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Introduction:

Acute high-risk abdominal surgery (AHA) is performed in hospitals worldwide. Ethiologies are heterogeneous, but overall emergency surgery carries a very high mortality rate (1)(2). In particular, emergency laparotomies performed on elderly people has a high mortality rate(3)(4). Different quality improvement programmes for that particular patient group have been suggested, but the quality of care and thereby mortality varies considerably between hospitals(5)(6). The use of postoperative intensive care and monitoring seem to be inadequate for this high risk population in a variety of hospitals(1)(7)(8). It is of paramount importance to identify the frailest and most acutely deranged patients, who are in risk of poor outcome, in order to allocate resources for improved monitoring and optimisation postoperatively. It has been shown that failure to rescue patients after having developed postoperative complications and inability to escalate care intensity affects the outcome. Organisation, teamwork and culture is important in the postoperative phase to be able to escalate care especially in standard care wards(9)(10). However, it is found difficult to predict which patients will experience complications in a standard care ward.

Different risk assessment tools have been proposed for patients undergoing AHA(11)(12). The APACHE-II score, even though developed for critical care, seems to be the one with best prediction of outcome. Risk assessment tools are important to support clinical decision making by the perioperative team as subjective clinical assessment often underestimates the risk for the patients in highest risk of complications and death(13). Good clinical decision-making is likely to improve the clinical outcome by allocating appropriate resources. Prognostic tools are also useful to inform patients about what to expect in the immediate postoperative phase and of long-term outcome. Especially in the elder population with increased risk of long hospitalisation and loss of function or independency this can be useful to give informed consent to treatment strategy. Furthermore, good risk assessment is

important to optimize palliative care after end-of-life decisions, which is often ignored in research, but highly relevant in clinical work.

During recent years use of prognostic biomarkers in other high mortality populations have received much attention for risk stratification(14). An ideal biomarker should be readily available upon decision-making, easy to measure, reliable and biologically stable. Furthermore it has to be able to accurately differentiate prognosis for patients to have value in the clinical decision-making and guide the treatment of the patient. It should be linked to the clinical outcomes.

We aim to identify serological AHA biomarkers that are prognostic or predictive for postoperative morbidity, mortality and length of hospitalisation.

Potential biomarkers:

Inflammation markers:

Soluble urokinase-type plasminogen activator receptor (SUPAR) is a relatively new potential biomarker for inflammation. It is related to mortality in critically ill patients(15) especially in combination with the APACHE II score(16)(17). SUPAR is thought to be related to the inflammation reaction and endothelial dysfunction affected in critically ill patients(18). It is furthermore related to cardiovascular disease and subclinical organ damage(19) and is shown to be relatively stable throughout the day without circadian variations. It is related to all-cause mortality in major cohorts from different parts of the world(20). It has been suggested that the biomarker can be used to strengthen risk stratification for patients presenting acutely at the emergency department(21).

IL-10, IL-6, Tumor Necrosis Factor alpha, IP-10 are other markers of inflammation that regulate the inflammatory response to disease. It is proposed that increased synthesis or reduced metabolization of IL-6 is related to poor outcome in sepsis(22).

Troponin:

Troponin is a well-established biomarker for myocardial infarction. Recent studies show that it is related to the APACHE II score and could be useful as a biomarker for overall mortality in critically ill patients with severe sepsis(23)(24)(25).

Endothelial dysfunction

Markers of endothelial function have been suggested to predict poor outcome. Syndecan-1, a transmembrane endothelial proteoglycan, which in serum is a marker of glycocalyx degradation, is related to mortality in trauma patients(27).

Endocrine and other markers

The endocrine system is highly involved in the surgical stress response and imbalances in hormonal response to stress could potentially predict outcome. Especially catecholamines are involved in the inflammatory response and related to endothelial dysfunction(27).

Furthermore, combination of different molecular biomarkers could be of interest to create the best possible molecular risk assessment tool(14).

Methods

Manuscripts will be prepared using the STROBE-ME statement guidelines(29).

Approvals and ethical considerations:

Danish legislation "Lov om behandling af personoplysninger" will be followed and the study will be reported to the Danish Data Protection Agency.

Data used in these studies are collected in the quality improvement program "Acute High-Risk Abdominal Surgery study – an optimized Perioperative Course (AHA)" (ClinicalTrials.gov Identifier: NCT01899885) approved by the Danish Data Protection Agency (jr.no. 2007-58-0015) **HVH-2013-052** in 2013. In this program patients were enrolled in a standardized protocol of perioperative optimization. The standardized protocol included collection of blood samples for future research.

Patients were informed about the AHA protocol and gave informed consent where possible. If patients were in a state where informed consent was not possible informed consent was sought from relatives.

As this was a qualitative improvement programme, it did not require approval by an ethical committee according to Danish law. Blood samples were drawn and collected with the standard preoperative tests and stored at -80C in a biobank.

We will establish a new research bio bank based on material from the samples collected in relation to the standard treatment according to the AHA-protocol (one EDTA-tube and one tube with coagulation enhancer). The research biobank will be reported to the Danish Data Protection Agency. After approval the biological material will be kept in the research biobank until the end of the study, at which time the samples have been used entirely or will be transferred back to the biobank from which they originally were taken. The present study is expected finalized by May 1, 2022.

Analysis of the samples from the biobank will require approval by the Ethics Committee including dispensation from obtaining consent from involved patients. Retrospective consent is impossible to obtain as a large proportion of patients have died since the data collection started, and analysis of the data set without the patients, who have died is worthless as a predictive tool. Research on this vulnerable population with high mortality and morbidity is very limited. It is therefore an important ethical aspect that the collected data can be used for research, which may be beneficial for future critically ill, emergency patients.

Approval for these studies will be applied from the Ethics Committee of the Capital Region of Denmark. Referral of data from the AHA quality improvement project to this study will be registered and approved by the Danish Data Protection Agency. The biological material and non-anonymous data, from both the research bio bank and the samples collected in relation to standard treatment, will the destroyed when the approval from the Danish Data Protection Agency expires.

We will search the national register for usage of biological tissue, "Vævsanvendelsesregistret", for patients who only wish their tissue is used for their own treatment. Samples from these patients will not be included in this project.

Risks, side effects and harms

Potential harms for the participants are limited as the research is focused on preoperative risk assessment and not long-term prediction of future diseases. There is therefore a low risk of findings that may affect the participants. The biomarkers we wish to study have in similar studies of critically ill patients not shown any random findings affecting the participants.

Population

We will include patients with a preoperative blood sample stored in the bio bank; the patients did undergo AHA at Hvidovre Hospital, Copenhagen, Denmark, from June 1st 2013 when the AHA study protocol was initiated.

Inclusion criteria were: Patients aged 18 or older with the suspicion for abdominal pathology requiring immediate emergency laparotomy or laparoscopy including reoperations after elective surgery and reoperations after previous AHA surgery.

We excluded the following procedures: appendectomies; negative laparoscopies and laparotomies; cholecystectomies; simple herniotomies following incarceration (without intestinal resection); reoperation due to fascial separation with no other abdominal pathology; internal herniation after roux-en-y gastric bypass surgery; sub-acute surgery (planned within 48 hours) for inflammatory bowel disease; and sub-acute colorectal cancer surgery.

We excluded, traumas, pregnant woman, uro-genital, gynaecological and vascular pathology except for mesenteric ischemia.

Data collection

Data on mortality was obtained using the Danish Civil Registration System(30). This register is maintained by the Danish government and assigns a unique personal identification number to all Danish citizens. It contains data on address, immigration emigration, gender, date of birth and exact day of death. It is updated within days of any change in information.

Other data were collected in the medical records of patients. Complications were defined using the Clavien-Dindo Classification for Surgical Complications(31).

Outcome measures

Primary outcomes are short and long-term mortality (30 days and 180 days respectively) and major complications during index hospitalization (Clavien-Dindo >2). Secondary outcomes are length of hospitalisation, length of intensive care admission as well as perioperative need for inotropic or vasopressor support and high volume fluid resuscitation.

Description of biological material

Blood samples were collected at the time of induction of anaesthesia. For AHA-patients a pre-operative optimisation protocol was introduced in the hospital throughout the data collection period, which stated that the patient was optimized with goal directed fluid therapy before induction of anaesthesia. The blood samples were usually taken from the arterial line used for invasive blood pressure monitoring but some were obtained from venous puncture with standard preoperative tests.

The blood samples were collected in tubes with EDTA as anti-coagulant for plasma and in tubes with coagulation enhancer for serum. After centrifugation for 10 mins at room temperature the plasma and serum, respectively were transferred to freezing tubes for subsequent long-term storage at -80C.

Biochemical analysis

A predefined biochemical analysis protocol for each biomarker will be followed. The methods used will be described in the specific published studies.

Statistical analysis protocol

Data will be analysed using the SPSS, STATA, R or SAS software. Tests will be two-sided with $p<0.05$ considered to be significant.

Missing data will be assessed using Little's test for data missing completely at random(32). If data is not missing completely at random multiple imputation will be performed(33).

All patients or random samples (with a size defined by a preoperative sample size estimation) from the AHA cohort with blood samples taken preoperatively will be included in the analysis. Baseline characteristics will be presented and for biomarkers with continuous data patients will be stratified into quartiles or tertiles. Characteristics of populations will be presented as frequencies for categorical variables and medians with inter-quartile rage for continuous variables. Differences between groups will be presented using appropriate statistical tests. Kaplan-Meier curves will be produced for the primary outcomes and compared using the log-rank test. COX-regression will be used to perform multivariate analyses adjusted for potential confounders with Bonferroni correction.

Receiver operating characteristic curves for each biomarker will be produced for the primary outcomes. Area under the curve will be measured to evaluate the prognostic value of the biomarkers.

Limitations of the study

Data collection during clinical work including blood samples requires resources and is susceptible to selection and information bias. It is possible that resources for collecting blood samples correctly where reduced for the most unstable patients at the time of induction of anaesthesia. Patients who were considered too frail to undergo surgery, where a palliative or conservative approach was chosen, were not included in this study. Postoperatively, patients in the AHA-cohort were assigned to postoperative intensive monitoring in the post-operative care unit if they had American Society of Anaesthesiologist physical performance score(34) above two. This stratification is susceptible to information-bias because of inter-observer variation(35).

The data is collected in a single medical facility with and optimized care protocol for AHA and can therefore not uncritically be applied to other populations.

Economical support and budget:

The clinical study as well as establishment of the biobank has been supported by a grant from the Capital Region of Denmark (Region H). The cost of biochemical analysis will be covered by the Department of Clinical Biochemistry, Hvidovre Hospital. Other expenses will be covered by the AHA research group, Department of Anaesthesiology and Intensive Care and Department of Surgical Gastroenterology, Hvidovre Hospital.

Publication of results:

Results from the studies will be published in medical journals indexed in the PubMed/EMBASE database.

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