthy controls, and the levels were significantly downregulated in critically ill patients admitted to ICU (Fig. 1A–C). However, interestingly, the levels of angpt-2 were found unchanged in both the moderate and severe groups *vs.* healthy controls (Fig. 1A, D). Similar to VEGF, PDGF-AA and PDGF-BB were also robustly upregulated in the moderate cases, and the levels significantly declined in severe *vs.* moderate cases (Fig. 2A–C).

Since these GFs are largely contributed by activated endothelial cells and platelets [31,32], we assessed the potential correlation of these GFs with the endothelial cell activation markers P-selectin and soluble CD40L (sCD40L) we recently reported in these patients [29]. The levels of P-selectin and sCD40L were profoundly induced in the moderate COVID-19 patients and levels significantly declined with the COVID-19 severity. Interestingly, there were significant positive correlations of VEGF, PDGFs, and EPO with P-selectin and sCD40L (Fig. 3A–F). Surprisingly, a significant inverse correlation between the level of angpt-2 and sCD40L and a trend of inverse correlation between angpt-2 and P-selectin was observed (Fig. 3G–H). These data indicate that induction of VEGF, PDGFs, and EPO in COVID-19 may occur due to SARS-CoV-2 –induced endothelial and platelet activation. The inverse correlations between angpt-2 and endothelial dysfunction markers indicate that elevation of angpt-2 may have occurred during the severe hypoxic condition of COVID-19 when plasma endothelial dysfunction markers decline.

3.3. SARS-CoV-2 infection stimulates the level of G-CSF but not GM-CSF

The SARS-CoV-2 infection leads to vascular inflammation and recruitment of the immune cell population through CSF [33]. Hence, we sought to assess the level of the immune cell population stimulating GFs in moderate and severe COVID-19 patients. Irrespective of COVID-19 severity, the level of G-CSF was strikingly upregulated compared to healthy controls (Fig. 4A–B). Surprisingly, in contrast, the level of GM-CSF in both moderate and severe cases was comparable to healthy controls (Fig. 4A, C). Similarly, SCF was also found to be unchanged in the recruited patients (Fig. 4A, D). These findings suggest that G-CSF may play an important role in immune cell infiltration after SARS-CoV-2 infection.

3.4. SARS-CoV-2 induces several growth factors related to tissue fibrosis

Pulmonary fibrosis and liver fibrosis are among critical complications in severe COVID-19 patients [34–36]. Therefore, it is imperative to understand the underlying pathomechanisms of tissue fibrosis observed in COVID-19. In this series, we assessed the GFs related to fibrosis at two different stages of COVID-19 pathogenesis. Interestingly, the levels of TGF-α, FGF-basic, and EGF were significantly upregulated in the moderate patients but were downregulated with the progression of severity (Fig. 5A–D). However, an elevated level of HGF was observed in the moderate patients, and the level remained elevated in the critically ill
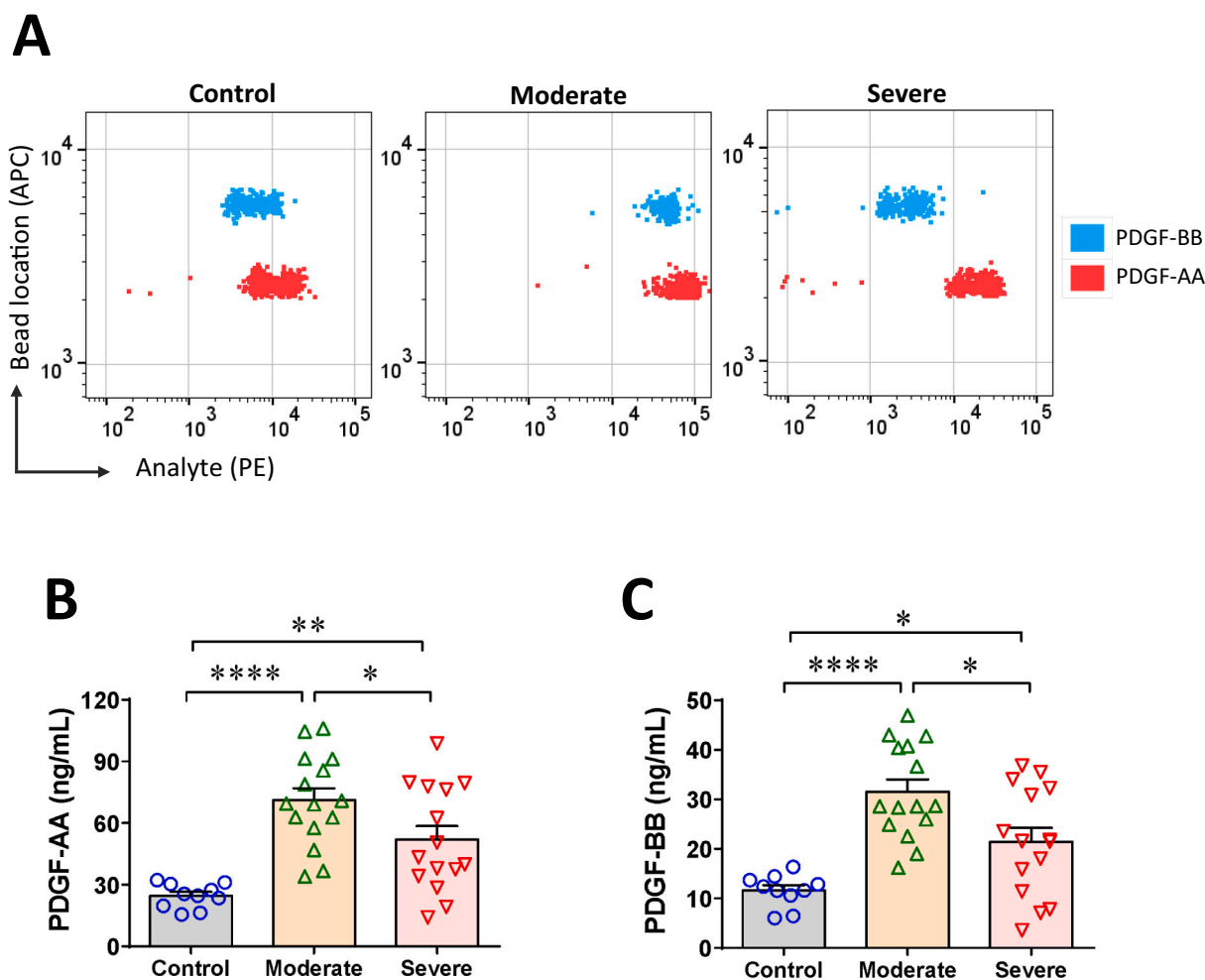

Fig. 2. SARS-CoV-2 infection rapidly induces plasma PDGF levels. (A) Representative scatter dot plots show the levels of plasma platelet-derived growth factor AA (PDGF-AA) and PDGF-BB in COVID-19 patients and healthy controls. (B) Bar diagrams show a profound induction of (A) PDGF-AA and (B) PDGF-BB in moderate cases and the levels significantly declined with disease severity. *, $P < 0.05$; **, $P < 0.01$; ****, $P < 0.0001$.
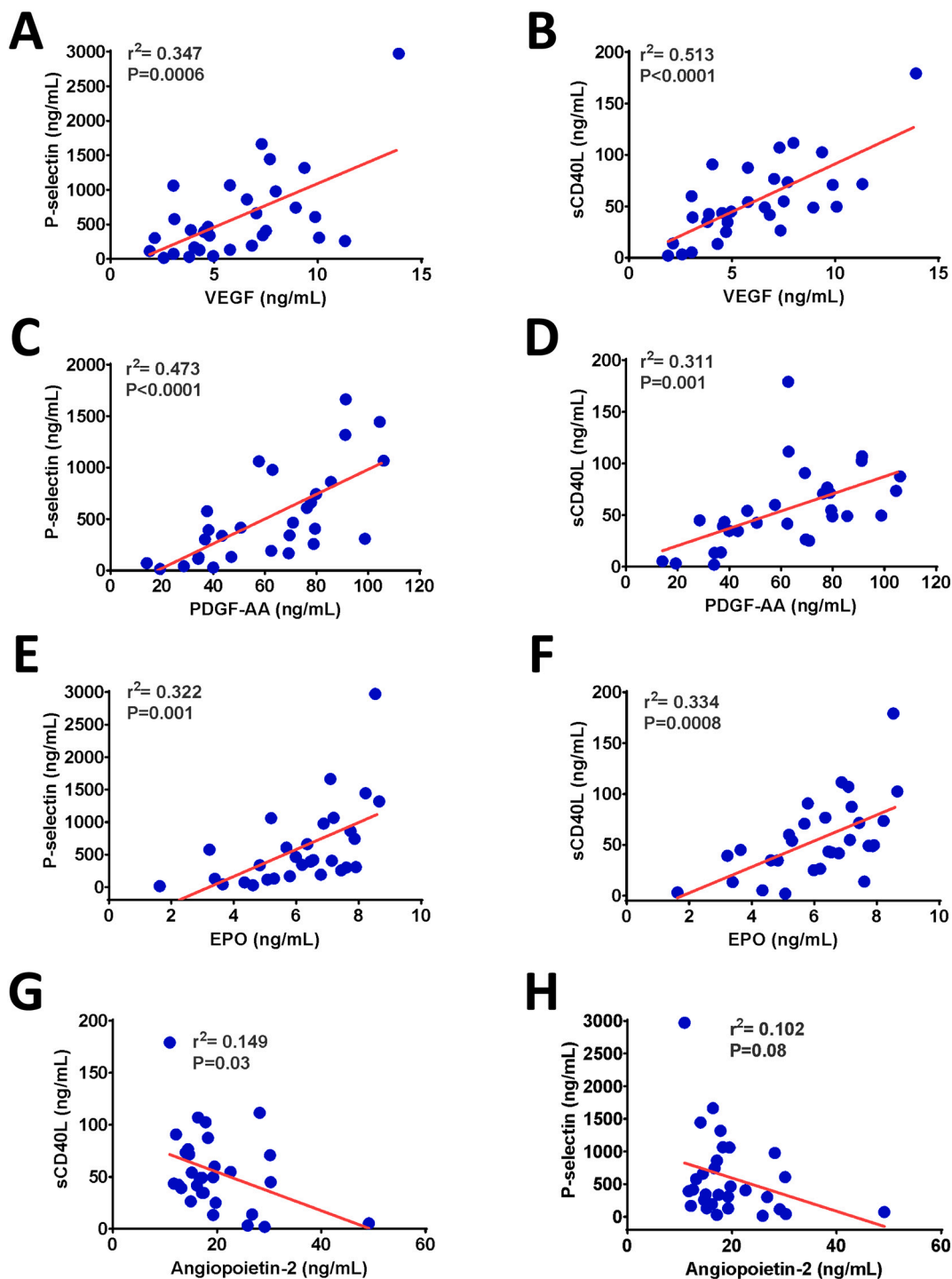
Fig. 3. Correlation of growth factors with endothelial dysfunction markers. The scatter plots from linear regression analysis show positive correlations of endothelial dysfunction markers (plasma P-selectin and CD40L) with (A–B) plasma vascular endothelial growth factor (VEGF), (C–D) platelet-derived growth factor AA (PDGF-AA) and (E–F) erythropoietin (EPO) and, (G–H) inverse correlations with angiopoietin-2.

patients (Fig. 5A, E). Overall, these findings indicate that assessed growth factors may regulate tissue fibrosis during COVID-19 pathogenesis.

3.5. PDGFs and EPO inversely correlate with thromboembolism and tissue injury markers

Several GFs play important roles in maintaining endothelial activity and vascular integrity and an imbalance in GFs may induce VTE [7]. We recently reported the significantly elevated levels of D-dimer [29] and tissue injury marker myoglobin [6], particularly in the ICU group of these patients. Therefore, next, we investigated the potential roles of selected GFs in thromboembolism and tissue injury or repair through their correlation with D-dimer and myoglobin in COVID-19 patients. First, we correlated the GFs with D-dimer levels and observed strong inverse correlations of PDGF-AA, PDGF-BB, EPO, and EGF with D-dimer in COVID-19 (Fig. 6A–D). Surprisingly, only angpt-2 was positively correlated (Fig. 6E), and the rest of the assessed GFs demonstrated no
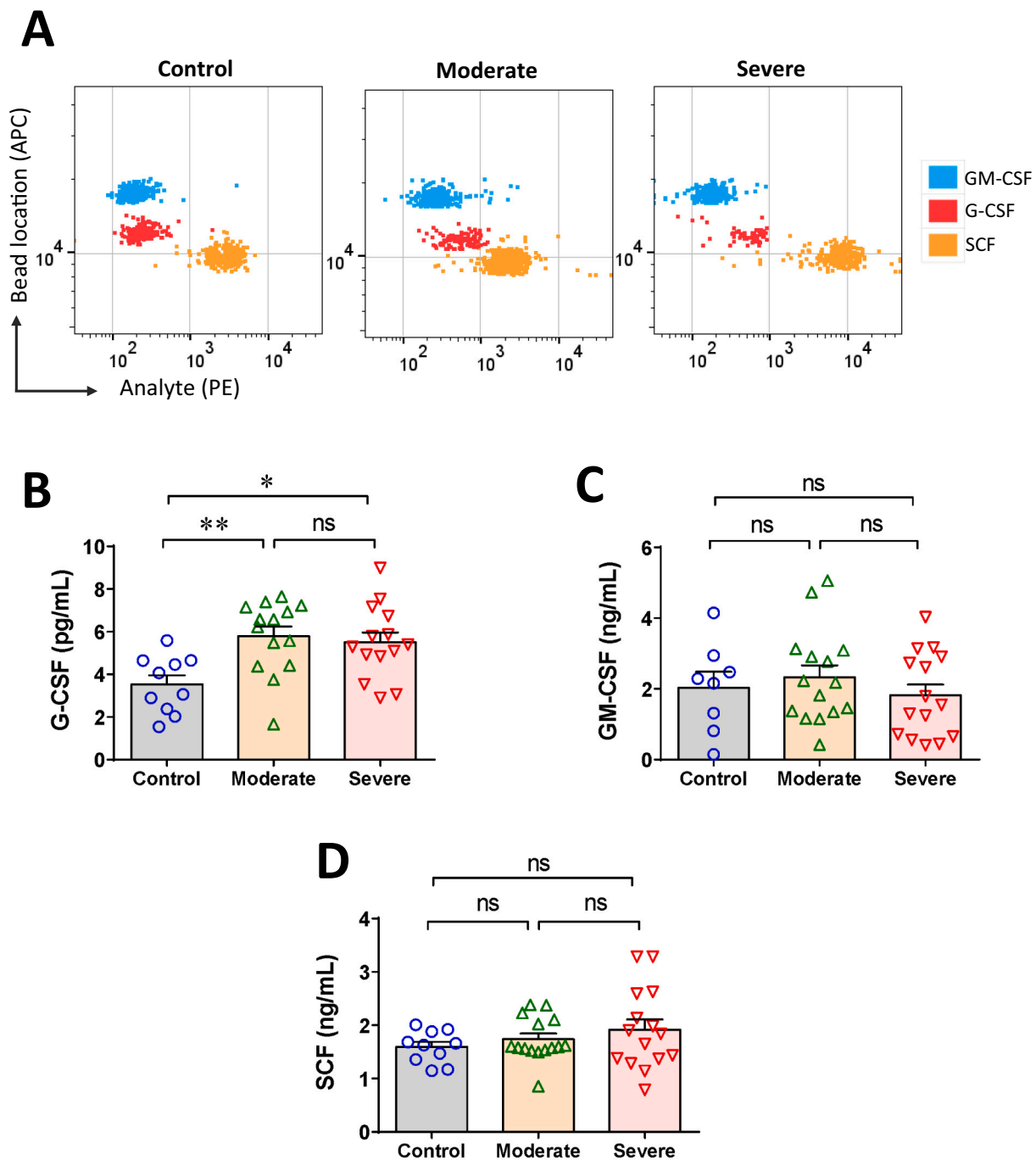
Fig. 4. SARS-CoV-2 infection promotes rapid secretion of G-CSF. (A) Representative scatter dot plots show the levels of plasma granulocytes-colony stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF) and stem cell factor (SCF) in healthy controls and COVID-19 patients. Bar diagrams show (B) a rapid elevation of G-CSF in moderate patients which remained elevated in severe patients, (C) comparable levels of GM-CSF and (D) a comparable level of SCF in COVID-19 patients *vs.* healthy controls. *, $P < 0.05$; **, $P < 0.01$.

correlation with D-dimer. Further correlation of these GFs with tissue injury marker myoglobin revealed their potential roles in tissue injury or repair. Consistent with the inverse correlation with D-dimer, the levels of PDGF-AA, PDGF-BB and EPO exhibited significant inverse correlations with the myoglobin levels in the COVID-19 (Fig. G–H). A trend of a negative correlation of EGF and a positive correlation of angpt-2 with myoglobin was also observed (Fig. I, J). These findings indicate that PDGF and EPO induction likely play protective; however, EGF and angpt-2 may play damaging roles in COVID-19.

4. Discussion

Here we show a profound elevation of VEGF, EPO and PDGFs at the moderate stage of COVID-19 which declines with the progression of disease severity. Importantly, these growth factors have shown positive correlations with endothelial activation markers plasma P-selectin and sCD40L in COVID-19. The level of G-CSF was found elevated in moderate and remained elevated in severe cases; however, in contrast, no changes were observed in GM-CSF. Moreover, tissue fibrosis-related GFs including TGF-α, bFGF and EGF were also found induced in the
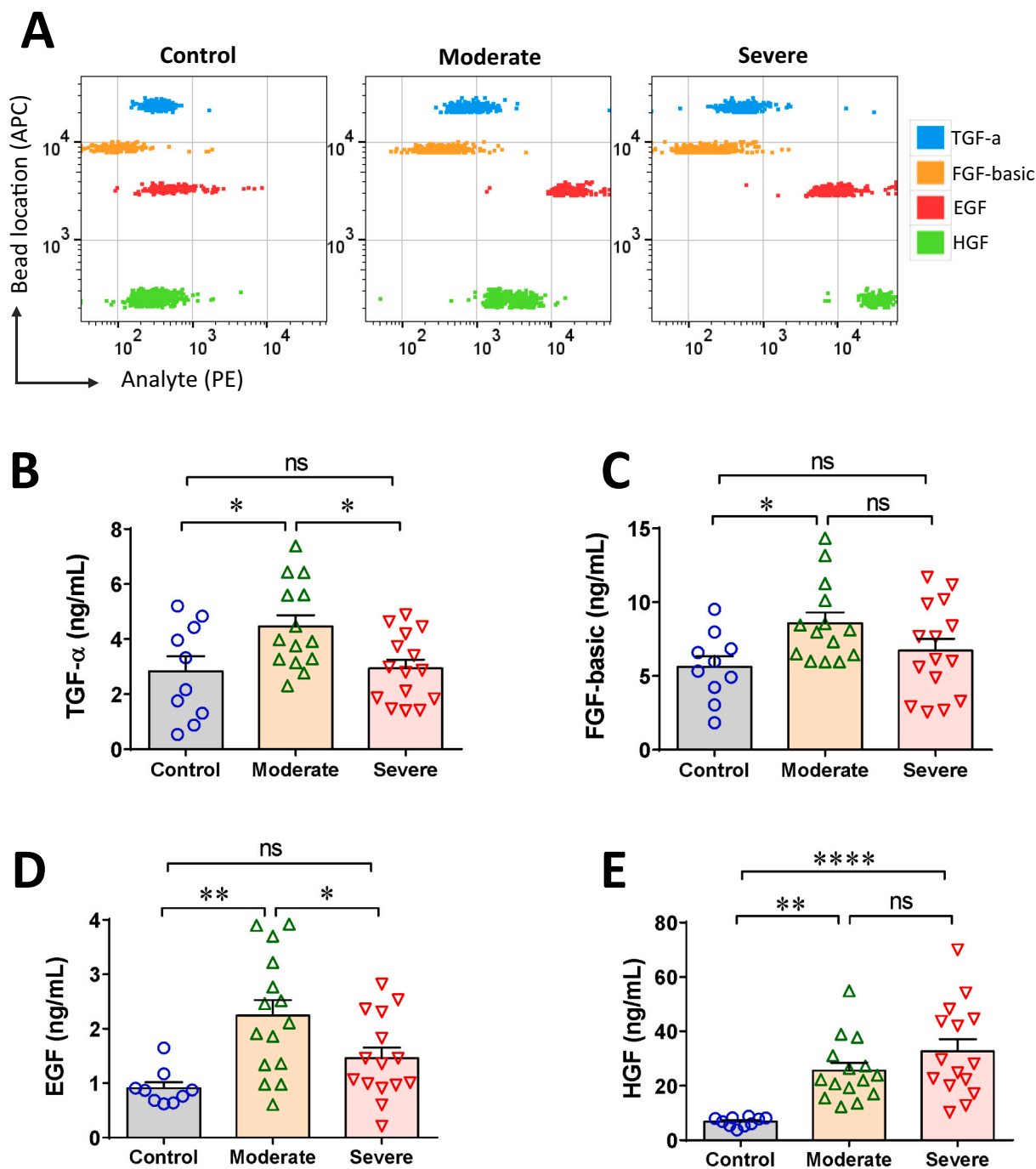
Fig. 5. Elevation of fibrosis-related plasma growth factors at the moderate stage of COVID-19. (A) Representative scatter dot plots show the levels of plasma transforming growth factor-α (TGF-α), basic- fibroblast growth factor (FGF-basic), epidermal growth factor (EGF) and hepatocyte growth factor (HGF) in COVID-19 patients and healthy controls. Bar diagrams show significant elevation of (B) TGF-α, and (C) FGF-basic, (D) EGF and (E) HGF in moderate COVID-19 cases. *, P < 0.05; **, P < 0.01; ****, P < 0.0001.

moderate stage of COVID-19. Interestingly, we show for the first time, that the levels of EGF, VEGFs, and EPO negatively correlate with D-dimer and tissue injury marker myoglobin in COVID-19. Our findings indicate that these biomarkers play roles in COVID-19 pathogenesis and may help better understand the pathomechanism of COVID-19.

The elevation of several GFs in moderate COVID-19 infection is generally consistent with the literature [5,37]. However, to our surprise, unlike cytokines, the levels of most of these GFs did not further elevate but declined with the increasing severity of COVID-19. Our data validates and extends a recent report showing differences in the circulating

levels of several GFs between moderate and severe COVID-19 patients [37]. The induction of most of the assessed GFs at the moderate stage and, their positive correlations with P-selectin and sCD40L suggest that they are elevated most likely due to platelet, epithelial and endothelial cell dysfunction post-SARS-CoV-2 infection. Moreover, the decline in GFs levels at the severe stage of COVID-19 likely occurs due to exhaustion of the platelet, epithelial, endothelial cells, and immune cell population, which are the primary sources of these GFs. The lower levels of GFs in severe patients may limit the overall healing process of these patients by limiting tissue repair caused by cytokine storms. Similarly,
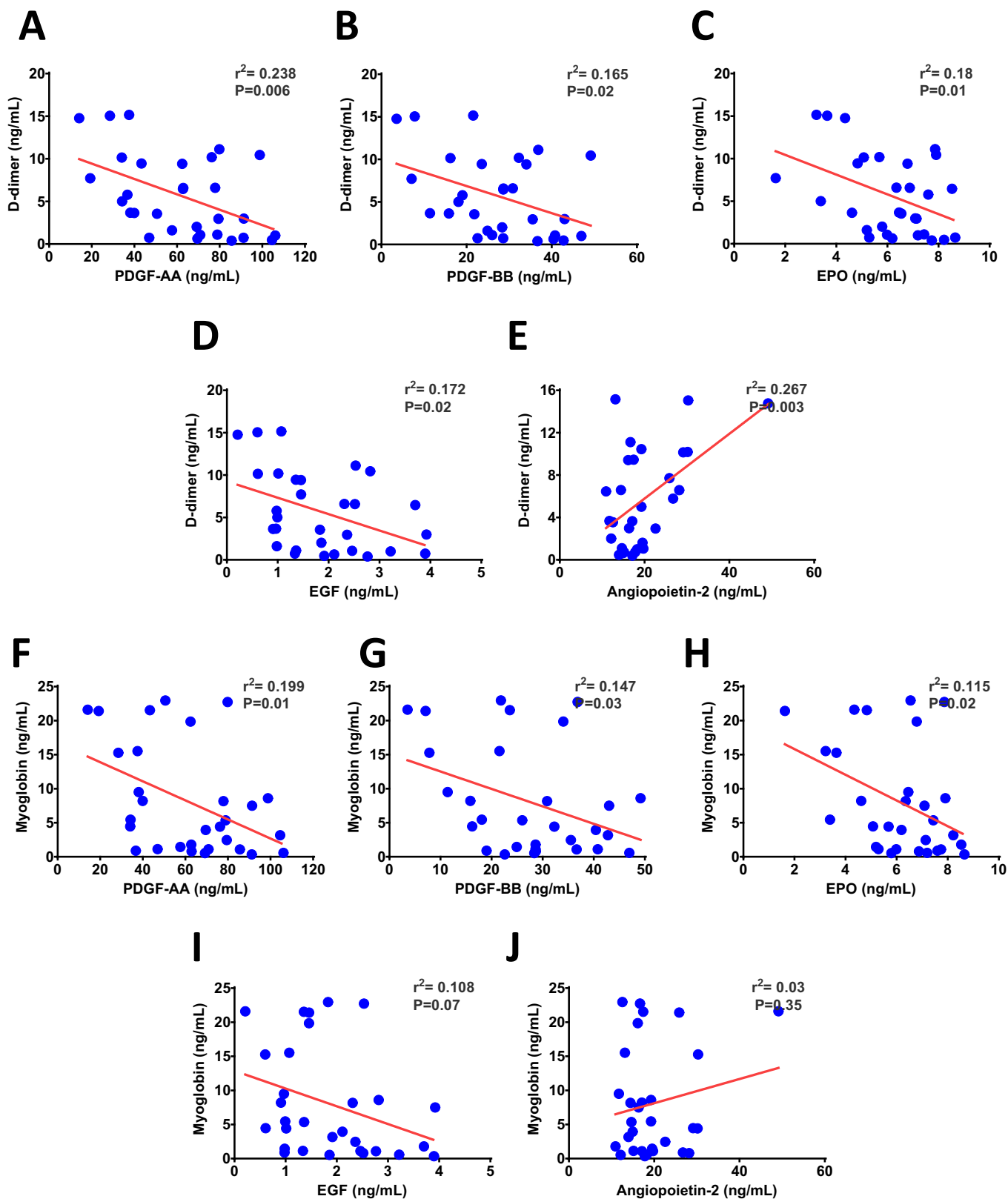
Fig. 6. Correlation of different growth factors with thromboembolism and tissue injury markers in COVID-19. The scatter plots from linear regression analysis show negative correlations of plasma (A) PDGF-AA, (B) PDGF-BB, (C) erythropoietin (EPO), (D) epidermal growth factor (EGF) and (E) a positive correlation of angiopoietin-2 with D-dimer. Scatter plots show negative correlations of plasma (F) PDGF-AA, (G) PDGF-BB, (H) erythropoietin (EPO), (I) epidermal growth factor (EGF) and (J) a positive correlation of angiopoietin-2 with myoglobin.

the high level of GFs in mild patients contributes to tissue repair and reduces the risk of developing severe disease.

VEGF has recently been implicated in the pathogenesis of severe COVID-19 [38]. Specifically, the dysregulated VEGF in COVID-19 patients is associated with an ARDS-related phenotype, characterized by pulmonary edema, thrombotic events, and translocation of the virus into the systemic circulation [39]. Though this retrospective study only recruited severe COVID-19 patients, these findings are consistent with our report of elevated VEGF in moderate and severe COVID-19. Since VEGF is reported to enhance inflammatory reaction, it may play an important role in the induction of cytokine storm in COVID-19 [39]. The positive correlations of plasma VEGF with p-selectin and sCD40L further reinforce the potential contributions of VEGF to endothelial dysfunction and thrombotic complications in COVID-19.

Severe COVID-19 patients demonstrate generalized tissue hypoxia with minimal or no dyspnea [40]. The reduced tissue oxygenation is implicated in the dysfunction of multiple organs in COVID-19. The elevated plasma EPO in our cohort may represent a compensatory response to a hypoxemia state by increasing the RBCs production. An activated renin-angiotensin system is a potent stimulus of EPO production in COVID-19 patients [41]. Nevertheless, a blunted EPO response is reported in advanced COVID-19 patients [41], which partly agrees with our findings in patients with severe SARS-CoV-2 infection. Consistent with the compensatory elevation of EPO in COVID-19, a negative correlation of EPO with D-dimer and myoglobin further indicates the protective roles of EPO in COVID-19. These findings necessitate the need for recombinant EPO as supportive therapy in COVID-19 patients [42].

Angpt-2 is an important factor for the angiogenesis signaling pathway and is generally activated in response to an inflammatory reaction in the endothelium, which is often seen in severe COVID-19 [11]. A study reported that angpt-2 could serve as a potent biomarker of endothelial dysfunction, particularly in COVID-19 patients admitted to ICU [12]. This study has reported a positive correlation of D-dimer and plasma E-selectin with angpt-2 in severe COVID-19. Our results are partly consistent with these findings as we observed a positive correlation of angpt-2 with D-dimer, however, inverse correlations with endothelial dysfunction markers plasma P-selectin and CD40L. Interestingly, similar to this study, we did not observe changes in angpt-2 levels between non-ICU *vs.* ICU patients. Another meta-analysis has reported the clinical values of angpt-2 in COVID-19 and suggests that elevated levels of circulating angpt-2 increase the risk of mortality in COVID-19 [43]. This finding is in line with our observation of positive correlation of angpt-2 with D-dimer in COVID-19. Though the direct involvement of angpt-2 in VTE is not clear, a recent study has shown the correlation of angpt-2 with pulmonary embolism severity, right ventricular dysfunction and admission to ICU [44]. These observations indicate that targeting angpt-2 may limit COVID-19 severity.

We found higher levels of both PDGF isoforms in COVID-19, which comply with our recent finding of elevated sCD40L in these patients [45]. These molecules are released by activated platelets and play a role in immune modulation and cell survival [46,47]. Interestingly, these factors work in conjunction in COVID-19 patients [37], indicating their collaborating roles in pathomechanisms of COVID-19. sCD40L is also a critical regulator of several proangiogenic factors, including FGF [48]. Specifically, sCD40L activates endothelial cells, vascular myocytes, and pericytes to produce FGF. Similar to PDGFs, FGF has a wide range of angiogenic, mitogenic, and pro-inflammatory activities. We found an elevation of bFGF in moderate, but not in severe COVID-19. A recent study found similar upregulation of plasma FGF and SCF in the early stages of SARS-CoV-2 infection [49]. However, in contrast to our findings, FGF was further upregulated in patients with fatal COVID-19 in the terminal stage. Similarly, in contrast to this study, we did not observe changes in the SCF levels during moderate and severe stages of COVID-19. We did not perform serial measurements of these markers in our patients to the eventual disease outcome. However, our data do not rule out a late-onset elevation of plasma FGF in patients with moderate COVID-19.

In compliance with the expressions of other GFs, TGF-α and EGF expressions were upregulated in the patients with moderate COVID-19 infection. Both factors contribute to tissues fibrosis and are implicated in lung injury during COVID-19 infection [50,51]. Thus, their overexpression in COVID-19 patients may contribute to disease severity. Accordingly, inhibition of TGF-α and EGF receptors may have therapeutic potential in COVID-19 infection [50,52].

Of assessed GFs, only HGF and G-CSF were elevated at the moderate stage of COVID-19 and remained significantly elevated in the severe stage of COVID-19. Consistent with our findings, a recent study has shown HGF as the best predictor of ICU admission and fatality [1]. HGF is considered a potent anti-inflammatory GF and plays a crucial role in lung tissue injury repair [26]. Hence elevation of HGF at moderate and severe stages of COVID-19 indicates that the body likely uses this GF as a counter mechanism to limit the pro-inflammatory effects of cytokine storms and pulmonary fibrosis induced by SARS-CoV-2. Other proinflammatory markers like CSFs play roles in immune cell mobilization upon viral infection [27]. The therapeutic potential of GM-CSF is being assessed in COVID-19 patients with acute respiratory hypoxic failure (NCT04326920) because GM-CSF mediates alveolar macrophage homeostasis and lung inflammation in COVID-19 [28]. Surprisingly, we did not observe any change in GM-CSF levels in either moderate or severe cohorts. Conversely, an elevated level of G-CSF was observed in these patients. Consistent with our findings, a recent report has shown an increased level of G-CSF, but not GM-CSF, in the COVID-19 patients at any stage of pathogenesis [49]. Hence, targeting G-CSF might be clinically more relevant than targeting GM-CSF in COVID-19.

Altogether, we report the induction of several GFs at the moderate stage of COVID-19. The robust correlations of these GFs with disease markers such as D-dimers and myoglobin indicate their roles in the COVID-19 pathophysiology. These factors may play critical roles in tissue injury and repair during COVID-19 pathogenesis and appear as attractive molecular targets for treating COVID-19 patients. The elevation of GFs at the moderate stage potentially helps patients better tolerate the clinical complications including tissue injury caused by severe SARS-CoV-2 infection. However, the lower levels of GFs in the severe cohort most likely limit the tissue repair and overall healing process. Supplementing the COVID-19 patients with some of these GFs including PDGF, EGF and EPO and limiting the levels of angpt-2 may be an attractive therapeutic approach to treat the severe complications of COVID-19.

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CRediT authorship contribution statement

AG and MNJ have performed experiments and collected data, MAS and MK helped with data interpretation and manuscript writing, RH helped with sample and clinical data collection and interpretation, and manuscript writing, RQ helped with data analysis and interpretation and, manuscript writing, and FA designed the study, acquired funding, supervised the project, performed data analysis and interpretation, wrote and revised the manuscript.

Declaration of competing interest

The authors declared no potential conflicts of interest concerning the research, authorship, and/or publication of this article.

Data availability

Data will be made available on request.

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