A Prospective Observational Study on Assessment of the Soluble Urokinase Plasminogen Activator Receptor in Adult Patients Undergoing Major Non-cardiac Surgery (SPARSE): Study protocol

(SPARSE: if our hypothesis is correct, the SuPAR will help to investigate whether the preoperative suPAR level can aid in identifying patients at high risk for postoperative complications, morbidity and mortality.)

BACKGROUND

Soluble urokinase plasminogen activator receptor

The biomarker soluble urokinase plasminogen activator receptor (suPAR) is the soluble form of the cell membrane-bound protein urokinase plasminogen activator receptor (uPAR), which is expressed mainly on immune cells, endothelial cells, and smooth muscle cells. uPAR is released during inflammation or immune activation, and therefore the suPAR level reflects the extent of immune activation in the individual. All human beings have a baseline level of suPAR that is individually determined and increases with age. Studies have shown that the suPAR level is
associated with morbidity and mortality in a number of acute and chronic diseases and in the general population.\textsuperscript{2-15} The suPAR level is elevated across diseases, and not solely associated with one specific disease. Therefore, suPAR is applicable as a prognostic marker and not as a diagnostic marker. This characteristic may be utilized for risk stratification in unselected patients.

Originally, uPAR was proven a receptor for urokinase (uPA) which splits plasminogen into active plasmin. Moreover, uPAR interacts with other proteins and plays a role in several important cell processes like migration, adhesion, angiogenesis, proliferation, and chemotaxis.\textsuperscript{1} The suPAR protein was discovered in 1991, when it was found to be a marker of cancer progression.\textsuperscript{1} In recent years, several studies have shown that suPAR is associated with a number of chronic diseases (including cardiovascular, hepatic, renal, and pulmonary diseases), and that the level is a predictor of a negative outcome of various infectious diseases (tuberculosis, HIV, malaria, sepsis, meningitis, pneumonia) and in critically ill patients.\textsuperscript{2-12, 15, 17-21}

Across diseases, the suPAR level discriminates non-survivors from survivors. suPAR reflects the level of chronic inflammation, and therefore it has been studied as a potential marker of development of diseases, and studies have shown that an elevated level predicts development of chronic diseases and cancer in the general population.\textsuperscript{2-15} The suPAR blood level is stable with no diurnal variation and no changes following fasting, while suPAR can be measured in blood, plasma, urine, cerebrospinal fluid, ascites fluid and pleural fluid.\textsuperscript{1} The level increases and decreases with progression and improvement of a disease, respectively, but more slowly compared to e.g. C-reactive protein (CRP). The normal suPAR plasma level is 2-3 ng/mL in healthy individuals, about 3-4 ng/mL in unselected patients in emergency departments, and about 9-10 ng/mL in critically ill patients.
**suPAR in intensive care**

In critically ill patients, the suPAR level is significantly increased. suPAR is an independent prognostic marker, and the change over time correlates with organ dysfunction. suPAR is elevated and has a prognostic value in patients with: SIRS (systemic inflammatory response syndrome),\(^{22,23}\) sepsis/septic shock,\(^{24-30}\) burn injuries,\(^{31}\) and traumatic brain injuries.\(^{32}\) The suPAR level reflects the body’s immune response to infections, and the level increases with the severity of the infection. In patients with organ dysfunction, the suPAR value is often a two-digit value. In particular hepatic and renal dysfunction affects the suPAR level.\(^{24-26}\) suPAR has been studied in patients with SIRS who were acutely admitted to the emergency department (n=902). The studies showed that suPAR is a stronger marker of 2-day, 30-day, and 90-day mortality than age, CRP, IL-6, creatinine, and procalcitonin. However, for diagnostic purposes, IL-6 and CRP are superior to suPAR in predicting a positive blood culture.\(^{22,23}\) A Greek multicenter study including 1914 patients with sepsis showed that suPAR is a strong predictor of mortality, and that a suPAR level above 12 ng/mL is linked to a >80% sensitivity for mortality and a negative predictive value of 94.5%.\(^{27}\) In addition, the prognostic value of suPAR in patients with sepsis is independent of relevant covariates like APACHE score, CRP, etc.\(^{27-30}\)

In patients with burn injuries and inhalation trauma requiring mechanical ventilation, the plasma suPAR level and bronchoalveolar lavage fluid level correlate to IL-6 and coagulation factors. An elevated plasma suPAR level is associated with prolonged Intensive Care Unit (ICU) stay and the duration of mechanical ventilation.\(^{31}\) The suPAR level is elevated in patients with traumatic brain injury. In trauma patients who suffered a brain injury within 12 hours prior to blood sampling, the mean suPAR level is 14.9 ng/mL ± 6.9 vs. 2.8 ng/mL ± 0.7 in control subjects.
these patients suPAR is associated with severity of the brain injury and with mortality.\textsuperscript{32}

**suPAR in surgery**

The suPAR level is elevated in patients with infections, chronic diseases, and cancer compared to healthy individuals. A high suPAR level is associated with increased mortality risk,\textsuperscript{33} poor prognosis,\textsuperscript{34-38} postoperative pneumonia,\textsuperscript{39} and prosthetic joint infection.\textsuperscript{40} SuPAR is a well-studied biomarker predicting prognosis, disease severity, and organ dysfunction and is being considered as a marker of the individual’s inflammatory status. It has been demonstrated that biomarkers are able to improve triage and are effective in identifying high and low risk patients among acutely admitted patients.\textsuperscript{41} Improving the preoperative risk stratification using biomarkers may optimize the patient's clinical outcome.\textsuperscript{42}

Available data on use of biomarkers in addition to risk stratification are observational data, and suPAR has mainly been studied in medical and oncological patients. Gastric surgery patients and orthopedic surgery patients were included in a study conducted in the emergency department at Hillerød Hospital, Denmark. The TRIAGE study included 5992 unselected patients and confirmed the prognostic value of suPAR regarding mortality, and found it similar in both medical and surgical patients.\textsuperscript{41} In the same study, it was shown that triage based on suPAR level was superior to the current triage system in predicting 30-day mortality: AUC 0.84 (0.82-0.87) vs. 0.62 (0.58-0.66), respectively. In multivariate analyses of 30-day mortality in relation to suPAR quartiles, adjusted for sex, age, CRP, leucocytes, and triage category, HR was 1.0, 2.2, 8.3, and 26.9 in the upper quartile. A high suPAR level has
been demonstrated in both tumor tissue and in blood, and in several cancers, the suPAR level is shown to correlate with a poor prognosis. In a few studies, suPAR has been studied as a potential biomarker in gastric surgery.

In a cohort of 518 elective colorectal cancer patients, preoperative measurement of the suPAR level was performed. In multivariate analyses adjusted for age, sex, tumor classification, and localization, suPAR was significantly associated with mortality, HR 1.74 (1.33-2.26; p<0.0001). In addition, the suPAR level was associated with tumor stage and localization; and in colon cancer patients the suPAR level was significantly higher compared to rectal cancer patients. The same cohort was also followed in another study, in which the suPAR plasma level was found to be an independent prognostic marker. To identify risk patients among elective colon cancer patients the suPAR level was studied. In patients receiving blood transfusion during surgery, the suPAR level was higher, and a significant association between the suPAR level and postoperative infections was shown. Occurrence of pneumonia was significantly associated with the suPAR level, but any significant association with other infectious complications could not be found. In patients with gastric cancer, the suPAR level was significantly higher compared to healthy controls (2.3 ng/mL ± 0.77), and the suPAR level was significantly higher in cancer patients with metastatic disease (7.0 ng/mL ± 6.1) than in patients with no metastases (4.8 ng/mL ± 4.4). In the group of patients with a suPAR value above 5.2 ng/mL, the mortality was significantly increased. In patients with rectal cancer and colon cancer, a similar prognostic value is found, indicating an increased mortality risk.

The diagnostic value of suPAR in prosthetic knee/hip joint infection has been examined in a study. The study included 80 patients of which 45 experienced prosthetic joint infection defined by presence of clinical signs (swelling, redness,
tenderness, and pus inside the joint) and a positive culture. In these patients, a significantly higher median suPAR level (6.8 ng/mL) was found compared to patients without infection, who had revision surgery done. Furthermore, suPAR was positively correlated with CRP, and the study showed that suPAR was more precise in diagnosing prosthetic knee/hip joint infection than CRP.

**AIM**

SPARSE is a single-center observational study aiming to investigate if suPAR measured preoperatively and immediately after surgery can predict the risk of future complications and post-operative mortality in adults following major non-cardiac surgery.

**METHODS**

**Design**

This is a prospective observational study designed in accordance with the declaration of Helsinki. The study will be register at Clinical Trials.gov and has been approved by the Institutional Review Board of the University Hospital of Larisa, under the reference number 60580/11-12-2018.

**Patient eligibility**

Consecutive patients who are scheduled to undergo elective major non-cardiac surgery with expected duration \( \geq 2 \) hours under general anesthesia will be screened for
inclusion. All operative approaches will be eligible for inclusion, including open, laparoscopic and robotic procedures. Patients fulfilling the following inclusion criteria will be included: age ≥ 18 years, and American Society of Anesthesiologists’ (ASA) physical status I to IV.

Exclusion criteria will be age <18 years, any infection within the previous 4 weeks, severe liver disease, patients on renal replacement therapy pre-operatively, patients who had previously received a transplant, patients with allergies, inflammatory or immune system disorders, and/or connective tissue disease including rheumatoid arthritis, ankylosing spondylitis, and systemic lupus erythematosus, administration of steroid, antipsychotic, or anti-inflammatory/immunomodulatory medication during the 3 months prior to surgery, administration of opioids during the past week, asthma, obesity (BMI ≥ 30 kg m⁻²), mental disability or severe psychiatric disease, alcohol or other abuse, legal incapacity or limited legal capacity, and subjects within the exclusion period of another study.

**Management of Anesthesia and Surgical Procedures**

Endotracheal intubation and anesthetic care will be performed according to our institutional routine. Intravenous induction of general anaesthesia will include midazolam 0.15-0.35 mg/kg iv over 20-30 seconds, fentanyl 1μg/kg, propofol 1.5-2 mg/kg, ketamine 0.2 mg/kg (intravenous bolus), and rocuronium 0.6 mg/kg. All drugs will be prepared in labelled syringes and induction will be achieved by administration of a predetermined iv bolus dose on the basis of the patient’s weight and/or age. Laryngoscopy and intubation will proceed in a standard fashion, while the position of the endotracheal tube will be confirmed by auscultation and
capnography/capnometry. The patients will then connected to an automated ventilator (Draeger Primus®; Drägerwerk AG & Co., Lübeck, Germany).

All patients will be ventilated using a lung-protective strategy with tidal volume of 7 mL/kg, positive end-expiratory pressure of 6-8 cmH₂O, plateau pressures <30 cmH₂O, and recruitment maneuvers repeated every 30 min after tracheal intubation.⁴⁴ Maintenance of general anesthesia will include desflurane 1.0 MAC with 40% oxygen and 60% air, while intraoperative dose changes will be left to the anesthesiologist in charge of the patient. Depth of anesthesia (bispectral index-BIS, Covidien, France) will be monitored, with the target ranging between 40 and 60.⁴⁵,⁴⁶ Normocapnia will be maintained by adjusting the respiratory rate as needed, while normothermia (37ºC) will be maintained throughout the intraoperative period.

All patients will be operated by at least two consultant surgeons who will be supervised by a Professor of Surgery.

**Sampling and laboratory measurements**

Participants will undergo sampling of peripheral venous blood, immediately after arrival to the OR, and at the Post-Anesthesia Care Unit (PACU). Blood samples drawn from all patients and EDTA plasma will be stored at -80º C until later measurement. Plasma suPAR levels will be determined using the suPARnostic® quick triage lateral flow assay (ViroGates, Denmark), according to the manufactures instructions.
Microcirculation flow analysis

In addition to routine hemodynamic data, sublingual microvascular flow will be measured using noninvasive technology. We will obtain measurements preoperatively (PRE), intraoperatively (INT), and postoperatively after arrival in the PACU (POST). At each time point, we will image the microcirculatory network of the sublingual mucosa with sidestream darkfield (SDF+) videomicroscopy which has an increased optical resolution resulting in one pixel recording an area of 0.56 μm². This device uses the new SDF+ technology that allows the red blood cells to be visualised with greater accuracy and detail, which is essential for enhancing the capabilities of the analysis application.

We will use the sublingual space as the site of imaging because it shares the same embryologic origin as the splanchnic mucosa and can reflect derangements in splanchnic blood flow; impaired sublingual microcirculatory blood flow is associated with increased mortality and worsening organ failure in patients with sepsis.47-51

Outcomes

The primary endpoint will be the presence of complications and/or admission to ICU and/or mortality within the first 60 postoperative days.

Secondary endpoints will be intraoperative complications (including desaturation [SpO₂ <92% for 3 minutes or more], need for unplanned recruitment maneuvers, hypotension [defined as systolic blood pressure <90 mmHg or mean arterial pressure <65 mmHg for 3 minutes or more or need of vasoactive drugs for correction], need for unplanned vasoactive drugs [need for vasoactive drugs not
planned before and/or continuous infusion], and/or acute new arrhythmia [atrial fibrillation, sustained ventricular tachycardia, supraventricular tachycardia, and/or cardiac arrest]), reintubation, hospital length of stay, and length of stay in ICU. Secondary endpoints will also include postoperative complications within 90 days after surgery defined as in the SURPAS studies, with the inclusion of atelectasis and ileus, resulting in 22 possible postoperative complications, as well as survival at hospital discharge, at 90 days, and at 1 year.

**Data Collection and Monitoring**

Data analysis will be based on predefined data points on a prospective data collection form. The staff will be blinded to measurements until the end of the study and all data are analyzed. Clinical monitoring throughout the study will be performed to maximize protocol adherence, while an independent Data and Safety Monitoring research staff will monitor safety, ethical, and scientific aspects of the study. Data collection will include demographics, anesthesia parameters, C-reactive protein, P-POSSUM score, ACS-NSQIP score, APACHE II, SOFA, and the Charlson Age-Comorbidity Index (Charlson score). We will use a SAS macro based on ICD-10 diagnoses to calculate the Charlson score; Two other simpler models adding predictive value to the ASA classification will be also used; the Surgical Mortality Probability Model and a similar model proposed by Glance and Donati.
Data management

The goal of the clinical data management plan is to provide high-quality data by adopting standardized procedures to minimize the number of errors and missing data, and consequently, to generate an accurate database for analysis. Remote monitoring is performed to signal early aberrant patterns, issues with consistency, credibility and other anomalies. Any missing and outlier data values are individually revised and completed or corrected whenever possible.

Sample size and predefined statistical analysis plan

Patients will be consecutively included during the study period, in a convenience sample and will be stratified into low-risk or high-risk groups, according to suPAR level (suPAR_{high} above and suPAR_{low} below 5.5 ng/ml), respectively. An a priori power analysis was done set to detect a difference of 0.50 on the suPAR (between suPAR_{high} and suPAR_{low} group, based on the researchers’ preference. The number of patients planned for recruitment was rounded up to 100 to allow for flexibility in the study. This sample size was estimated to achieve 80 percent power to detect the suggested difference. The sample size calculation was made using G*Power 3.1. The association between ASA classification, suPAR level, CRP and the rate of postoperative complications will be analyzed with logistic regression and Cox regression analyses, estimating odds ratios (ORs) and hazard ratios (HRs).
Ethics and dissemination

The study will be performed according to national and international guidelines. The study will not begin until approval has been obtained from the Hospital’s Institutional Review Board, according to local regulation. Prospective written informed consent will be or will be not requested before inclusion of all eligible patients based on the Review Board’s decision; if a waiver of consent is decided by the Review Board, it will follow local guidelines.

Competing interests

None declared.
References


42. Biccard BM, Devereaux PJ, Rodseth RN. Cardiac biomarkers in the prediction of risk in the non-cardiac surgery setting. Anaesthesia 2014;69:484-93.


45. Kertai MD, White WD, Gan TJ. Cumulative duration of "triple low" state of low blood pressure, low bispectral index, and low minimum alveolar concentration of volatile anesthesia is not associated with increased mortality. Anesthesiology 2014;121:18-28.


56. Januel JM, Luthi JC, Quan H, et al. Improved accuracy of co-morbidity coding over time after the introduction of ICD-10 administrative data. BMC Health Serv Res 2011;11:194.


