Increased plasma heparanase activity in COVID-19 patients

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Abstract

Background: Several reports suggest endothelial dysfunction and loss of endothelial barrier function in COVID-19. It is well established that the endothelial glycocalyx-degrading enzyme heparanase (HPSE) contributes to vascular leakage and inflammation. Low molecular weight heparins (LMWH) serve as an inhibitor of heparanase. We hypothesize that heparanase contributes to the pathogenesis of COVID-19, and that HPSE may be inhibited by the use of LMWH.

Methods: Heparanase activity and heparan sulfate levels were measured in plasma of healthy controls (n=10) and COVID-19 patients (n=48).

Findings: Heparanase activity and heparan sulfate levels were significantly elevated in plasma of COVID-19 patients. There was an association between heparanase activity and disease severity including the need for intensive care and mechanical ventilation, lactate dehydrogenase levels and creatinine levels. Use of prophylactic low molecular weight heparin in non-ICU patients was associated with a reduced HPSE activity.

Interpretation: Prophylactic doses of low molecular weight heparin reduces heparanase activity in COVID-19. In addition to HPSE inhibition, low molecular weight heparin contributes to anti-coagulation and may exert anti-inflammatory effects. Since there is no other clinically applied heparanase inhibitor currently available, treatment of COVID-19 patients with low molecular weight heparins should be explored.

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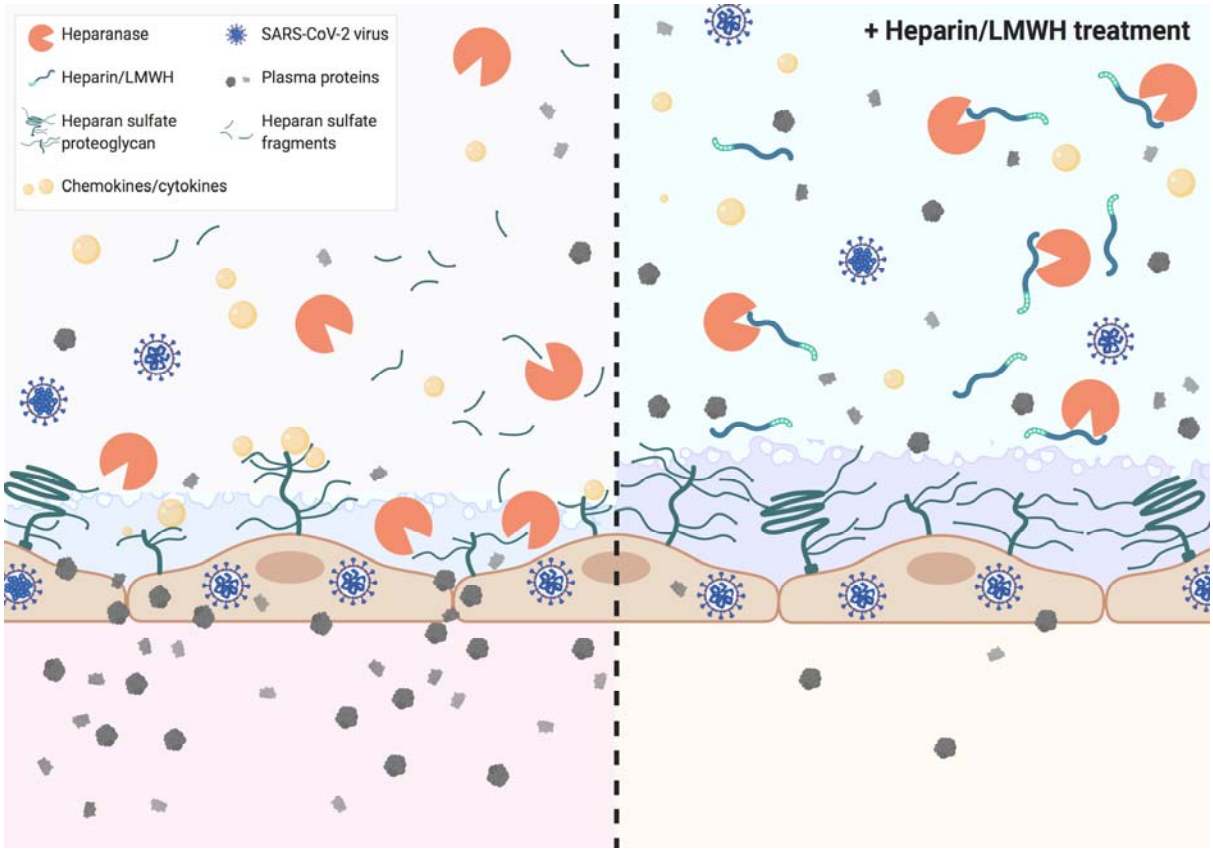
Keywords:

COVID-19, Heparanase, Glycocalyx, Inflammation, Vascular leakage, Heparin

Graphical Abstract



Research in context

Evidence before this study

Severe forms of COVID-19 present with acute respiratory disease syndrome (ARDS) and proteinuria, which are both associated with a leaky vasculature. An increased activity of the endothelial glycocalyx degrading enzyme heparanase (HPSE) compromises endothelial barrier function. HPSE activity-mediated loss of endothelial barrier function has been established for several inflammatory disease conditions including infection/sepsis, ARDS and proteinuric kidney diseases.

Added value of this study

This study demonstrates that HPSE activity is increased in COVID-19 patients, especially in those with severe disease like ICU patients. Additionally, use of prophylactic doses of low molecular weight heparin (LMWH) is associated with a reduced heparanase activity in COVID-19 patients.

Implications of all the available evidence

Increased HPSE activity in COVID-19 contributes to vascular leakage as manifested by ARDS and proteinuria. LMWH inhibits HPSE activity, contributes to anti-coagulation and exerts anti-inflammatory effects. Since LMWH is the only clinically approved HPSE inhibitor, treatment of COVID-19 patients with therapeutic doses of LMWH should be explored.

Introduction

The coronavirus disease-2019 (COVID-19) pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has impacted widely on global health.[1] Severe COVID-19 usually manifests as pneumonitis or acute respiratory distress syndrome (ARDS).[2,3] Moreover, severe COVID-19 can lead to multi-organ dysfunction. Case series showed that upon hospital admission 59% of COVID-19 patients had proteinuria[4], and 22% of the non-ventilated patients and 90% of the ventilated patients developed acute kidney injury (AKI).[5] Therefore, patients with more severe COVID-19 disease show a higher incidence of AKI, highlighting AKI as a negative prognostic factor for the survival of COVID-19 patients.[5,6]

Endothelial barrier function is crucial in the regulation of fluid and protein extravasation, particularly in the lungs[7,8] and in the kidneys.[9,10] An important role for endothelial cell dysfunction in the pathogenesis of the complications of COVID-19 has been proposed by several studies.[11,12] As pulmonary edema occurs when fluid leaks into alveoli, dysfunction of the endothelium is likely to contribute to pulmonary edema in COVID-19. Furthermore, it has been well established that proteinuria occurs when the endothelial barrier function in the glomerulus is compromised.[9,10,13]

Endothelial cells are covered with a thick layer of negatively charged glycosaminoglycans (GAGs), termed the glycocalyx. Heparan sulfate (HS) is the predominant sulfated GAG in the glycocalyx. Due to its negative charge and sulfation pattern, HS contributes to the charge-dependent barrier function and mediates the binding of chemokines and selectins/integrins on leukocytes to the endothelial cell surface under inflammatory conditions.[14] Degradation of HS by heparanase (HPSE), the only known mammalian HS-degrading enzyme, disrupts the endothelial glycocalyx. As such, shedding of the glycocalyx and subsequent loss of endothelial barrier function, as observed in ARDS and proteinuric kidney diseases, can be attributed to increased HPSE activity.[7,15-17] In addition to declining barrier function, HPSE generates a pro-inflammatory glycocalyx that promotes the binding of chemokines, cytokines and leukocytes to the endothelial cell surface. Inhibition of HPSE

and/or HPSE deficiency appears to be beneficial in experimental lung and kidney diseases.[7,15-18] Notably, heparins that are currently debated to be beneficial for COVID-19 patients[19] (*i.e*. low molecular weight heparin (LMWH)) have inhibitory effects on HPSE activity.[20,21]

Taken together, we hypothesize that increased HPSE activity is one of the driving forces in severe COVID-19 manifestation, including ARDS and proteinuria/AKI, and that HPSE may be inhibited by the use of LMWH in COVID-19.

Methods

Human Samples

This study was performed according to the latest version of the declaration of Helsinki and guidelines for good clinical practice. The local independent ethical committee approved the study protocol (CMO 2020-6344, CMO 2020-6359, CMO 2016-2923). All patients admitted to the Radboud university medical center (Radboudumc) with a PCR-proven SARS-CoV-2 infection was asked for informed consent for participation in this study. After obtaining informed consent, ethylenediaminetetraacetic acid (EDTA) blood was collected and centrifuged for 10 minutes at 2954 *xg* at room temperature (RT), plasma was collected and stored at -80 °C for later analysis. Demographic data, medical history and clinical laboratory measurements were collected from the medical file and processed in encoded form in electronic case report forms using Castor electronic data capture (Castor EDC, Amsterdam, the Netherlands).

HPSE activity assay

The activity of HPSE in EDTA plasma was determined by an in-house developed heparanase (HPSE) activity assay. Nunc maxisorp flat bottom 96 plates (Thermo scientific) were coated with 10 ug/ml HSBK (Sigma-aldrich) in optimized HS coating buffer, overnight in a humidified chamber at RT. Subsequently, plates were washed with 0.05% PBS-Tween 20 (Sigma-Aldrich) (PBST) and blocked for minimal 2 hours with 1% bacto-gelatin (Difco laboratories) in PBS at RT. Plates were washed with PBST, followed by a final washing step with PBS prior to 2 hours incubation at 37°C with 4 times diluted plasma sample in HPSE buffer. Samples were randomly distributed over plates. The HPSE buffer consisted of 50 mM citric acid-sodium citrate (Merck) buffer supplemented with 50 mM NaCl (Merck), 1 mM $CaCl_2$ (Sigma-Aldrich) and 1mM DTT (Sigma-Aldrich) at final pH 5.0. Next, plates were washed with PBST and incubated with primary mouse anti-rat IgM HS antibody JM403 (Amsbio, cat. no. #370730-S, RRID: AB_10890960, 1 µg/ml in PBST) for 1 hour at RT. Subsequently, plates were washed with PBST and incubated with secondary goat anti-

mouse IgM HRP antibody (Southern Biotech, cat. no. #1020-05, RRID: AB_2794201, dilution 1:10000 in PBST) for 1 hour at RT. Finally, plates were washed with PBST and 3,3',5,5'-tetramethylbenzidine (TMB) substrate (Invitrogen) was added and reaction was stopped by addition of 2M sulfuric acid, and absorbance was measured at 450 nm. The HPSE activity in plasma was related to a standard curve of recombinant human HPSE (R&D systems, Cat#7570-GH-005) in healthy control EDTA plasma.

For the *in vitro* HPSE inhibition experiment with dalteparin (Pfizer, Fragmin 12,500 IU/0.5 ml), the HPSE activity was determined using the HPSE activity assay described above. For inhibition 0-1 IU/ml dalteparin was used with a constant amount of 150 ng/ml recombinant human HPSE (R&D systems, Cat#7570-GH-005).

HS competition assay

HS in EDTA plasma samples was quantified by an in house developed HS competition assay.[22,23] Importantly, this assay is specific to HS, therefore the measurement is not affected by the presence of LMWH use. Plates were coated with HSBK and blocked with bacto-gelatin as outlined for the HPSE activity assay. Uncoated plates, blocked with bacto-gelatin, were washed with PBST. The plasma samples were 4 times diluted in PBST containing primary mouse anti-rat IgM HS antibody JM403 (Amsbio, cat. no. #370730-S, RRID: AB_10890960, 1.3 µg/ml) and incubated for 1 hour at RT. Samples were randomly distributed over plates. Subsequently, the samples were transferred from the uncoated plates to the HSBK-coated plates and incubated for 1 hour at RT. Plates were washed with PBST and incubated with secondary goat anti-mouse IgM HRP antibody (Southern Biotech, cat. no. #1020-05, RRID: AB_2794201, dilution 1:10,000) for 1 hour at RT. Plates were developed and measured as outlined for the HPSE activity assay. The amount of HS detected in plasma is expressed in arbitrary units since HS from bovine kidney was coated and used to prepare the standard curve.

Statistical analysis

Values are expressed as mean±SEM. D'Agostino & Pearson normality test is performed to test for normality of data. Significance was determined by Fisher's exact test to compare categorical variable, by Student's t-test or Mann Whitney test to compare two groups and by Kruskal-Wallis test followed by Dunn's test to compare more than two groups using GraphPad Prism V.8.4.2 (La Jolla, USA). P values less than 0.05 were considered as statistically significant.

Results

Demographics and baseline characteristics of COVID-19 patients

Plasma was collected from 48 PCR-confirmed COVID-19 patients admitted to the ICU (n= 14) or to designated COVID-19 clinical wards (n= 34). More men than women were included (Table 1). ICU patients had a significantly higher C-reactive protein concentration than non-ICU patients. The non-ICU patients were further aggregated in those receiving prophylactic LMWH (LMWH+) (in general dalteparin 5000 IU subcutaneously once daily) (n=17) and those receiving either alternative anticoagulation (n=8; vitamin K antagonist n=6, direct oral anticoagulant n=2) or patients for whom the sample collection was performed before initiation of any standard medical intervention (LMWH-) (n=9). Day of sampling was significantly different between ICU and non-ICU groups as well as between LMWH- and LMWH+ groups. The LMWH group had significantly higher median platelet count and D-dimer concentrations compared to LMWH- group, whereas the concentrations of the inflammatory markers CRP and serum ferritin were similar between LMWH+ and LMWH-.

Plasma HPSE activity is elevated in COVID-19 patients

Several (experimental) disease models have shown that increased HPSE activity can lead to endothelial barrier dysfunction, which may be involved in the development of ARDS and proteinuria/AKI.[7,24,25] Measurement of plasma HPSE activity levels in COVID-19 patients and healthy controls revealed that HPSE activity was significantly elevated in COVID-19 patients in comparison to healthy controls (Figure 1A). In line with the increased HPSE activity, HS plasma levels were also significantly elevated in COVID-19 patients compared to healthy controls (Figure 1B). Overall, these results suggest that SARS-CoV-2 infection causes an increase in the activity of HPSE in plasma and an increase in circulating HS.

HPSE activity associates with COVID-19 disease severity

Next, we investigated whether HPSE activity levels were associated with COVID-19 disease severity. More specifically, potential associations were assessed between HPSE activity

levels and the need for intensive care or for mechanical ventilation, lactate dehydrogenase (LDH) values as a measure of tissue damage and creatinine values as a measure of kidney function. Plasma HPSE activity was significantly higher in both non-ICU and ICU patients compared to healthy controls, and HPSE levels in ICU patients were significantly higher than in non-ICU patients (Figure 2A). HS levels in plasma were also significantly higher in both non-ICU and ICU patients compared to healthy controls (Figure 2B). HPSE activity was also significantly elevated in the plasma of patients in need of mechanical ventilation (Figure 2C). Importantly, plasma HPSE activity was increased in patients with elevated LDH values (>280 U/l) compared to patients with LDH values within the normal range (Figure 2D). Patients with elevated serum creatinine values (>110 µmol/ for men and >90 µmol/ for women) also displayed increased plasma HPSE activity (Figure 2E). These findings reveal that patients with severe COVID-19 disease, such as those admitted to the ICU department, have higher plasma HPSE activity levels than patients with moderate, such as non-ICU patients, COVID-19 disease.

Use of LMWH is associated with lower HPSE activity in plasma of COVID-19 patients

Recent studies show a high rate of thromboembolic complications in patients with severe COVID-19. Autopsy studies in COVID-19 patients have identified the presence of coagulation in the microvasculature, which might also contribute to organ failure.[26,27] Prophylactic treatment with LMWH is therefore recommended for patients hospitalized with COVID-19[28], whereas some experts recommend higher doses for critically ill patients. As LMWH possesses HPSE inhibiting properties, the effect of prophylactic LMWH on HPSE activity in plasma of COVID-19 patients was analyzed. Markedly, non-ICU patients who received LMWH displayed significantly lower HPSE activity compared to non-ICU patients without LMWH prophylaxis (Figure 3A). According to literature a single injection of 5000 units dalteparin would result in an estimated concentration of around 0.37 U/ml *in vivo.*[29] We found a dose dependent inhibition of recombinant HPSE at concentrations between 0.0025 and 0.05 U/ml and full inhibition starting from 0.25 U/ml dalteparin *in vitro* (Figure 3B). These

data suggest that the applied prophylactic LMWH dose is already effective in inhibition of HPSE activity within plasma of moderately diseased, but not severely ill, COVOID-19 patients.

Treatment with chloroquine does not reduce HPSE activity in plasma of COVID-19 patients

Regarding treatments under debate for COVID-19, chloroquine (CQ) has been suggested to be beneficial for the clinical outcome.[25,30,31] Notably, it has been described that CQ can prevent the cathepsin-L-mediated activation of pro-heparanase into active HPSE in the lysosome.[20] Pro-heparanase can also be activated extracellularly as well as in the lysosome[32], suggesting that CQ can only partially inhibit HPSE activation. In line with this, COVID-19 patients with CQ treatment did not display significantly different HPSE activity levels compared to patients without CQ treatment (see S figure 1).

Discussion

COVID-19 appears to be a disease that leads to endothelial dysfunction and disruption of the endothelial barrier, which may underly development of ARDS and proteinuria/AKI.[11,33] Here, we report increased HPSE activity and HS levels in plasma of COVID-19 patients, which were also associated with severity of the disease.

Several mechanisms are currently proposed to explain pulmonary edema and ARDS in COVID-19. One suggested mechanism focuses on the kallikrein/kinin system, which is involved in the local inflammation and vascular leakage in the lung.[34,35] It has been suggested that over-activation of the bradykinin pathway occurs due to consumption of angiotensin converting enzyme-2 (ACE2) during viral entry.[35] Interestingly, endothelial cell surface GAGs, such as HS, regulate activation of bradykinin pathways whereas degradation of HS by bacterial heparinases resumes proteolytic bradykinin generation.[36] These findings suggest that increased HPSE activity observed in COVID-19 patients could induce activation of the bradykinin pathway, thereby additionally contributing to vascular leakage and local inflammation. Another possible mechanism involved in endothelial dysfunction in COVID-19 patients is activation of the renin-angiotensin system.[37] Increased angiotensin II levels have been reported in COVID-19 patients.[38] Angiotensin II induces vasoconstriction, inflammation, fibrosis and proliferation, which in turn can cause thrombosis, ARDS and AKI. Importantly, we have previously shown that Angiotensin II is a potent inducer of HPSE expression.[39,40] Moreover, it is feasible that endothelin-1 (ET-1), one of the downstream mediators activated by angiotensin II[41,42] is also increased in COVID-19 and it is known that ET-1 can induce HPSE expression as well.[43]

Besides the role of HPSE in compromising the endothelial glycocalyx and in turn the endothelial barrier function, HPSE and circulating HS fragments play an important role in inflammation.[44] HPSE can activate macrophages inducing secretion of MCP-1, TNF-α and IL-1, independent of HS-degrading activity.[45] HS fragments released by HPSE activity also

induce a pro-inflammatory response by binding to TLR2 and TLR4.[45,46] Moreover, cleavage of HS by HPSE releases HS bound molecules, such as chemokines and cytokines, promoting inflammation.[47] Cells exposed to HPSE show an enhanced response to pro-inflammatory cytokines like IFN-γ and LPS.[17,48,49] Interestingly, cytokines such as IL-1β, IL-6, TNF-α and MCP-1 appear to be elevated in COVID-19 patients[50-52] and can induce HPSE expression[15], suggesting the formation of a HPSE-mediated positive feed forward loop for inflammation in COVID-19. Notably, HPSE appears to have a direct effect in shaping the cytokine milieu, since HPSE deficiency reduces expression of a wide range of cytokines including TNF-α, IL-6, IFN-γ in experimental models.[15] Therefore, inhibition of HPSE activity may also partially dampen the inflammatory response observed in COVID-19 patients.

Heparin and LMWH are able to inhibit HPSE due to their structural similarity with HS.[21] Potential beneficial effects of prophylactic as well as therapeutic doses of LMWH in COVID-19 patients have been described.[53-56] Our findings suggest that prophylactic doses of LMWH were able to inhibit HPSE activity in moderately diseased, non-ICU patients, emphasizing the importance of non-anticoagulant properties of LMWH against COVID-19. In ICU patients, however, HPSE activity appears to remain elevated despite general use of prophylactic LMWH in the ICU. Considering that HPSE activity was significantly higher in ICU patients compared to non-ICU patients, therapeutic LMWH dose instead of prophylactic dose might be needed to reduce HPSE activity of COVID-19 patients in the ICU. In addition to inhibition of HPSE, LMWH have other non-anticoagulant functions that may be beneficial for patients with COVID-19, such as neutralization of chemokines/cytokines, interference with leukocyte trafficking, neutralization of extracellular cytotoxic histones, neutralization of high molecular weight kinogen, and reduction of viral entry.[36,57-59]

In summary, this cross-sectional study shows that HPSE activity and HS levels are significantly elevated in plasma of COVID-19 patients, which is associated with the severity of COVID-19. Targeting of HPSE activity could be beneficial for the clinical outcome of COVID-19 patients, since it is well established that increased HPSE activity compromises

304 the endothelial glycocalyx and endothelial barrier function and contributes to the
305 establishment of a pro-inflammatory cytokine milieu. Considering the fact that no specific
306 clinically approved heparanase inhibitors are currently available, prospective studies
307 evaluating the clinical outcome of COVID-19 patients treated with therapeutic doses of
308 LMWH are urgently needed.

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Declaration of Competing Interests

The authors have declared that no conflict of interest exists.

Author contributions

BB, CY, QdM and JvdV designed the experiments, analyzed data and wrote the manuscript. BB, CY and MdG performed the experiments. AdN, IG, NAFJ, LABJ, MGN and FLvdV were co-investigators on CMO 2020-6344, which provided COVID-19 patient samples. BB, CY, IJ, NR, RD and JvdV were co-investigators on CMO 2020-6359, which provided COVID-19 patient samples. MK and PP were co-investigators on CMO 2016-2923, which facilitated COVID-19 ICU patient sampling. MLMH created the graphical abstract and wrote the manuscript. TN and LH helped with analysis of the clinical data. JvdV has full access to all the data in the study and takes responsibility for the integrity of the data. All authors critically reviewed and edited the manuscript. BB and CY share first authorship and AdN and IG are co-second authors, listed in alphabetical order.

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Figures

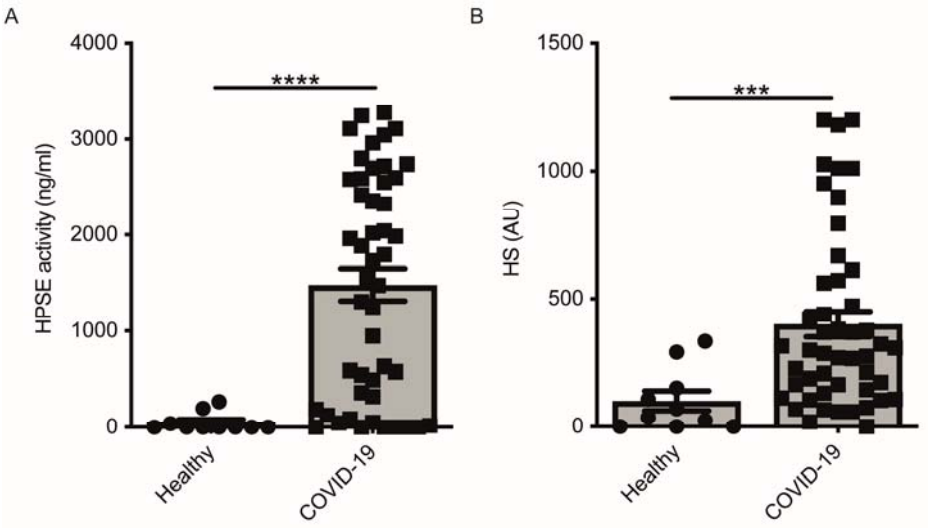


Figure 1. COVID-19 patients display increased HPSE activity and elevated levels of heparan sulfate in plasma. A. HPSE activity was increased in plasma of COVID-19 patients compared to healthy controls. HPSE activity was measured using an in-house developed ELISA with a specific anti-HS antibody. **B.** HS levels were increased in plasma of COVID-19 patients compared to healthy controls. HS levels were measured by an in-house developed competition ELISA using a specific anti-HS antibody. Data were presented as mean±SEM and tested for normal distribution with D'Agostino & Pearson omnibus normality test and statistical differences were calculated using Mann Whitney test (n=10 healthy; n=48 COVID-19, *** $p<0.001$, **** $p<0.0001$). HPSE, heparanase; HS, heparan sulfate; Healthy, healthy controls; COVID-19, coronavirus disease-19 patients; AU, arbitrary units.

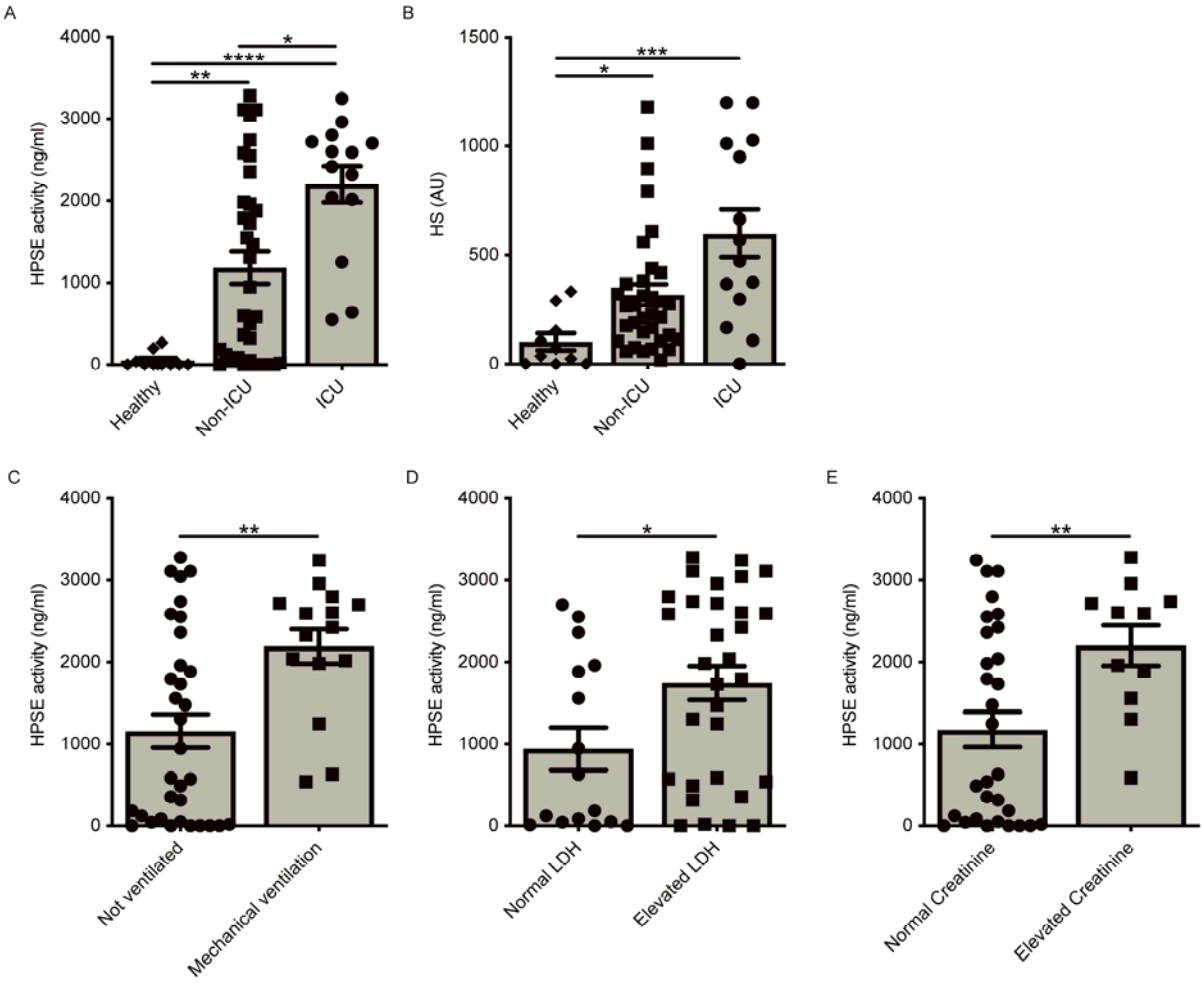


Figure 2. Increased plasma HPSE activity associates with COVID-19 disease severity. **A.** Plasma HPSE activity was significantly higher in ICU and non-ICU patients compared to healthy controls, and higher in ICU patients compared to non-ICU patients. (n=10 healthy; n=34 non-ICU; n=14 ICU) **B.** HS levels were significantly increased in plasma of ICU and non-ICU patients compared to healthy controls (n=10 healthy; n=34 non-ICU; n=14 ICU) **C.** HPSE activity was significantly higher in plasma of patients with mechanical ventilation compared to patients without mechanical ventilation. (n=33 not ventilated; n=15 mechanical ventilation) **D.** HPSE activity was significantly higher in plasma of patients with elevated LDH (>280 U/l) values compared to patients with normal LDH levels. (n=15 normal LDH; n=26 elevated LDH). **E.** HPSE activity was significantly higher in plasma of patients with elevated creatinine (>110 µmol/ for men and >90 µmol/ for women) values compared to patients with normal creatinine values. (n=30 normal creatinine; n=11 elevated creatinine; patients with

history of renal disease were excluded from this analysis). HPSE activity was measured using an in-house developed ELISA with a specific anti-HS antibody. Data were presented as mean±SEM and tested for normal distribution with D'Agostino & Pearson omnibus normality test and statistical differences were calculated using Kruskal Wallis test followed by Dunn's multiple comparison test, unpaired one-tailed Student's t-test or unpaired one-tailed Mann Whitney test (* p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001). HPSE, heparanase; HS, heparan sulfate; LDH, lactate dehydrogenase; Healthy, healthy controls; non-ICU: COVID-19 patients in normal hospital ward; ICU, COVID-19 patients in ICU; AU, arbitrary units.

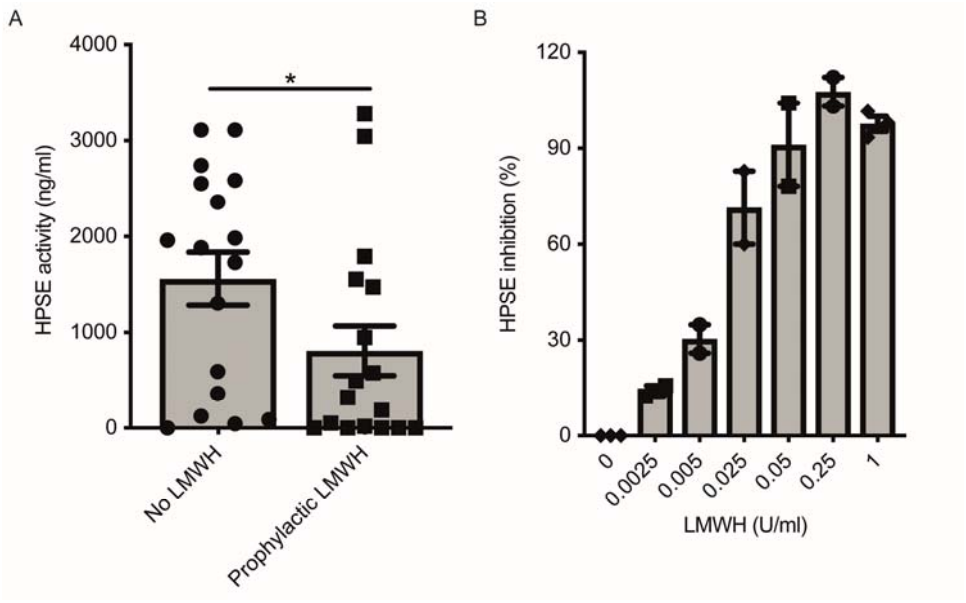
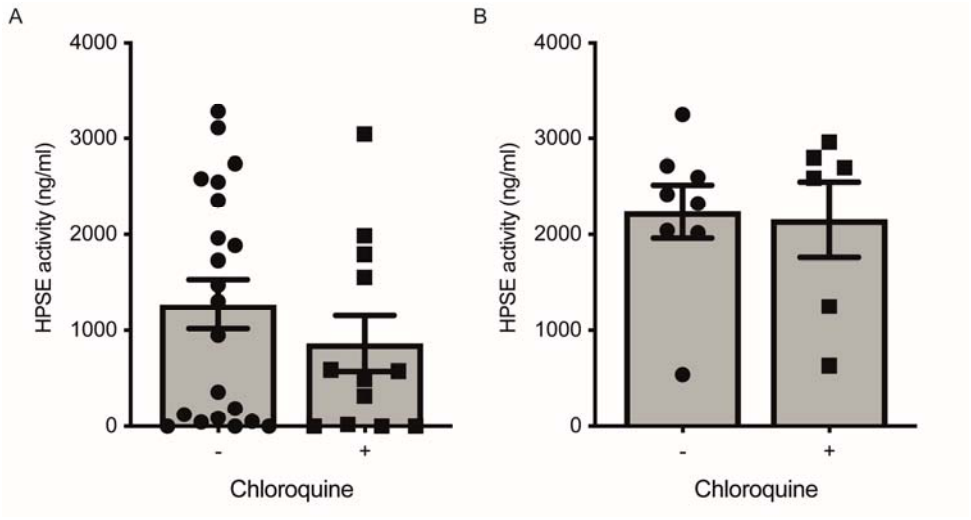


Figure 3. LMWH reduces plasma HPSE activity in moderately diseased COVID-19 patients. A. LMWH reduces HPSE activity in plasma of non-ICU patients with COVID-19, which was measured using in house developed HPSE activity assay. (n=17 for both groups, p<0.05). Data were presented as mean±SEM and tested for normal distribution with D'Agostino & Pearson omnibus normality test and statistical difference was calculated using unpaired one-tailed Mann Whitney test. **B.** LMWH inhibits recombinant HPSE activity *in vitro* in a dose dependent manner. HPSE activity was measured using an in-house developed ELISA with a specific anti-HS antibody. (n=3)



Supplementary Figure 1. HPSE activity was similar in the plasma of chloroquine treated COVID-19 patients. A. Plasma from non-ICU patients with or without CQ treatment had similar HPSE activity levels. (n=21 CQ-; n=12 CQ+). Data was tested for normal distribution with D'Agostino & Pearson omnibus normality test and statistical difference was calculated using unpaired one-tailed Mann Whitney test. **B.** Plasma from ICU patients with or without CQ treatment had similar HPSE activity levels. (n=8 CQ-; n=6 CQ+).

543 **Table**

544 **Table 1. Demographics and baseline characteristic of COVID-19 patients***

Characteristics	All patients	ICU	Non-ICU			p_1	p_2
			LMWH-	LMWH+	Total		
	n=48	n=14	n=17	n=17	n=34		
Sex, male, n (%)	37 (77)	11 (78.5)	13 (76.5)	13 (76.5)	26 (76.5)	1.0000	1.0000
Age, median (IQR), yr	67.5 (57.3-74.75)	62.5 (53.0-69.5)	69 (62.5-76.5)	69 (53.5-77.0)	69 (58.8-77.0)	0.6792	0.2428
Hospital stay, median (IQR), days	9 (5-16)	26 (16-…)[&]	5 (5-8)	9 (6-12)	7 (5-9)	0.0443	0.0001
Day of sampling (IQR)	2 (1-3)	3 (1-6)	1 (1-2)	2 (2-4)	2 (1-3)	0.0089	0.1378
Coexisting disorder, n (%)							
Heart disease	8 (16.7)	3 (21.4)	3 (17.6)	2 (11.8)	5 (14.7)	1.0000	0.6757
Lung disease	16 (33.3)	4 (28.6)	2 (11.8)	10 (58.8)	12 (35.3)	0.0104	0.7460
Diabetes	6 (12.5)	2 (14.3)	3 (17.6)	1 (5.9)	4 (11.8)	0.6012	1.0000
Hypertension	18 (37.5)	8 (72.7)	5 (29.4)	5 (29.4)	10 (29.4)	1.0000	0.1032
Malignancy	9 (18.8)	1 (7.1)	4 (23.5)	4 (23.5)	8 (23.5)	1.0000	0.2501
Immunocompromised	7 (14.6)	0 (0)	2 (11.8)	5 (29.4)	7 (20.6)	0.3983	0.0898
Renal disease	4 (8.3)	2 (14.3)	0 (0)	2 (11.8)	2 (5.9)	0.4848	0.5659
COVID-19 treatment, n (%)							
(hydroxy)chloroquine	19 (39.6)	6 (42.9)	4 (42.9)	9 (52.9)	13 (38.2)	0.1571	1.0000
Remdesivir	1 (2.1)	1 (7.1)	0 (0.0)	0 (0.0)	0 (0.0)	NA	0.1153
CT severity score*, median (IQR)	NA	NA	9.0 (7.3-13.0)[+]	13.0 (9.3-16.5)[+]	11.5 (8.0-15.0)[++]	0.0743	NA
Laboratory, median (IQR)							
WBC, $x10^9$/l*	6.8 (5.2-9.1)[+]	6.7 (5.9-7.9)	6.0 (4.0-8.9)[+]	7.4 (4.8-9.8)	6.8 (4.5-9.8)[+]	0.5077	0.9722
Platelets, $x10^9$/l*	213 (158-279)[+]	220 (176-281)	164 (137-219)[+]	250 (189-330)	201 (149-286)[+]	0.0306	0.9706
C-reactive protein, mg/l*	93 (55-170)[++]	171 (120-258)[+]	81 (54-122)[+]	78 (41-136)[++]	79 (49-122)	0.6786	0.0007
Ferritin, µg/l	973 (567-1658)	1363 (872-1917)	788 (259-1018)	950 (571-1854)	871 (472-1369)	0.2214	0.1149
D-dimer, ng/ml*	1090 (315-2075)[+++++++]	1870 (313-5275)[+]	369 (199-883)[+]	1335 (1130-2775)[+++++]	940 (311-1510)[++++++]	0.0008	0.1199
Lactate dehydrogenase, U/l*	309 (258-407)[+]	306 (273-399)	302 (238-440)[+]	317 (259-362)	314 (257-414)[+]	0.8430	0.9907
Creatinine, µmol/l*[#]	92.0 (71.0-115.5)[++++]	101.0 (73.0-145.5)[++]	94.0 (78.0-115.8)	85.0 (62.3-97.8)[++]	89.5 (70.0-110.8)[++]	0.2500	0.2814

***** Data are presented as median (IQR) or percentage (%). P values comparing LMWH- with LMWH+ patients (p_1) or ICU patients with non-ICU patients (p_2) are calculated with Fisher's exact test, unpaired two-tailed Student's t test or unpaired two-tailed Mann Whitney test. ICU, intensive care unit; COVID-19, coronavirus disease-2019; IQR, interquartile range; LMWH-, patients without prophylactic LMWH; LMWH+, patients with prophylactic LMWH. Measurements with missing values are indicated with * and the number of $^+$ signs indicates the number of missing patients per characteristic and group. & 75% quartile is unknown due to prolonged hospitalization of some patients. # 4 patients with history of renal disease were excluded.