Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Proline-specific peptidase activities (DPP4, PRCP, FAP and PREP) in plasma of hospitalized COVID-19 patients

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ABSTRACT

Background: COVID-19 patients experience several features of dysregulated immune system observed in sepsis. We previously showed a dysregulation of several proline-selective peptidases such as dipeptidyl peptidase 4 (DPP4), fibroblast activation protein alpha (FAP), prolyl oligopeptidase (PREP) and prolylcarboxypeptidase (PRCP) in sepsis. In this study, we investigated whether these peptidases are similarly dysregulated in hospitalized COVID-19 patients.

Methods: Fifty-six hospitalized COVID-19 patients and 32 healthy controls were included. Enzymatic activities of DPP4, FAP, PREP and PRCP were measured in samples collected shortly after hospital admission and in longitudinal follow-up samples.

Results: Compared to healthy controls, both DPP4 and FAP activities were significantly lower in COVID-19 patients at hospital admission and FAP activity further decreased significantly in the first week of hospitalization. While PRCP activity remained unchanged, PREP activity was significantly increased in COVID-19 patients at hospitalization and further increased during hospital stay and stayed elevated until the day of discharge.

Conclusion: The changes in activities of proline-selective peptidases in plasma are very similar in COVID-19 and septic shock patients. The pronounced decrease in FAP activity deserves further investigation, both from a pathophysiological viewpoint and as its utility as a part of a biomarker panel.

1. Introduction

At the end of 2019, the first outbreak of infections with the severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) emerged in Wuhan. The disease caused by this virus was named 'coronavirus disease 2019' (COVID-19) and can elicit a protracted pneumonitis but also kidney, cardiovascular and neurological complications and thromboembolic phenomena of unclear pathogenesis [1]. By end of December 2021, more than 5 million people have died due to complications of COVID-19 [2].

In this study, we investigated the activity of proline-specific peptidases in plasma of COVID-19 hospitalized patients. The peptidases studied are dipeptidyl peptidase 4 (DPP4), fibroblast activation protein alpha (FAP), prolyl oligopeptidase (PREP) and prolylcarboxpeptidase (PRCP). These peptidases are present in a soluble form in plasma and are often linked to functions in the immune system and to inflammatory diseases [3,4]. These four enzymes preferentially cleave peptides after proline residues. The exact position of the proline in the peptide and the preferred *in vivo* substrates differ between the enzymes [3].

DPP4 is a ubiquitously expressed glycoprotein that exists either as a

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membrane-localized enzymatically active protein on endothelial, epithelial and immune cells or as a soluble form present in plasma and body fluids. In plasma, DPP4 is mostly known for its cleavage of the glucagon-like peptide (GLP)-1 and -2 and glucose dependent insulinotropic peptide (GIP), which makes it a validated therapeutic target for the treatment of type 2 diabetes [5–8]. In addition, it is known that DPP4 is able to cleave a number of chemokines, mitogenic growth factors and neuropeptides [9–16]. Because DPP4 has been characterized as the receptor for the MERS (Middle East respiratory syndrome) coronavirus [17], it was also suggested as a candidate receptor for SARS-CoV-2 [8]. However, using surface plasmon resonance and ELISA, Xi et al. did not find any specific binding between DPP4 and the SARS-CoV-2 spike protein and it is now clear that DPP4 is not a receptor for SARS-CoV-2 [18].

Like DPP4, the closely related FAP is expressed as a cell membrane bound glycoprotein that also appears as a soluble catalytically active enzyme in plasma [19]. However, in contrast to DPP4, FAP is expressed at only low levels if at all in normal adult tissue. It is involved in many cellular processes including tissue remodeling, cardiac and liver fibrosis, wound healing, inflammation and tumor growth where it plays a role in extracellular matrix degradation [20–22].

PREP is a ubiquitously expressed oligopeptidase localized in the cytoplasm of many cell types. For a long time, PREP was mainly regarded as a peptidase involved in neuropeptide metabolism. However, the last two decades experimental work indicated that PREP's physiological role depends on its location: inside or outside the cell, the type of cell or tissue and the physiological or pathological conditions[23]. Now, it is known that PREP is not only involved in the processing of several neuropeptides but also in the generation or breakdown of several peripheral bioactive peptides [24–26].

The fourth proline-selective enzyme studied here, PRCP, is a lysosomal enzyme present in many tissues and cell types, including several immune cells. In plasma and other body fluids, it is able to modulate the activity of bio-active peptides [27,28]. PRCP is mostly studied for its role in metabolic disorders like obesity and diabetes because it is involved in the cleavage of the neuropeptide α-MSH 1-13 and the adipokine (pyr)-apelin-13 [29,30]. Interestingly, both PREP and PRCP participate in the angiotensin cleavage pathway, sharing substrate specificity with Angiotensin converting enzyme 2 (ACE2), the entrance receptor for SARS-CoV-2 [31]. PREP and PRCP along with ACE2 can generate Angiotensin 1-7 (Ang(1-7)) from Angiotensin II (Ang II). Ang(1-7), an active peptide from the renin-angiotensin system (RAS), acts as a vasodilator that also protects the lungs from acute lung injury [26].

Recently, we showed that proline-specific peptidase activities (DPP4, FAP, PREP and PRCP) are dysregulated in plasma of patients experiencing septic shock [4]. A significant discrimination between septic shock patients and an intensive care unit (ICU) control group could be made, which makes these proline-specific peptidases potential diagnostic and/or prognostic biomarkers in sepsis and septic shock. This was not surprising because two of these enzymes, PREP and PRCP, are involved in blood pressure regulation [26,31] and inflammatory pathways [3], which are both disturbed in sepsis. Septic shock is defined as a subset of sepsis in which underlying circulatory and cellular metabolism abnormalities are profound enough to substantially increase mortality. Sepsis involves both pro-and anti-inflammatory responses in combination with alterations in other immunologic and non-immunologic pathways [32]. Similar to sepsis, patients with critical COVID-19 also experience multiple organ dysfunction (e.g., acute respiratory distress syndrome, myocardial injury or acute renal injury). Severe COVID-19 is accompanied by an excessive activation of the immune system, resulting in the production of many inflammatory factors, also referred to as "a cytokine storm". This over-activation of the immune system is also often seen in patients experiencing septic shock [32]. Therefore, it is interesting to study the same set of proline-specific peptidases (DPP4, FAP, PREP and PRCP) in the context of COVID-19.

So far, plasma activities of DPP4, FAP, PREP and PRCP have not been characterized in COVID-19 patients with longitudinal follow-up measurements. In a well-defined group of hospitalized COVID-19 patients and non-COVID-19 controls, we studied whether the specific plasma activities of DPP4, FAP, PREP and PRCP are dysregulated in COVID-19 patients at the time of hospital admission or during their hospital stay. We further explored whether these peptidases hold promise as biomarkers.

2. Methods

2.1. Materials

The substrates Z-Gly-Pro-AMC, Gly-Pro-pNA and Z-Pro-Phe were obtained from Bachem Feinchemikalien (Bübendorf, Switzerland). The PREP inhibitor KYP-2047 and FAP inhibitor UAMC-1110 were synthesized in house, as published [33,34].

2.2. Study design

This study is a post-hoc analysis within the COVID-19 Immune Repertoire Sequencing (IMSEQ) study, a prospective cohort study conducted at the Antwerp University Hospital (UZA). The study design is described separately (clinical trials.gov NCT04368143). The research complied with all the relevant national regulations, institutional policies and in accordance with the tenets of the Helsinki Declaration, and was approved by the University Hospital Antwerp/University of Antwerp ethics committee (Belgian registration number: 20/12/135) and the ITM IRB. All individuals gave their written informed consent.

Disease severity was assessed using the WHO COVID-19 disease severity categorization [35]. In short, the classifications are as follows: 1) mild: symptomatic patients without evidence of viral pneumonia or hypoxia, 2) moderate: patients with clinical evidence of pneumonia with respiratory rate not exceeding 30 breaths per minute and oxygen saturation not below 90% on room air, 3) severe: patients with clinical evidence of pneumonia with respiratory rate exceeding 30 breaths per minute and/or oxygen saturation below 90% on room air and/or signs of severe respiratory distress and 4) critical: patients with acute respiratory distress syndrome (ARDS), sepsis or septic shock and/or acute thrombosis.

2.3. Sample collection

Heparin plasma samples were collected from 56 hospitalized patients with laboratory-confirmed (PCR test) COVID-19 shortly after hospital admission (=baseline, 1 to 5 days after hospital admission) and thereafter at irregular time points until discharge. Additionally, plasma was taken from a control group of 32 healthy volunteers, recruited at the Institute of Tropical Medicine Antwerp (ITM). In the healthy volunteer group, two blood collections were performed approximately four weeks apart (day 0 and around day 28). At moment of sampling, none of the participants (both COVID and control group) were vaccinated against SARS-CoV-2 (sampling period was between 2nd of April 2020 and 6th of January 2021).

2.4. DPP4 measurement

DPP4 activity was measured colorimetrically using the substrate Gly-Pro-*p*NA as described earlier [36]. The release of *p*NA from the substrate was measured kinetically at 405 nm during 10 min at 37 °C, pH 8.3. Use of this method results in the selective measurement of DPP4 as demonstrated previously [36].

2.5. FAP and PREP measurements

FAP and PREP activity was measured fluorometrically using Z-Gly-Pro-AMC in a combined FAP/PREP assay as described earlier [37]. The

release of AMC from the substrate was measured kinetically during 30 min at 37 °C, pH 8. Because Z-Gly-Pro-AMC is cleaved by both FAP and PREP, plasma was pre-incubated with a specific PREP inhibitor (KYP-2047) or a FAP inhibitor (UAMC110) before the addition of substrate to measure FAP and PREP, respectively.

2.6. PRCP measurement

PRCP activity was determined by measuring the hydrolysis of Z-Pro-Phe by use of a reversed-phase high-performance liquid chromatography technique, as described earlier [38]. Samples were incubated for 2 h with Z-Pro-Phe at pH 5 at 37 °C, before stop solution (10% perchloric acid and 20% acetonitrile solution in purified water (v/v)) was added. The enzymatically formed Z-Pro was tracked by its UV absorbance at 210 nm after separation on a Shimadzu HPLC apparatus. Quantification was performed by peak height measurements.

2.7. Statistics

Statistical analysis was performed using SPSS software version 27 (IBM, New York, United States). GraphPad Prism version 9 was used for data plotting. DPP4, FAP, PREP and PRCP activities were not normally distributed in the COVID group as assessed by Shapiro-Wilk's test ($p < 0.05$). Therefore, non-parametric tests were used to assess differences between groups or timepoints. The specific statistical tests used in this study are mentioned in the legends underneath the figures. P values of < 0.05 were considered as statistically significant. * = $p < 0.05$, ** = $p < 0.005$, *** = $p < 0.0005$.

3. Results

3.1. Patient characteristics

The patient sample subset analyzed in this study resulted from patient enrollment at the Antwerp University Hospital between April 2020 and February 2021. During this enrollment, a total of 56 patients with laboratory confirmed COVID-19 were recruited. On average, in this study, patients with SARS-CoV-2 infection were hospitalized for 19 ± 12 days (range 3–61 days). Thirty-two clinically healthy subjects were included as well. Of these 32 individuals, 14 were previously exposed to SARS-CoV-2 (i.e., the exposed group, evidenced by a positive COVID-19 PCR test, at least 2 months before inclusion or by a positive serological test result), while the other 18 did not have indications of SARS-CoV-2 infection history (based on absence of known high-risk contact, absence of matching clinical symptoms, or negative PCR test in the event of a high-risk contact or matching clinical symptoms). Mean participant age and sex are summarized in Table 1.

3.2. Case-control analysis

3.2.1. Control versus COVID-19

First, we analyzed the control group for differences in peptidase activities between the SARS-CoV-2 exposed and non-exposed group. For none of the four peptidases (DPP4, FAP, PREP and PRCP) a significant difference was measured between the exposed and non-exposed group (Supplementary Fig. S1). Within our healthy control cohort, historical exposure to SARS-CoV-2 seems to have no effect on the activity levels of the proline-specific peptidases studied. Both healthy control subgroups (exposed and non-exposed) were thus merged into a single control group in our case-control analysis.

Table 1
Summary Sex and Age.

	Control (n = 32)	COVID-19 (n = 56)
Sex		
Male	34% (n = 11)	64% (n = 36)
Female	66% (n = 21)	36% (n = 20)
Mean Age	44	59

Because there is an unbalanced composition of control and COVID-19 group regarding sex and age (see Table 1), we analyzed if there are statistical differences in peptidase activities between men and women (Supplementary Fig. S2) and whether associations exist with age (Supplementary Fig. S3). The activities for the four peptidases do not differ in male or female patients, however, a weak negative association between age and FAP activity was observed (Spearman's rank order correlation test with $r_s = -0.233$, $p = 0.029$). FAP activity decreases with increasing age, so this must be considered in the case-control analysis. No associations with age were found for the other three peptidases.

The results of the case-control analysis are shown in Fig. 1. A Mann-Whitney *U* test was utilized to determine differences in selected enzyme activity between COVID-19 patients (shortly after admission) and healthy volunteers. Both DPP4 and FAP activity are significantly decreased in COVID-19 patients ($p = 0.013$ and $p < 0.0001$ respectively). PREP activity on the other hand is increased ($p = 0.002$) and PRCP activity remains unchanged ($p = 0.917$). Because there is a weak negative relationship between age and FAP activity and the mean age is higher in the COVID-19 group, we carried out a one-way ANCOVA test to adjust for age. After adjustment for age, there was still a statistically significant difference in FAP activity between COVID-19 patients and healthy volunteers ($p < 0.0001$).

3.2.2. Effect of severity of COVID-19 on peptidase activities

The severity of illness was classified following the WHO guidelines as asymptomatic/mild, moderate, severe, or critically ill. The classification was done on the patient's worst disease presentation during the entire hospital stay. No statistically significant differences in median DPP4, FAP, PREP or PRCP activity were measured between the severity classifications (Supplementary Fig. S4).

To evaluate if DPP4, FAP, PREP or PRCP activities can be used as diagnostic biomarkers, we computed receiver operating characteristic (ROC) curves (Supplementary Fig. S5) based on measurements on day of patient inclusion (shortly after hospital admission). The areas under the curve (AUC) values were 0.339, 0.201, 0.711 and 0.500 for DPP4, FAP, PREP and PRCP, respectively. These low values indicate that the individual peptidase activities cannot discriminate between healthy and COVID-19 within the present study population.

3.3. Longitudinal study

Subsequently, we analyzed the peptidase activities in function of time. This was done both for the control group (day 0 and around day 28) and for COVID-19 patients longitudinally sampled during their hospital stay, using the Wilcoxon signed-rank test. In the control group, there were no statistically significant differences measured in median DPP4, FAP, PREP or PRCP activity between day 0 and day 28 (Supplementary Fig. S6). For the COVID-19 patients, we grouped 12 patients who were all characterized by hospitalization for more than one week and having at least three serial timepoints available: at inclusion (shortly after the day of admission), around week 1 after inclusion, and the day of discharge (which varied for each patient, ranging from 15 to 46 days) (Fig. 2).

For DPP4 and PRCP no significant differences in median activity were observed between the three timepoints. For FAP activity a significant decrease in activity between day of inclusion and week 1 ($p = 0.012$) and a significant increase between week 1 and day of discharge ($p = 0.021$) was measured. FAP activity tends thus to decrease during disease course and normalizes when the patient recovers. In line with the higher PREP activity in patients versus controls, PREP activity significantly increased between day of inclusion and week 1 ($p = 0.003$), but in contrast with FAP, the activity of PREP stayed elevated at day of discharge ($p = 0.034$).

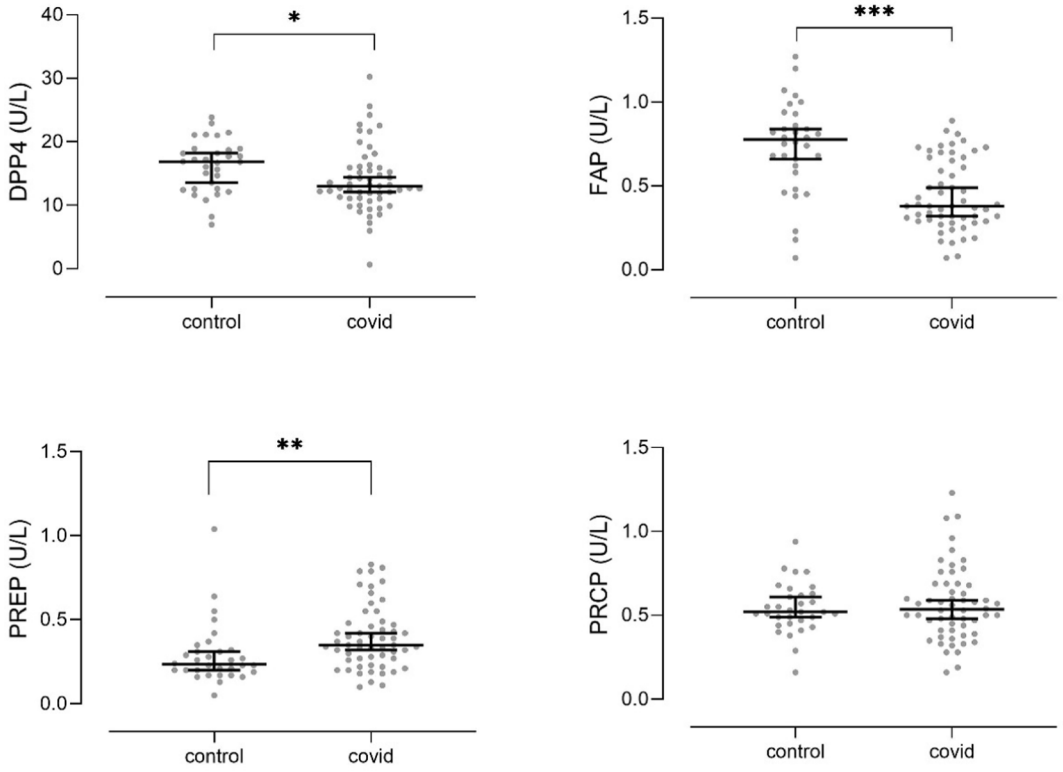

Fig. 1. Peptidase activities in control versus COVID-19 patients. Soluble DPP4, FAP, PREP and PRCP activities (median with interquartile range) in plasma of healthy volunteers (n = 32) and patients hospitalized with COVID-19 (n = 56). Analysis was performed on baseline measurements (shortly after hospital admission for the COVID-19 group and day 0 for the control group). The statistical difference between the two groups was analyzed using a Mann-Whitney *U* test. * = $p < 0.05$, ** = $p < 0.005$, *** = $p < 0.0005$.

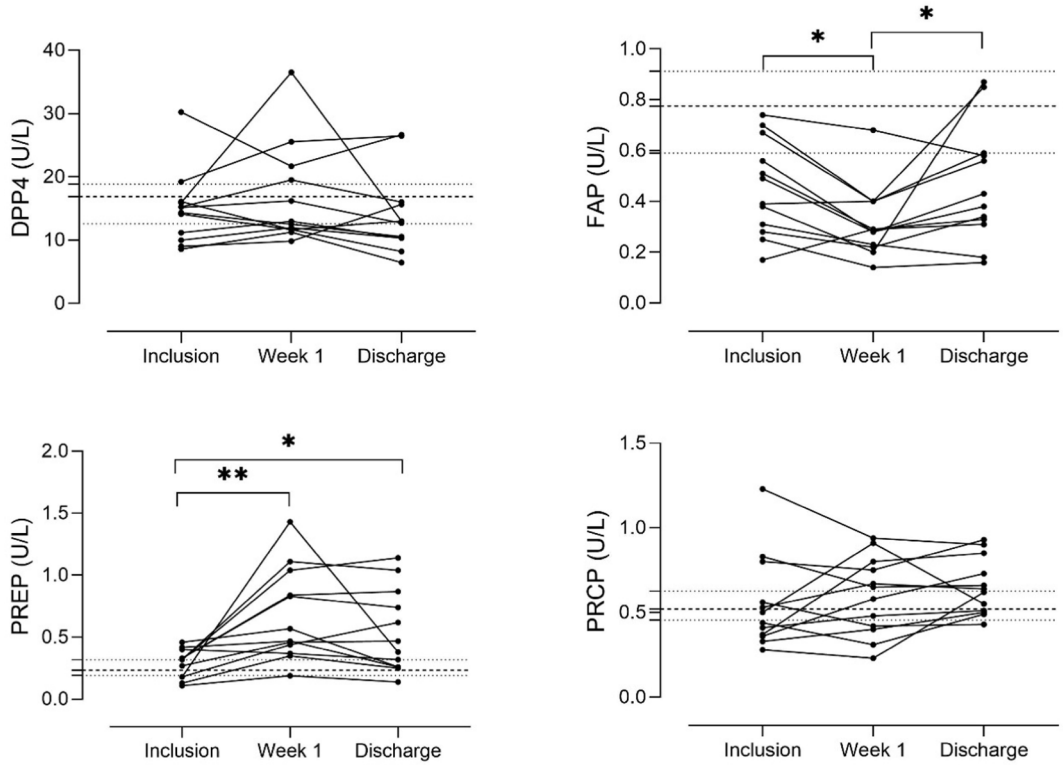

Fig. 2. Soluble DPP4, FAP, PREP and PRCP activities (individual measurements) on day of inclusion (ranging from 1 to 5 days after admission in hospital), 1 week after inclusion (ranging from 7 to 12 days after admission) and day of discharge (ranging from 15 to 46 days after admission) (paired measurements, n = 12). — = median activity of healthy control group, ····· = interquartile range of healthy control group. Statistical difference between time points was measured with the Wilcoxon Signed Rank test. * = $p < 0.05$, ** = $p < 0{,}005$, *** = $p < 0{,}0005$.

3.4. Trendline for five intensive care patients

For five COVID-19 patients who were admitted to the intensive care unit (ICU), samples were collected at more than 5 timepoints. For these patients, individual timelines were made to visualize the trend in peptidase activities during their ICU stay (Fig. 3). In general, these trendlines are in line with the above-described results. For some patients, DPP4 tends to decrease during ICU stay and in general the activities are lower than the median DPP4 activity measured in healthy volunteers. FAP activity decreases during ICU stay and normalizes again, presumably associated with the recovery of the patients. PREP activity increases first and seems to normalize after two or three weeks. This normalization to baseline for PREP was not detected in the larger group of patients when measuring PREP activity at day of inclusion, week 1 and day of discharge (Fig. 2). This could be due to the longer hospital stay of these ICU patients. Finally, PRCP activity remains stable during ICU stay and does not differ from the median PRCP activity in healthy controls. More information about the clinical background of these patients can be found in Supplementary Table 1.

3.5. Discussion and conclusion

DPP4, FAP, PREP and PRCP are all proline cleaving peptidases that are present as an active soluble form in plasma. DPP4 and FAP are both extracellular membrane proteins that can be shed from the cell membrane. PREP is a cytoplasmatic protein and PRCP is localized in lysosomes. Each of these proline-specific peptidases in plasma have been studied separately as biomarker for several diseases and conditions before. However, the simultaneous measurement of these peptidases in human circulation is rather exceptional. In Vliegen et al. [4] we compared the activity of all four enzymes in plasma of patients admitted to the ICU because of sepsis with those of ICU patients who underwent major intracranial surgery. Large differences were found, and the ROC curves yielded area under the curve (AUC) values for FAP, PREP, and DPP4 of 0.94, 0.88, and 0.86, respectively. PRCP had a lower prognostic value with an AUC of 0.71.

In contrast to sepsis, the individual peptidase activities did not yield high AUC values in the context of COVID-19. However, there are some interesting similarities that could be made with sepsis. Just as in sepsis, we observed lowered FAP and DPP4 activities while PREP activity was elevated in COVID-19 patients. PRCP activity remained unchanged and also in sepsis PRCP activity was only slightly elevated and therefore not suited as biomarker. These similarities are not surprising because both sepsis and critical COVID-19 illness are associated with a dysregulated answer of the immune system towards an infection.

The most pronounced observation in this study is the decreased activity of FAP in COVID-19 patients. In addition, when looking at the evolution longitudinally, we observed decreasing FAP levels during the initial disease course (approximately week 1–2 after hospital admission). Our measurements support the hypothesis that decreasing FAP levels are associated with worsening of the disease and normalization of FAP with recovery of the patients. Decreased soluble FAP activity has been reported in cases of inflammatory conditions such as arterial thrombosis [39–41] and sepsis [4] as well as several cancer types [42]. FAP is known as a protein that is largely absent in tissues of healthy persons and is upregulated in conditions such as cancer, fibrosis and tissue remodeling. In patients with liver cirrhosis it has been suggested that elevated circulating FAP levels originate from activated stellate cells and activated myofibroblasts in the liver [20]. While the origin of FAP in plasma of healthy persons is a matter of debate, several sources have been implicated, including, but not limited to human bone marrow-derived mesenchymal stem cells (MSCs)[43–45]. MSCs are multipotent stromal cells which can differentiate into a variety of cells, including osteoblasts, chondrocytes, myocytes and adipocytes. They also play a role in the support of hematopoietic stem cell function and it could be that during this process FAP is shed and ends up in the plasma.

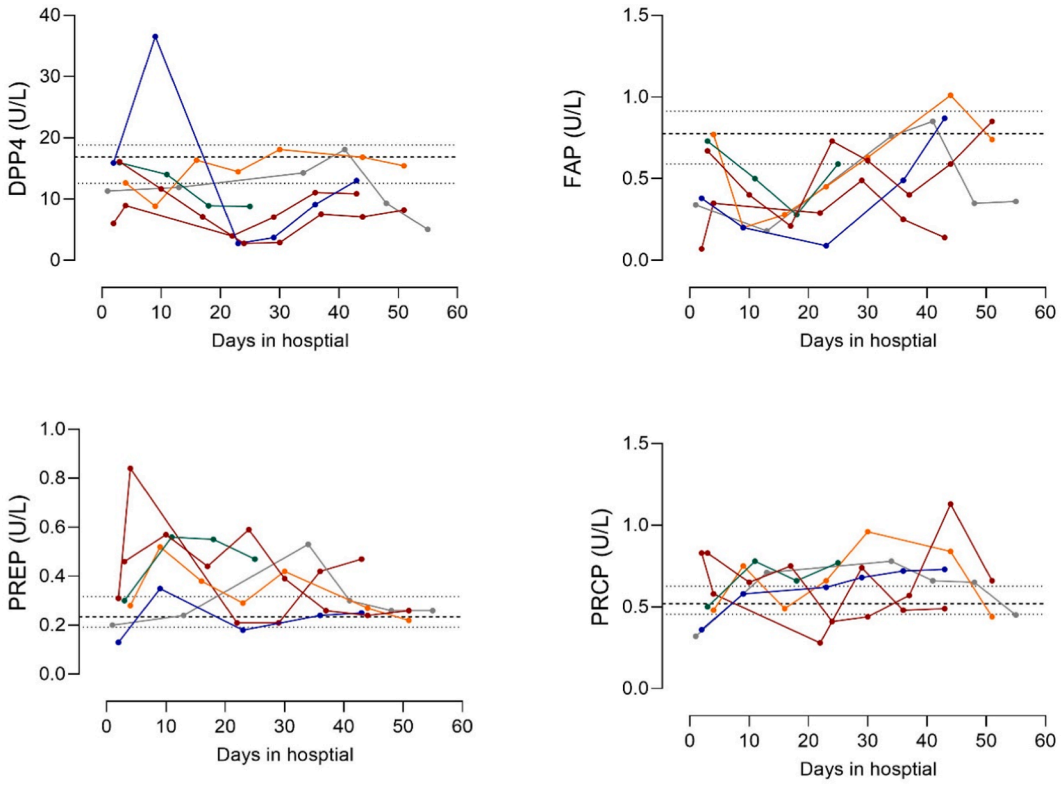

Fig. 3. Individual trendlines for five intensive care patients. For five patients admitted at ICU, trendlines were made for soluble DPP4, FAP, PREP and PRCP activity. (red = patient 1, blue = patient 2, green = patient 3, yellow = patient 4, grey = patient 5, — = median activity of healthy control group, ····· = interquartile range). Patient 5 deceased during hospital stay. More information about patients can be found in supplementary table.

Interestingly, MSCs can suppress or enhance the immune response, depending on the type and intensity of inflammatory stimuli [46]. Because of their immunosuppressive role, MSCs have been widely used in preclinical and clinical trials for various diseases and have shown great potential in the treatment of sepsis and COVID-19 [47]. Knowing that soluble FAP could originate from MSCs and that MSCs have immunosuppressive roles during over-stimulation of our immune system, as is the case with COVID-19 and sepsis, it is not surprising that FAP activity is dysregulated in these conditions. In addition, a recent study showed that FAP is expressed in natural killer (NK) cells in healthy persons [48] and it has been shown that NK cells are exhausted during the cytokine storm in COVID-19 [49]. If FAP originates from NK cells, it is plausible that FAP plasma levels decrease during periods of NK cell suppression. However, it must be mentioned that FAP expression in NK cells is rather low and that the percentage of NK cells in circulation is small compared to other lymphocytes.

Less pronounced than FAP, DPP4 activity is also decreased in plasma of COVID-19 patients, as is the case in various inflammatory conditions such as rheumatoid arthritis [50], multiple sclerosis [51], inflammatory bowel disease [52] and septic shock [4] and several cancer types [42]. It has been suggested that the process of shedding DPP4 from the cell surface is inhibited in inflammation [53]. However, at this point the origin and exact mechanism of DPP4 secretion and/or shedding from cell membranes in plasma remains poorly understood [53]. Moreover, in contrast to FAP, DPP4 is expressed in many cell types in healthy individuals. Liver epithelium and lymphocytes are often cited as the most likely source of soluble DPP4 [53]. More recently, a study in mice revealed important contributions from both endothelial cells and bone marrow-derived cells to plasma DPP4 [7]. Interestingly, high circulating DPP4 levels were found to be independently associated with the presence and severity of non-alcoholic fatty liver disease (NAFLD) [54]. As NAFLD often is associated with type 2 diabetes and obesity, both high-risk factors for severe COVID-19, we considered the influence of these factors on the DPP4 levels in COVID-19 patients. However, in our study population no significant differences were observed.

PREP is a cytoplasmatic protein present in most cells and tissues, however, there is not much known about its presence and translocation to the extracellular environment. We can hypothesize that the elevated PREP activity in plasma of patients with sepsis and COVID-19 originates from cell damage associated with acute lung injury or even multiple organ failure. There is evidence that increased PREP activity regularly occurs in the acutely injured lung and even contributes to the injury development [55]. In patients with severe COVID-19, the counter regulatory renin-angiotensin system (RAS) axis including ACE2/(Ang(1-7) and Mas receptor seems dysregulated [55]. It could be that the elevated PREP levels in plasma of COVID-19 patients that we observe in our study, are a reflection of an upregulated PREP expression that in its turn results from a disturbed RAS axis in the injured lung tissue.

Plasma PRCP concentrations have been shown to be increased in obesity, diabetes, and cardiovascular dysfunction [56,57]. However, in our study PRCP activity in COVID-19 patients remains unchanged.

An in depth discussion on the pathophysiological role of these enzymes falls outside the scope of the present study, but is described in recent literature [23,55,58–61]. Moreover, for each of these enzymes, specific pharmacological inhibitors are available, allowing more functional studies and investigations on their potential as therapeutic targets in lung diseases and COVID-19 in particular.

In conclusion, we observe a similar pattern in the proline-specific peptidase activities in COVID-19 patients compared with patients experiencing septic shock: decreased DPP4 and FAP activity and elevated PREP activity.

Although the differences in activities of DPP4, FAP and PREP between the COVID-19 and healthy groups were significant, the present study population does not allow to make firm conclusions on their value as stand-alone diagnostic or prognostic biomarkers. The most pronounced and remarkable observation in this study was the decreased FAP activity in COVID-19 patients. This observation raises questions about the origin and function of soluble FAP in plasma in both healthy and ill patients. Further research is necessary on this topic.

CRediT authorship contribution statement

A. Vujkovic, E. Vlieghe, K. Vercauteren, P. Van der Veken, S. Kumar-Singh, D. Hendriks, I. De Meester: Conceptualization. L. van Petersen, I. Brosius, C. Theunissen, S. van Ierssel, M. van Frankenhuijsen: Recruitment and selection of study participants and blood sampling. A. Hotterbeekx, F.H.R. De Winter and A. Vujkovic: Sample processing, A. Bracke, K. Claesen M. De bruyn, E. De Hert, G. Vliegen: Sample measurements. K. Claesen, A. Bracke, A. Hotterbeekx, A. Vujkovic, F.H.R. De Winter: Processing and interpretation of data. A.Bracke, I. De Meester: Drafting of the manuscript. All authors: Critical revision of the manuscript for important intellectual content.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2022.03.005.

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