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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Urinary cytokines correlate with acute kidney injury in critically ill COVID-19 patients

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ABSTRACT

Background: Acute kidney injury is common in COVID-19 patients admitted to the ICU. Urinary biomarkers are a non-invasive way of assaying renal damage, and so far, urinary cytokines are not fully investigated. The current study aimed to assess urinary cytokine levels in COVID-19 patients.
Methods: Urine was collected from COVID-19 patients (n = 29) in intensive care and compared to a preoperative group of patients (n = 9) with no critical illness. 92 urinary cytokines were analyzed in multiplex using the Olink Target 96 inflammation panel and compared to clinical characteristics, and urinary markers of kidney injury.
Results: There were strong correlations between proinflammatory cytokines and between urinary cytokines and urinary kidney injury markers in 29 COVID-19 patients. Several cytokines were correlated to kidney injury, 31 cytokines to AKI stage and 19 cytokines correlated to maximal creatinine.
Conclusions: Urinary inflammatory cytokines from a wide range of immune cell lineages were significantly upregulated during COVID-19 and the upregulation correlated with acute kidney injury as well as urinary markers of kidney tissue damage.

1. Introduction

Coronavirus infectious disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is associated with significant morbidity and mortality in intensive care [1].The systemic inflammatory response is thought to contribute the severity of the disease and mortality [2]. The SARS-CoV-2 virus has been postulated to attach to alveolar epithelial cells, and potentially other tissues [3] and activate both innate and adaptive immune systems, resulting in a massive release of cytokines [4]. Therefore, there are speculations whether severe COVID-19 infection may be due to improper release of cytokines and a number of studies have shown up-regulated cytokines in plasma [5,6,7]. It has been suggested that patients with severe COVID-19 should be screened for hyperinflammation to identify the subgroup of patients that would benefit from immunosuppression [6]. In addition to respiratory failure, infection with SARS-COV-2 may also affect other organs. Acute kidney injury is relatively common in patients admitted to the ICU due to COVID-19. The pathogenesis is unclear but postulated mechanisms include cytokine storm syndrome or direct cellular injury due to the virus [7]. Despite the noninvasive method, and possibility to assess renal inflammation, cytokine urine excretion has so far not been investigated. Based on the hypothesis that increased expression of cytokines and renal inflammation may be an important cause of renal dysfunction in severe COVID-19 we aimed to investigate the urinary secretion of cytokines in critically ill COVID-19 patients admitted to intensive care units at a tertiary care hospital in Sweden.

2. Materials and methods

This study is a sub-study of a larger prospective observational study and was performed at the general intensive care unit (ICU), a mixed COVD-19 ICU at Uppsala University Hospital, a tertiary care hospital in Sweden. The study was approved by the National Ethical Review Agency (EPM; No. 2020–01623). Informed consent was obtained from the

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patient, or next of kin if the patient was unable to give consent. The Declaration of Helsinki and its subsequent revisions were followed. The protocol of the study was registered (ClinicalTrials ID: NCT04316884).

2.1. Data collection and patient groups

Adult patients with COVID-19 admitted to the ICU between March 13, and April 14, 2020 with informed consent and urinary samples were included in the study. Clinical data were prospectively recorded. Simplified acute physiology score 3 (SAPS3) [8] was recorded at admittance. Acute kidney injury (AKI) was assessed according to the creatinine criteria of the KDIGO [9] definition since we have previously shown that the urinary volume criteria is not strongly associated with severe AKI or long-term kidney damage in COVID-19 [10]. Blood and urine samples were collected on admission to the ICU and daily during the ICU stay. Control samples were collected immediately preoperatively for patients admitted for cytoreductive surgery for peritoneal carcinosis. Urine TIM-1/KIM-1/HAVCR and Lipocalin-2/NGAL were analyzed by commercially available sandwich immunoassays (DY1750B and DY1757, R&D Systems, Minneapolis, MN, USA). The assays were calibrated against highly purified recombinant human proteins and the total coefficients of variation (CV) of the assays were approximately 6%. Urine albumin and urine creatinine were analyzed on a BS380 chemistry analyzer (Mindray, Shenzhen, China). The urine albumin (2 K989-21) and enzymatic creatinine (8L24-41, IDMS calibrated) reagents were from Abbott Laboratories (Abbott Park, IL, USA). The total CV were 1.4% at 8.5 mmol/L and 1.5% at 4.2 mmol/L for U-creatinine and 4.0% at 31 mg/L and 1.1% at 95 mg/L for U-albumin. All assays were performed blinded without knowledge of clinical data.

2.2. Proteomic urine profiling

Urine cytokine levels of 92 different analytes were analyzed at the Clinical Biomarkers Facility (SciLifeLab, Uppsala, Sweden) using the Target 96 Inflammation panel from OLINK Proteomics® (Uppsala, Sweden). The first urine sample taken after admittance to ICU was used together withcontrol urine collected immediately after catheter placement in preoperative controls. The method has been described elsewhere [11] but briefly, one μL urine, diluted 1:4, was mixed with 3 μL incubation mix containing probes (consisting of paired antibodies labelled with unique corresponding DNA oligonucleotides) in microtiter plate wells. The mixture was incubated at 8 °C overnight and then 96 μL extension mix containing PEA enzyme and PCR reagents was added, and the samples were incubated for 5 min at room temperature. The plate was transferred to the thermal cycler for 17 cycles of DNA amplification. A 96.96 Dynamic Array IFC (Fluidigm, South San Francisco, CA, USA) was prepared and primed according to the manufacturer's instructions. In a separate plate, 2.8 μL of sample mixture was mixed with 7.2 μL detection mix from which 5 μL was loaded into the right side of the primed 96.96 Dynamic Array IFC. The unique primer pairs for each analyte were loaded into the left side of the 96.96 Dynamic Array IFC, and the protein expression program was run in the Fluidigm BioMark™ HD real-time PCR platform. The extracted values, called normalized protein expression (NPX) values, are relative and expressed on the log2 scale. In the logarithmic phase of the curve, one increase of the NPX value corresponds to a doubling of the protein content and high NPX value therefore means a high protein concentration. The signal specificity is high, since binding by both the two protein specific probe in close proximity is required to produce a signal. The average intrassay % CV for the 92 biomarkers in this study was 4%.

2.3. Statistical analysis

All reported data were analyzed using Microsoft Excel (Redmond, WA, USA) and R version 4.0.3. Continuous variables are presented as median (IQR) or as mean ± SD as appropriate. Categorical variables are presented as number of observations (percent of total number of observations). Comparison of continuous variables were made using Wilcoxon rank sum test and Kruskal-Wallis ANOVA and *t*-test and one-way ANOVA as appropriate. Correlations were calculated using Spearman's rank correlation. The effect of increasing AKI stage on biomarkers was analyzed using a mixed model anova taking repeated samples into account. P-values were corrected for the False Discovery Rate (FDR) and a FDR < 0.05 was considered significant.

3. Results

A total of 38 patients were included in the study, 29 COVID-19 patients admitted to ICU and a control group of nine elective abdominal surgery patients.

COVID-19 patients were admitted to intensive care after a median time of 10 days from symptom onset. The age was similar between groups (57 ± 3 years in COVID-19 compared to 63 ± 4 in controls). The majority of critically ill COVID-19 patients were men (73% vs 44% in controls) and all patients with AKI stage 3 were men. The most common comorbidities in decreasing order were hypertension, chronic pulmonary disease and diabetes mellitus. In the control group all had malignant disease, followed by hypertension and diabetes mellitus. SAPS3 on admission was 53 ± 2 in COVID-19 patients (Table 1).

Of 29 COVID-19 patients 19 (66%) developed AKI based on serum creatinine at some point during the course of intensive care. 10 patients (34%) did not develop AKI, 9 (31%) developed AKI stage 1, 6 (21%) stage 2, and 4 (14%) stage 3. None of the preoperative controls had reduced kidney function when the samples were collected, which was before surgery. All patients with AKI stage 3 required renal replacement therapy compared to none in AKI stage 1 and AKI stage 2. Nine COVID-19 patients died, 3 (33%) patients with AKI stage 0, 1 (11%) with AKI stage 1, 3 (50%) with AKI stage 2 and 2 (50%) with AKI stage 3 (Table 1). Baseline and maximal values for creatinine, as well as urinary albumin to creatinine ratio, the kidney damage markers KIM-1 and NGAL are reported in table 2.

Out of 92 analyzed cytokines, 28 where below the limit-of-detection for the majority of samples and were hence excluded from further analysis. The remaining 64 cytokines were subsequently analyzed. The admission level of 31 cytokines were associated with maximum AKI stage during intensive care (Table 3).

Many of the proinflammatory cytokines showed strong correlations with each other. The correlation to maximal creatinine was significant for 19 analytes, where of 18 were positively correlated and one was negatively correlated. Urinary NGAL and KIM-1, as well as urinary albumin to creatinine ratio correlated well with cytokine expression, and clustered among the cytokines on hierarchical cluster analysis (Fig. 1).

4. Discussion

The main result was a general increase of proinflammatory cytokines in the urine of COVID-19 patients compared to patients admitted for elective surgery. There was not only a correlation between elevated urinary cytokines and acute kidney injury based on serum creatinine but also between urinary cytokines and urinary kidney tissue injury markers NGAL, KIM1 and ACR. Further, urinary cytokines did not correlate significantly with mortality, indicating some degree of specificity for the kidney injury beyond the degree of disease severity and general organ dysfunction. Although AKI is generally associated with increased mortality the homogenously severe phenotype of COVID-19 in present study may weaken this association [10].

Proinflammatory cytokine release compared to the cytokine storm phenomenon has been reported in COVID-19 [12]. Previously, a plasma-cytokine profile in COVID-19 has been reported to include increased IL2, IL7, GCSF, interferon gamma, IP10, MCP1, MIp1-alfa and TNF-alpha [13]. Urinary cytokines may reflect systemic release, as seen in plasma, but may also be more closely associated with kidney injury.

Table 1
Patient demographics and comorbidities in the cohort of critically ill COVID-19 and preoperative controls with no critical illness or reduction of kidney function. Data are presented as Mean ± SEM, or n (%).

	HIPEC	AKI stage 0	AKI stage 1	AKI stage 2	AKI stage 3
	n = 9	n = 10	n = 9	n = 6	n = 4
Men, n (%)	4 (44%)	7(70%)	6(67%)	5(83%)	4(100%)
Age	63 ± 4	48 ± 2,1	53 ± 3,7	54 ± 7,2	61,5 ± 2,8
Body weight	84 ± 6	84,2 ± 2,7	88,5 ± 4,1	94,8 ± 13,9	82,5 ± 3,5
BMI	27 ± 2	29,3 ± 1,2	27 ± 1,6	31,2 ± 1,7	28,9 ± 1,1
Height, cm	167 ± 2	171,3 ± 4,4	173 ± 2,7	176 ± 28,4	166 ± 3,8
SAPS3	53 ± 2	60,5 ± 6,5	51 ± 4,3	67 ± 8,7	67 ± 0,4
Comorbidities, n (%)					
Pulmonary disease	0	4(40%)	3(33%)	2(33%)	0
Hypertension	2(22%)	6(60%)	4(44%)	4(67%)	3(75%)
Heart failure	1(9%)	0	0	0	0
Ischemic heart disease	0	0	0	3(50%)	0
Vessel disease	0	1(10%)	1(11%)	3(50%)	0
Thromboembolic event	0	0	1(11%)	1(17%)	0
Liver failure	0	0	0	0	0
Malignant disease	9(100%)	0	1(11%)	0	1(25%)
Diabetes mellitus	1(11%)	1(10%)	2(22%)	4(67%)	1(25%)
ACEi-/ARB treatment	2(22%)	4(40%)	5(55%)	4(67%)	2(50%)
Renal replacement therapy	0	0	0	0	4(100%)
Mortality	0	3(30%)	1(11%)	3(50%)	2(50%)

Table 2
Biochemical findings in 29 critically ill COVID-19 patients. Data is given as median (IQR) or n(%) as appropriate.

Baseline plasma creatinine (mmol/L)	68 (62–81)
Maximum plasma creatinine (mmol/L)	112 (90–185)
Urinary Albumin to creatinine ratio (mg/mmol)	7.8 (2.7–22.8)
Urinary KIM-1 (ng/mL)	5.3 (2.7 – 10.6)
Urinary NGAL (ng/mL)	33 (13 – 130)
Continuous renal replacement therapy in ICU	4 (14%)

Further, patients with sepsis show increased rate of cytokine elimination by the kidneys, which may be associated with increased urinary cytokines but not related to kidney tissue damage [14]. The present data show a general up-regulation of proinflammatory cytokines in the urine of COVID-19 patients which is consistent with previous studies in plasma [15]. The coordinated upregulation of both IL6 and IL18, while IL10 was below the detection limit, indicates activation of the NLRP3/IL1beta inflammasome [16]. This is consistent with early experimental reports [17] and provides a pathway for the upregulation of IL6 [18]. The proinflammatory cytokine IL-6 was identified as a severity marker in early studies, which is consistent with the current findings of high urinary IL-6 in COVID-19 patients admitted to the ICU as compared to controls [12]. Further, ferritin and IL6 levels have been reported to be increased in non-survivors as compared to survivors, indicating that inflammation is associated with mortality [19].

In the present study, many of the studied urinary cytokines showed good correlation with the kidney injury markers NGAL, KIM-1 as well as the incidence of AKI compared to controls. However, several cytokines, including IL-6 did not correlate with increasing stage of AKI, rather the difference in these may reflect COVID-19 compared to control. This is interesting since earlier studies found bidirectional damage between lungs and kidneys, where injured renal tubular epithelium upregulates IL-6 which promotes additional pulmonary dysfunction. In turn, renal tissue hypoxia damages renal tubular epithelial cells causing production of IL-6 [20]. This mechanism would be of interest considering the marked hypoxia of COVID-19 patients. Although studies of urinary cytokines are less common than investigations concerning systemic cytokines our findings are consistent with previous findings for specific urinary analytes, such as IL-18, in other critically ill populations [21].

One analyte, uPa, was downregulated in COVID-19 patients and has earlier been found down-regulated in patients with ARDS [22]. uPA plays an important role in anticoagulation by activation of plasminogen to plasmin which primary function is facilitating fibrinolysis. COVID-19-patients are more likely to develop thrombotic complications [23], thus the decreased levels of uPA might reflect this hypercoagulative state. This may be a salient finding in relation to AKI since fibrin thrombi formation in glomerular and peritubular capillaries has been described [24]. The fact that no correlation was seen between uPa and urinary kidney injury markers in the present study may indicate that although levels are decreased increasing injury may be associated with increasing uPa filtration into the urine.

We found the urinary kidney injury markers KIM1 and NGAL to be strongly correlated to many of the urinary cytokines. This is consistent with inflammatory kidney injury where both cell damage and inflammation markers are increased. Most of the up-regulated cytokines in the current study tend to be of innate immune system origin, which is in line with studies considering cytokine storm as a pathogenic pathway of severe disease in COVID-19 [6].

CD68-macrophages has also been found in tubulointerstitium, which validate the assumption of proinflammatory cell migration into renal parenchyma in COVID-19 patients with AKI [25]. VEGFA, MCP1, CCL23 and IL-8 also correlated strongly with both levels of NGAL and KIM1 and grade of AKI. Interestingly, earlier studies showed possibilities to predict renal recovery and 3-month patient mortality by analyzing levels of urinary IL-8, IL-10, VEGF and MCP-1 in patients with AKI [26]. However, a strong correlation between elevated levels of NGAL/KIM1 and increased urinary cytokines has been shown in elderly [27].

Strengths of the present study is being prospective with a small selection bias and the use of a preoperative control group without critical illness or AKI. Secondly, the patients were collected during the first wave of COVID-19 before the wide-spread use of corticosteroids or antivirals. A weakness of the study is the preoperative controls not being well-matched, in particular that they are all admitted for peritoneal carcinosis, and their cytokine patterns may differ from that of healthy individuals. A second weakness is that the number of included patients is relatively small, which has the effect that patients are not evenly distributed between the different stages of AKI. Although this is ameliorated by using the creatinine definition it may still make the analysis more prone to confounding, which we see in the form of cytokines that do not consistently increase with worsening AKI.

In conclusion, the present study shows marked increases in a wide number of proinflammatory cytokines elevated in urine in COVID-19 patients that were closely correlated to urinary kidney damage markers as well as correlated to the development of AKI.

Authorship contributions

Table 3
Urinary cytokines detected using the Proximity extension assay in urine of preoperative control patients (n = 9) compared to critically ill COVID-19 patients (n = 29) by level of AKI. Additional cytokines did not reach level of detection in sufficient samples for analysis: NT3, ST1A1, IL-5, GDNF, ASA, TNF-Beta, IL-20RA, IL2RB, IL-1alfa, IL-2, TSLP, TNFSF14, FGF23, IL-10RA, IL22RA1, beta-NGF, TRANCE, IL-24, IL-13, IL-10, IL-20, IL-33, IFN-gamma, IL-4. Group-FDR: False Discovery rate based on Wilcoxon Signed Ranks test between Controls and COVID-19 day 1 samples. AKI-FDR: False Discovery rate based on the significance for increasing AKI-stage in a mixed model ANOVA taking repeated samples in some patients into account. N = is given as patients/samples.

Assay	Control	Group FDR	COVID-19 AKI 0	AKI 1	AKI 2	AKI 3	AKI-stage FDR
	n = 9/9		n = 10/25	n = 9/24	n = 6/14	n = 4/12	
ARTN	0.3 ± 0.3	0.017	0.7 ± 0.2	0.8 ± 0.6	0.9 ± 0.7	1 ± 0.6	NS
AXIN1	0.3 ± 0.3	NS	0.5 ± 0.5	0.3 ± 0.4	0.3 ± 0.2	0.8 ± 0	NS
CASP-8	3.3 ± 1	NS	1.8 ± 0.7	1.2 ± 0.4	1.2 ± 0.9	2.2 ± 1.1	NS
CCL11	3 ± 1.2	NS	3.4 ± 0.7	2.8 ± 1.1	3.1 ± 1.9	4.9 ± 0.1	0.042
CCL19	2.4 ± 1	0.063	3.6 ± 2.7	2.7 ± 2.3	2.6 ± 2	6.6 ± 2.8	0.001
CCL20	2.9 ± 0.8	NS	3 ± 1.2	2.5 ± 0.8	2.7 ± 1	6.8 ± 2.9	0.001
CCL23	1.4 ± 0.5	0.039	2.2 ± 1.2	1.7 ± 1	1.9 ± 1.1	5 ± 1.8	NS
CCL25	3 ± 1.8	NS	5 ± 1.3	3.9 ± 2.2	3.5 ± 2.6	6.1 ± 0.1	NS
CCL28	0.1 ± 0.1	0.010	0.7 ± 0.5	0.6 ± 0.4	0.7 ± 0.5	1.2 ± 0.1	0.023
CCL3	1.3 ± 0.6	0.006	4.1 ± 2.5	3.9 ± 3.1	2.6 ± 1.6	8.2 ± 2.3	0.009
CCL4	1.6 ± 0.8	NS	2.6 ± 0.8	2.3 ± 1.2	1.9 ± 1.5	4.8 ± 0.1	NS
CD244	2.3 ± 0.8	NS	2.4 ± 0.7	1.7 ± 0.4	1.7 ± 0.8	1.7 ± 1.4	NS
CD40	13 ± 0.9	0.009	14 ± 0.6	13.7 ± 0.7	13.4 ± 1.2	14.3 ± 0.2	NS
CD5	3.1 ± 0.8	NS	3.6 ± 1	2.9 ± 0.6	2.8 ± 1.3	4.6 ± 0.5	NS
CD6	1.5 ± 0.5	NS	1.5 ± 0.5	1.3 ± 0.3	1.2 ± 0.6	2.5 ± 0.5	0.012
CDCP1	1.3 ± 1	0.019	1.7 ± 0.8	0.9 ± 0.9	1.3 ± 1.1	3.4 ± 0.5	NS
CSF-1	9.8 ± 1.3	0.017	11 ± 0.4	10.6 ± 0.7	10.4 ± 1.3	11 ± 0.3	NS
CST5	3.9 ± 1.3	NS	4.4 ± 1.6	3.8 ± 1.7	3 ± 2	5.1 ± 1.6	NS
CX3CL1	1.3 ± 0.8	NS	2 ± 0.8	1.4 ± 0.8	1 ± 1.5	2.5 ± 0	0.0002
CXCL1	4.5 ± 1.5	NS	4.8 ± 1.7	3.9 ± 1.1	4.2 ± 2.1	8.5 ± 1.2	0.008
CXCL10	5 ± 1.6	NS	6.7 ± 1	5.5 ± 1.8	5.7 ± 2.4	8.4 ± 0.3	0.012
CXCL11	1 ± 1.2	NS	2.7 ± 1	1.7 ± 1.4	2.1 ± 2.3	4.2 ± 0.4	0.009
CXCL5	2 ± 0.7	NS	1.6 ± 0.9	1.6 ± 1.1	1.6 ± 1	4.7 ± 4.6	0.0004
CXCL6	5.3 ± 1.5	NS	3.9 ± 1.9	3.4 ± 1.8	3.1 ± 1.7	6.3 ± 3.1	0.002
CXCL9	1.7 ± 0.8	NS	2.6 ± 1.5	2.1 ± 1.3	2.3 ± 1.7	5.8 ± 2.1	NS
DNER	8.2 ± 0.9	NS	7.9 ± 0.7	7.6 ± 0.5	7.2 ± 1.4	8 ± 0.8	0.001
EN-RAGE	2.2 ± 1.3	NS	1.8 ± 1.8	2.1 ± 1.7	2.1 ± 1.3	4.5 ± 2.6	NS
FGF-19	0.4 ± 0.2	0.008	1.5 ± 1.3	1 ± 1.1	0.6 ± 0.5	1.9 ± 0.7	0.006
FGF-21	0.7 ± 0.2	0.023	3.3 ± 3.1	2.8 ± 2.4	1.7 ± 0.9	6.2 ± 6.3	NS
FGF-5	1 ± 0.5	NS	1.5 ± 0.8	1.4 ± 1.1	1.2 ± 1	2 ± 0.7	NS
Flt3L	5.3 ± 1.2	NS	5.5 ± 1	4.7 ± 1.3	3.9 ± 2.3	5.5 ± 1.4	0.010
HGF	7.1 ± 0.9	0.017	8.2 ± 0.7	7.6 ± 1	7.7 ± 1.2	9.6 ± 1.4	0.001
IL-10RB	6.2 ± 1.1	NS	6.1 ± 0.7	5.4 ± 0.5	5.3 ± 1.6	5 ± 1.6	0.043
IL-12B	1 ± 0.3	NS	0.8 ± 0.2	0.7 ± 0.3	0.8 ± 0.5	0.8 ± 0.9	NS
IL-15RA	0.6 ± 0.4	0.019	1 ± 0.6	0.9 ± 0.8	0.6 ± 0.5	1.6 ± 0.7	0.064
IL-18	4.3 ± 1.5	0.019	6.1 ± 0.8	5.3 ± 1.4	5 ± 2	8.4 ± 0.8	NS
IL-18R1	6.5 ± 1.6	0.010	7.6 ± 1.7	7.7 ± 1.5	7.4 ± 2.5	8.2 ± 1.7	0.079
IL-6	1.4 ± 0.9	0.023	3.3 ± 1.4	2.1 ± 0.9	2.3 ± 1.3	5.6 ± 2.3	0.001
IL-7	0.5 ± 0.3	NS	0.5 ± 0.2	0.4 ± 0.2	0.5 ± 0.5	1.2 ± 0.2	0.001
IL-8	3.4 ± 1.3	0.026	5 ± 2	4.8 ± 1.4	5 ± 2.1	9.4 ± 7.6	0.001
LIF	2.4 ± 1.5	NS	3.5 ± 1	3.2 ± 1.1	3.5 ± 1.6	6.4 ± 2.5	0.009
LIF-R	1.9 ± 1	NS	2.8 ± 1.4	2.2 ± 1.1	2.2 ± 1.4	3.6 ± 1.1	0.038
MCP-1	10 ± 1.6	0.042	11.5 ± 1	10.4 ± 1.5	10.5 ± 2.1	13.4 ± 1.8	NS
MCP-2	3.6 ± 1.2	NS	6.9 ± 0.6	5.5 ± 1.8	5.6 ± 2.8	7.1 ± 1.4	0.0004
MCP-3	0.3 ± 0.7	0.019	2.1 ± 0.9	1.8 ± 1.1	2 ± 1.3	4.4 ± 0.9	NS
MCP-4	9.5 ± 0.9	NS	9.6 ± 0.9	9.2 ± 0.9	9.3 ± 2.1	10.9 ± 1.1	NS
MMP-1	2.8 ± 1.3	0.015	4.7 ± 2.5	4.5 ± 2.3	5.1 ± 2	7.7 ± 5.2	NS
MMP-10	3.1 ± 1	0.008	3.9 ± 1.8	3.4 ± 1.4	3.6 ± 1.5	4.6 ± 4	0.015
OPG	5.9 ± 0.9	0.019	7.5 ± 1.2	6.8 ± 1.7	6.8 ± 1.4	8.8 ± 0.4	0.002
OSM	0.9 ± 0.5	NS	1.9 ± 1.3	1.6 ± 0.8	1.2 ± 0.8	4 ± 3.3	NS
PD-L1	4.5 ± 1.1	0.010	6.1 ± 1	5.5 ± 1.5	5 ± 1.6	6.6 ± 0.4	NS
SCF	3.1 ± 1.3	0.017	5.3 ± 1.8	4.9 ± 1.9	4.1 ± 2.2	4.1 ± 1.7	NS
SIRT2	0.9 ± 0.2	0.019	2.5 ± 1.4	2 ± 1.3	2.1 ± 1.8	2.1 ± 0.1	NS
SLAMF1	1.5 ± 0.8	0.006	1.6 ± 0.6	1.4 ± 0.8	0.9 ± 0.6	1.3 ± 0.4	0.042
STAMBP	2.8 ± 0.8	NS	2.3 ± 0.7	1.6 ± 0.7	1.7 ± 0.8	2.7 ± 0.1	NS
TGF-alpha	2.9 ± 0.8	NS	2.6 ± 1.1	1.8 ± 0.9	2.1 ± 1.3	3.1 ± 0.7	NS
TGF-beta-1	1.4 ± 0.7	NS	2.2 ± 1.5	1.6 ± 0.5	2.3 ± 1.5	3.4 ± 0.5	0.027
TNF	−0.4 ± 0.2	NS	0.2 ± 1.1	0.6 ± 1.8	−0.3 ± 0.4	1.7 ± 1.2	NS
TNFRSF9	8 ± 1.3	NS	8.5 ± 0.8	7.7 ± 1.5	7.1 ± 2.2	9.7 ± 1.2	0.008
TRAIL	1.3 ± 0.7	NS	2.1 ± 1	1.4 ± 0.5	1.3 ± 0.9	3.4 ± 0.2	NS
TWEAK	2.6 ± 1.7	0.020	5.9 ± 0.9	5.1 ± 2	4.8 ± 3.2	7.3 ± 1.5	0.036
uPA	12.7 ± 0.6	0.017	11.2 ± 1.8	10.5 ± 1	10.6 ± 1.9	11.8 ± 1.6	NS
VEGFA	8.1 ± 0.9	0.015	10.4 ± 1.6	9.7 ± 1.5	9.2 ± 1.7	12.2 ± 1.3	NS
X4E-BP1	3.4 ± 0.8	0.039	5.3 ± 2.8	4.6 ± 2.9	3.8 ± 2.3	6.9 ± 2.3	NS

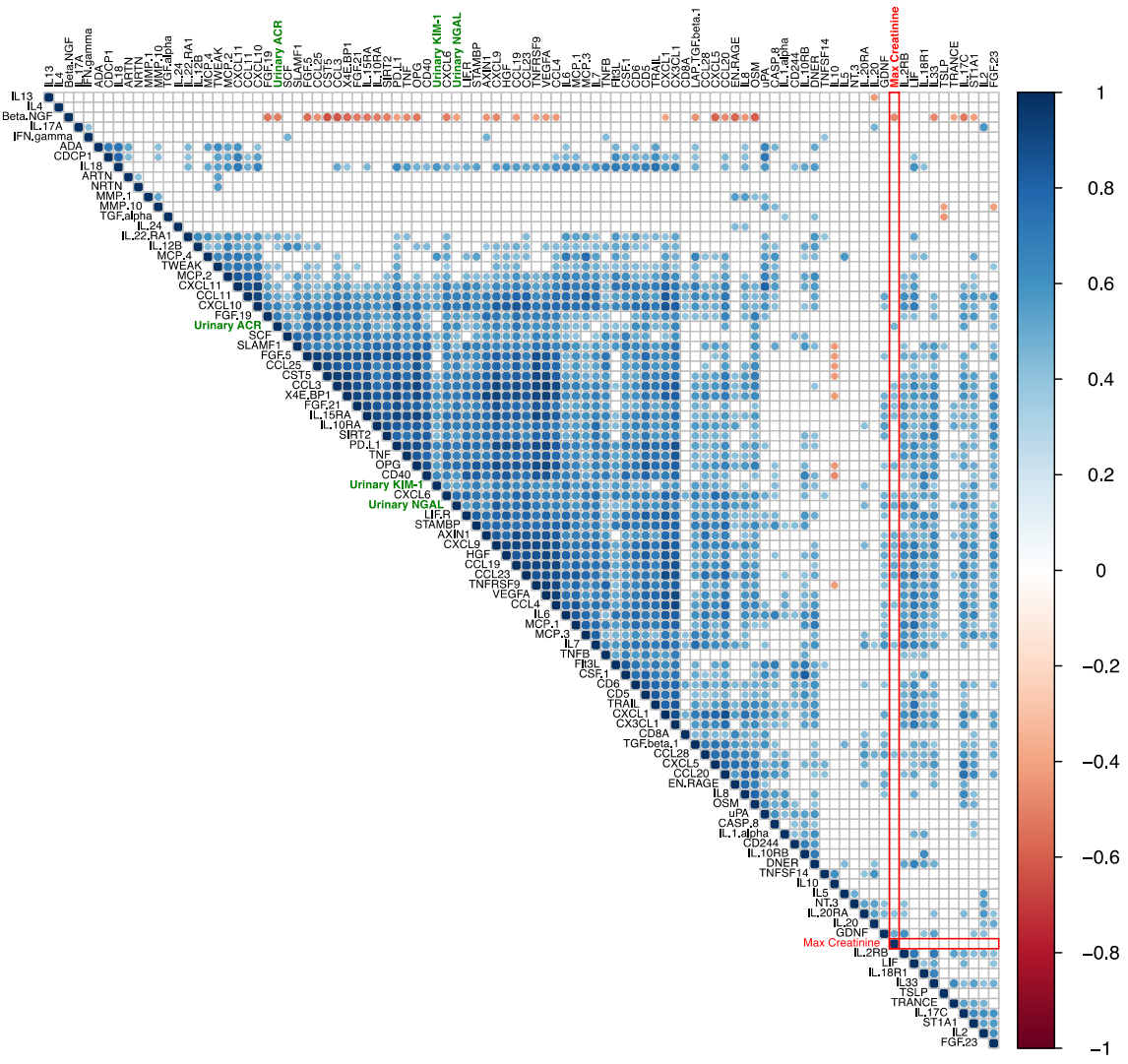

Fig. 1. Correlation between urinary cytokines detected using the proximity extension assay (black), the urinary kidney damage markers (green) albumin/creatinine-ratio (ACR), kidney injury molecule-1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), and acute kidney injury (AKI) in the form of maximal plasma creatinine (red) in critically ill COVID-19 patients (n = 29). Correlations were calculated using Spearman's correlation, blue indicates positive correlation, red indicates negative correlation. Non-significant correlations are white.

All authors participated in conception and design of the study. All authors had access to the data and participated in data collection and interpretation. AG, HA and MH analyzed data and drafted the manuscript. All authors contributed to manuscript revision and gave approval of the final version.

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A. Gradin: Resources, Formal analysis, Visualization, Writing - original draft, Writing - review & editing. **H. Andersson:** Resources, Formal analysis, Visualization, Writing - original draft. **T. Luther:** Investigation, Resources, Writing - review & editing. **S. Bülow Anderberg:** Investigation, Resources, Writing - review & editing. **S. Rubertsson:** Conceptualization, Supervision, Writing - review & editing. **M. Lipcsey:** Conceptualization, Methodology, Resources, Project administration, Supervision, Writing - review & editing. **M. Åberg:** Conceptualization, Resources, Writing - review & editing. **A. Larsson:** Conceptualization, Resources, Writing - review & editing. **R. Frithiof:** Conceptualization, Methodology, Supervision, Project administration, Funding acquisition, Resources, Writing - review & editing. **M. Hultström:** Conceptualization, Methodology, Supervision, Project administration, Funding acquisition, Resources, Visualization, Writing - review & editing.

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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