Soluble ST2 concentrations associate with in-hospital mortality and need for mechanical ventilation in unselected patients with COVID-19

Torbjorn Omland,1,2 Christian Prebensen,2,3 Christine Jonassen,4 My Svensson,2,5 Jan Erik Berdal,2,3 Ingebjørg Seljeflot,2,6 Peder Langeland Myhre 1,2

ABSTRACT

Objective Soluble ST2 (sST2) reflects inflammation, endothelial dysfunction and myocardial fibrosis, is produced in the lungs and is an established biomarker in heart failure. We sought to determine the role of sST2 in COVID-19 by assessing pathophysiological correlates and its association to in-hospital outcomes.

Methods We enrolled 123 consecutive, hospitalised patients with COVID-19 in the prospective, observational COVID-19 MECH study. Biobank samples were collected at baseline, day 3 and day 9. The key exposure variable was sST2, and the outcome was ICU treatment with mechanical ventilation or in-hospital death.

Results Concentrations of sST2 at baseline were median 48 (IQR 37–67) ng/mL, and 74% had elevated concentrations (>37.9 ng/mL). Higher baseline sST2 concentrations were associated with older age, male sex, white race, smoking, diabetes, hypertension and chronic kidney disease. Baseline sST2 also associated with the presence of SARS-CoV-2 viraemia, lower oxygen saturation, higher respiratory rate and increasing concentrations of biomarkers reflecting inflammation, thrombosis and cardiovascular disease. During the hospitalisation, 8 (7%) patients died and 27 (22%) survivors received intensive care unit (ICU) treatment. Baseline sST2 concentrations demonstrated a graded association with disease severity (median, IQR): medical 43 (36–59) ng/mL; ICU 67 (39–104) ng/mL and non-survivors 107 (72–116) ng/mL (p<0.001 for all comparisons). These associations persisted at day 3 and day 9.

Conclusions sST2 concentrations associate with SARS-CoV-2 viraemia, hypoxaemia and concentrations of inflammatory and cardiovascular biomarkers. There was a robust association between baseline sST2 and disease severity that was independent of, and superior to, established risk factors. sST2 reflects key pathophysiology and may be a promising biomarker in COVID-19.

Trial registration number NCT04314232.

INTRODUCTION

Patients hospitalised with COVID-19 are at risk of poor outcome. A recent meta-analysis of the first wave of the COVID-19 pandemic estimated that one-third of patients require intensive care unit (ICU) treatment, and among these, ~40% die during the hospitalisation.1 Cardiovascular disease (CVD) and accompanying comorbidities, such as obesity, diabetes and hypertension, are among the most important risk factors for severe trajectories from COVID-19.2

Soluble ST2 (sST2) is part of the interleukin-1 receptor family and a major player in immune and inflammatory responses through mechanical stress, inflammatory cytokines or necrosis.3 sST2 has become an established biomarker in heart failure (HF), reflecting myocardial inflammation, remodelling and fibrosis.4 The lungs are a relevant source of sST2 in HF and seems to play an active role in the pathological ST2 response.5 Elevated sST2 is associated with increased risk of poor outcome in acute6 and chronic7 HF
and after myocardial infarction. The prognostic information seems to be independent of established cardiac biomarkers such as N-terminal pro-B-type natriuretic peptide (NT-proBNP), cardiac troponin T and emerging biomarkers like growth differentiation factor-15 (GDF-15) and galectin-3. Accordingly, sST2 is recommended for additive risk stratification for HF in the 2017 US guidelines. sST2 also seems to have a prognostic value for additive risk stratification for HF in the 2017 US guidelines. However, the prognostic importance in COVID-19 is not clear.

The COVID-19 pandemic has caused considerable strain on ICUs and overwhelmed the capacity of hospital systems worldwide. Thus, efficient patient triage and early identification of high-risk patients is important. Our aim was to assess the pathophysiological correlates of sST2 and the association between serial measurements and outcome, in patients hospitalised for COVID-19.

**METHODS**

The COVID-19 Mechanisms (COVID MECH) Study prospectively enrolled unselected adult patients hospitalised for COVID-19 from 18 March to 4 May 2020 at a teaching hospital affiliated with the University of Oslo, Akershus University Hospital, with a primary catchment area of 560,000, corresponding to 11% of the population of Norway. Details about the study design and inclusion process has previously been published. Inclusion criteria were a positive SARS-CoV-2 real-time PCR nasopharyngeal swab and COVID-19 symptoms as the main reason for admission. Study-specific consent forms were signed by all participants or by the next of kin if the patient was unable to consent. It was not possible to involve patients or the public in the design, conduct, reporting or dissemination plans of our research due to the nature of the sudden COVID-19 pandemic. Data may be obtained on request and are not publicly available.

Clinical information was obtained from medical records by the investigators, and details about study conduction and classification of comorbidities have previously been published. The primary endpoint in the COVID-19 MECH study was the composite of death and/or admission to the ICU for >24 hours at any point during their hospitalisation. For capacity reasons during the pandemic, only patients requiring mechanical ventilation were treated in the ICU, while patients not requiring mechanical ventilation were returned to a medical ward after evaluation by an intensive care physician. Data for 30-day all-cause mortality was available for all patients.

**Laboratory methods**

Blood samples were collected by trained nurses at baseline (immediately after admission in the emergency department or early in the ICU stay for nine patients admitted directly to the ICU), day 2–5 (target day 3) and day 6–12 (target day 9). Routine clinical biomarkers, that is, white cell count, lymphocyte count, D-dimer, C-reactive protein (CRP), lactate dehydrogenase and creatinine were immediately analysed and estimated glomerular filtration rate (eGFR) calculated at the central laboratory. Biobank samples of plasma and serum were temporarily stored at 4°C, centrifuged at 2000 g for 10 min and then transferred into aliquots that were frozen and stored at −80°C. Serum samples from the biobank were used to measure interleukin-6, procalcitonin, ferritin, cardiac troponin T (cTnT), NT-proBNP and GDF-15 by the Elecsys assay on a Cobas e801 platform (Roche Diagnostics, Rotkreuz, Switzerland). SARS-CoV-2 RNA was detected in plasma by reverse transcription and quantitative PCR on a QuantStudio 7 PCR system (Thermo Fisher Scientific, Waltham, Massachusetts, USA) targeting the viral E-gene as previously described. sST2 was measured in serum samples using the Presage ST2 assay (Critical Diagnostics, San Diego, California, USA) with a quantification range from 2.35 to 200 ng/mL and a coefficient of variation of 4.3% and 4.5% (high and low concentration). Concentrations >200 ng/mL (n=3) were entered as 200 ng/mL. According to the manufacturer, concentrations in a healthy reference cohort were median 18.8 ng/mL and 95th percentile 37.9 ng/mL.

**Statistical methods**

Baseline characteristics are presented as mean±SD and n (%) and compared by linear regression for trend across quartiles of sST2. Biomarker concentrations are reported as median (quartiles (Q) 1–3) and compared using the non-parametric Kruskal-Wallis test and the Cuzick’s trend test. Correlations between sST2 and other biomarkers were assessed using Spearman rank correlation. Unadjusted and adjusted logistic regression analyses of the association between baseline sST2 and outcome (medical ward vs ICU vs in-hospital mortality) were performed. Covariates were a priori selected, that is, age, sex, race, CVD, body mass index (BMI) and eGFR. Due to the risk of collinearity and overfitting the regression models, other biomarkers were not included in the main adjusted analysis. However, in an exploratory logistic regression model, we also added the following biomarkers to the multivariable model: interleukin-6, CRP, procalcitonin, ferritin, D-dimer, cTnT and NT-proBNP. Non-parametric receiver operating curve (ROC) was analysed to assess the discriminatory performance of sST2 for combined ICU admission or mortality and for mortality only. Changes in biomarkers from baseline to day 3 and from baseline to day 9 were assessed by multilevel mixed-effects linear regression adjusted for age, sex, race, CVD, BMI and eGFR. To assess differences in biomarker changes according to outcome, an interaction term was included in the model. Concentrations of sST2 at day 3 and day 9 were associated with outcome using multivariable logistic regression with the same covariates as in for the baseline analysis. All biomarkers were log-transformed before included in regression analysis. All statistical analyses were performed using Stata Software (V.16, StataCorp).
**RESULTS**

In total, 201 patients were hospitalised with COVID-19 in the study period, and among these, 78 patients were not included in the COVID-19 MECH study due to direct discharge from the emergency department or unwillingness to participate in the study. Biobank blood samples were available from 123 of the 135 hospitalised patients with COVID-19 enrolled in the study. These were aged 59.6±15.2 years, 71 (58%) men and 68 (55%) Caucasians. Overall, 74 (60%) had ≥1 comorbidity, comprising hypertension (32%), obesity (27%), CVD (15%) diabetes (17%), chronic kidney disease (7%) and chronic obstructive pulmonary disease (5%). Symptom duration was 9±4 days; 81% had fever, 80% had cough and 70% had dyspnoea.

### Baseline sST2, clinical characteristics and other biomarkers

Baseline sST2 concentrations were median 48 (IQR 37–67) ng/mL and 91 (74%) had concentrations above the 95th percentile of healthy controls. Stratified by quartiles (Q), increasing concentrations of sST2 were associated with higher age, male sex, white race, smoking, diabetes mellitus, hypertension and chronic kidney disease (table 1). Oxygen saturation was lower and respiratory rate higher at admission with increasing sST2: mean 94% and 22/min in Q1 versus 89% and 30/min in Q4, respectively (p<0.001 for both). Increasing levels of sST2 by quartiles were significantly associated with biomarkers reflecting inflammation, coagulation, renal function, cardiac biomarkers and with SARS-CoV-2 viraemia (table 2). SARS-CoV-2 viraemia was most prevalent in patients with sST2 concentrations in the highest quartile: 63% in Q4 vs 31% for Q1–3. The strongest correlations with the established biomarkers were seen for procalcitonin (rho=0.62, p<0.001), IL-6 (rho=0.43, p<0.001), cTnT (rho=0.43, p<0.001) and ferritin (rho=0.40, p<0.001) (table 2). sST2 also correlated with GDF-15: rho=0.53, p<0.001.

### Baseline sST2 and hospital outcome

During the hospitalisation, eight patients (7%) died: four at the ICU and four with treatment restrictions in medical wards. No patients died after hospital discharge, within 30 days of study inclusion. Treatment in the ICU (all with mechanical ventilation) was given to 27 (22%) survivors. Baseline concentrations of sST2 increased in proportion to the severity of the hospital outcome (median, IQR): 43 (36–59) ng/mL for patients treated in medical wards, 67 (39–104) ng/mL for ICU-treated patients and 107 (72–116) ng/mL for non-survivors: p<0.001 for both medical ward versus ICU and medical
ward vs non-survivors (table 3; figure 1). This association persisted after adjusting for demographics, BMI, CVD and eGFR (table 3). Concentrations of IL-6, CRP, procalcitonin and ferritin were higher with increasing severity, while there were no significant associations for D-dimer, cTnT and NT-proBNP. In a regression model adding all measured biomarkers to demographics, BMI, CVD and eGFR, sST2 was the only variable associated with ICU admission or death (online supplemental table 1). In exploratory analysis also including GDF-15, both sST2 (p=0.05) and GDF-15 (p=0.015) remained associated with outcome.

The area under the receiver operating curve (ROC AUC) for discriminating combined ICU admission and hospital mortality (n=35) was 0.69 (0.57–0.80) and for discriminating hospital mortality alone (n=8) 0.84 (0.72–0.96) for baseline sST2.
Changes in sST2 and hospital outcome

Repeated measures of sST2 concentrations were performed at day 3 (n=94; 61 medical ward, 25 ICU and eight non-survivors) and day 9 (23 medical ward, 20 ICU and 6 non-survivors). The main reason for missing follow-up samples was discharge from the hospital. Two of the eight non-survivors died between day 3 and day 9 and had therefore missing samples at day 9. The overall median sST2 concentration was 47 (IQR 36–69) ng/mL at day 3 and 49 (IQR 36–83) ng/mL at day 9, which was not significantly different from the baseline concentrations (p=0.14 and p=0.29, respectively). The change in sST2 from baseline to day 3 and day 9 was different in patients with a primary outcome (ICU treatment or death) compared with those treated at medical ward (p for interaction=0.003). Patients treated in the medical ward had significant decline in sST2 concentrations to day 3 (−12%, 95% CI −19% to −5%, p=0.002) and day 9 (−23%, 95% CI −31% to −13%, p<0.001), while patients who were treated in the ICU or died had no significant change (p=0.84 to day 3 and p=0.51 to day 9). When excluding the two patients who died before day 9, the results were consistent (p for interaction=0.003). Compared with patients treated at the medical ward, concentrations of sST2 at day 3 and day 9 were higher in non-survivors or ICU-treated patients (p<0.001 and p=0.001, respectively) and ICU-treated patients only (p<0.001 and p=0.004, respectively) in adjusted models (figure 1).

DISCUSSION

In this prospective study of patients hospitalised for COVID-19, we found higher concentrations of sST2 in patients treated in the ICU and the highest concentrations in non-survivors. The association with poor outcome was independent of established risk factors, inflammatory and cardiovascular biomarkers. At day 3 and day 9, the sST2 elevation persisted in patients who were treated in the ICU or died.

In a recent proteomics study measuring 1420 proteins in patient with COVID-19, sST2 came out among the top 3 candidate proteins predicting mortality, which supports our findings of sST2 as an important prognostic marker in COVID-19.19 sST2 has been proposed as a promising biomarker in COVID-19, reflecting inflammatory status and disease severity.20 21 In our study, concentrations of ST2 correlated with inflammatory biomarkers, cardiovascular biomarkers, SARS-CoV-2 viraemia and hypoxaemia, reflecting key pathophysiological pathways in COVID-19. Being part of the interleukin family, the association with inflammation is not surprising and in line with previous findings from sepsis and acute respiratory distress syndrome (ARDS).11 13 14 sST2 is released by endothelial cells and various immune cells, and function as a decoy receptor to IL-33. The alveolar epithelial cells in the lungs are a source of ST2 in HF.5 The interplay between sST2 and IL-33 plays an important role in CVD, and accumulating evidence suggests that sST2 is a biomarker of vascular health.5 Furthermore, experimental studies have demonstrated that ST2/IL-33 activate endothelial cells and promotes inflammation.22 23 We found an association between sST2 and D-dimer, and it is well established that endothelial dysfunction and thromboembolism are cornerstones in COVID-19 pathogenesis.24 Overall, the integration of these pathways by sST2 may contribute to the strong, progressive association with the need for mechanical ventilation and in-hospital mortality. However, the exact mechanisms in COVID-19 causing the elevation in sST2 remains largely unknown and should be assessed in experimental studies. Interestingly, sST2 was the only biomarker associated with adverse outcome in models adjusting biomarkers reflecting myocardial injury and stress, inflammation and thrombosis.

We recently reported that GDF-15 was associated with poor outcome in COVID-19.15 Concentrations of GDF-15 correlated moderately with sST2 (r=0.53) and did not have the same predictors. Moreover, the association with outcome for sST2 and GDG-15 was independent of each other and other cardiovascular and inflammatory biomarkers. In contrast to sST2 concentrations that remained stably elevated throughout the hospitalisation, concentrations of GDF-15 continued to increase to day 3 and day 9 in patients with poor outcome.15 These differences indicate that sST2 and GDG-15 reflect separate disease processes in COVID-19 and may provide complementary pathophysiological information.

Limitations

The modest sample size and the lack of an external validation cohort are the main limitations in our study. Particularly, the number of patients with available samples at day 9 was low. This was primarily due to early discharge from the hospital, and we did not collect blood samples from patients after discharge. Still, given the coherent findings from proteomics analysis of COVID-1919 combined with
the robust prospective design of our study, we believe our findings are clinically relevant. However, the findings should be validated in more diverse populations and settings before being implemented in clinical practice. Although clinical characteristics and ICU admissions were comparable with other COVID-19 cohorts, the in-hospital mortality for COVID-19 was relatively low in our hospital during the first wave of the pandemic, and this may limit the generalisability to centres with higher mortality rates.

Our multivariable regression models may be overfitted given the limited number of events compared with the number of covariates, but we believe the consistent results with the unadjusted analysis strengthen these findings. We analysed eight inflammatory and cardiovascular biomarkers and present their association to outcome in the current study. We did not use statistical methods to account for multiple comparisons, as our main objective was to investigate sST2 as a prognostic marker in COVID-19. Of note, the association between sST2 and outcome remained significant in all models when using a Bonferroni corrected p value (0.05/8=0.006) as the significance level.

CONCLUSIONS

We found an association between increasing levels of sST2 and disease severity among patients hospitalised for COVID-19. The association was independent of, and superior to, established risk factors. sST2 associated with SARS-CoV-2 viraemia and biomarkers of inflammation, CVD and thrombosis. sST2 remained elevated in patients with poor outcome at day 3 and day 9 of the hospital stay. sST2 seems to be a clinically useful predictor in COVID-19, but our findings should be confirmed in larger studies before implementation to patient care.

Twitter Peder Langeland Myhre @pmyhre

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Patient consent for publication Consent obtained directly from patient(s)

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Data availability statement Data may be obtained from a third party and are not publicly available.

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ORCID iD

Peder Langeland Myhre http://orcid.org/0000-0002-5871-1804

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