

ALBUMIN MASS BALANCE IN LIVER TRANSPLANTATION

An open exploratory study

Product: *None*

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2 SUMMARY

PROTOCOL IDENTITY AND AIM

| | |
|-----------------|--|
| NCT-number | NCT xxxxx |
| Protocol title: | Albumin mass balance in liver transplantation. |
| Study aim: | Assess the losses of albumin from circulation during and after liver transplantation and identify predictors of albumin loss |

METHODOLOGY

| | |
|----------------------------|--|
| Study design: | Open explorative physiological study |
| Primary research question: | Is cumulative perioperative albumin shift from plasma positive at postoperative day 7? |
| Effect parameter: | Albumin mass balance |
| Safety parameters: | Vital parameters |

INVESTIGATED POPULATION

| | |
|-----------------|---|
| Study subjects: | Patients undergoing liver transplantation |
| Number: | 240 |

TIME PLAN

| | |
|-------------------------|---------|
| First patient included: | Q1 2018 |
| Last patient included: | Q1 2021 |
| Last patient finalized: | Q2 2021 |

3 ABBREVIATIONS

| Abbreviation | Unit | Explanation |
|---------------------|-------------|---------------------------------------|
| ASR | mg/kg/day | Absolute Synthesis Rate |
| Bq | | Becquerel, radiation dose |
| CPAS | g | Cumulative perioperative albumin loss |
| CRF | | Case report form |
| D ₅ -Phe | | Deuterium labeled phenylalanine |
| FSR | %/day | Fractional Synthesis Rate |
| MELD | | Model for End-Stage Liver Disease |
| MPE | | Mol percent excess |
| POD | | Post operative day |

4 ADMINISTRATIVE INFORMATION

4.1 PRIMARY INVESTIGATOR

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5 BACKGROUND

5.1 ALBUMIN IN VOLUME RESUSCITATION

Albumin is a major constituent of plasma proteins, contributing to the colloid osmotic pressure in the capillaries [Kaysen 1998, Margaron 2002]. The association between massive volume overload and poor outcome in surgery and critical illness has contributed to the popularity of colloid solutions for volume resuscitation for the last 50 years. It might seem logic to use exogenous albumin in situations of circulatory instability, in particular when plasma albumin concentration is low. The background is that volume resuscitation employing a colloid like

albumin may be achieved with less than half the fluid volume as compared to isotonic crystalloid solutions, which has been demonstrated repeatedly in healthy subjects [Groeneveld 2011]. In situations characterized by low blood pressure, the immediate effect upon blood pressure of a colloid like albumin is also very convincing in a short time perspective [Margaron 2002]. However, in blinded randomized studies of patients with severe sepsis or septic shock, the volume needed to resuscitate is not dramatically different between groups given a colloid based regimen as compared to a crystalloid based regimen [Annane 2013, Finfer 2004, Myburgh 2012, Perner 2012]. In addition, there is no consistent outcome advantage for the patients randomized to be given a colloid containing protocol [Delaney 2011, Dellinger 2013, Perel 2013].

The explanation sought for this discrepancy between the situation in healthy subjects and patients with an assumed increase in capillary leakage on one hand, and the short term obvious effect seen on for example blood pressure after colloid administration, has been efforts to select the patients that are most compromised in terms of circulatory shock [Caironi 2014].

Today, after the recommendation against the use of hydroxyethyl starches as a synthetic colloid of the European Medical Agency for safety reasons, albumin containing solutions remain the only alternative when the use of a colloid is preferred. This is also reflected in existing international guidelines that recommend considering the use of albumin containing solutions when resuscitation with crystalloid solutions only seems insufficient [Rhodes 2017]. This is despite the absence of solid evidence for this treatment strategy in long term outcomes.

5.2 ALBUMIN IN LIVER TRANSPLANTATION

End-stage liver failure is associated with high mortality, unless treated by liver transplantation. Almost 100 liver transplantations are performed yearly at Karolinska University Hospital Huddinge. Of these, 5-10 recipients are children, and 10-20% of the patients will stay in the ICU for more than 3 days after the liver transplantation. Some of these patients have large losses in drains and in children these losses can amount to 10-30% of total body weight per day. There is very sparse literature or evidence for guidance in the treatment of these patients. At our unit, fluid losses are largely treated by infusions containing albumin, but there is little scientific support for this routine. A more systematic assessment of these patients would be of great value for future guidelines.

In a recent pilot study on selected patients undergoing liver transplantation (EPN D-nr 2015-1048-31/2), we found a cumulated loss of albumin until the 3rd post-operative day (POD3) of 48 ± 33 g. There was a wide variation but in contrast to patients undergoing major abdominal surgery [Norberg 2016] the loss seemed to persist from end of surgery to end of study. Furthermore, albumin synthesis rate was recovered at POD3 in this cohort of patients. This pilot study demonstrated the feasibility of a concept using albumin mass balance and measurement of synthesis rate of liver export proteins (albumin and fibrinogen) to characterize albumin losses from the plasma compartment and albumin physiology. The pilot study excluded patients with major peroperative bleeding, re-operations and other risk factors for a complicated post-operative course. Therefore, in order to extend both the internal and external validity of our observational study, we plan to recruit consecutive patients undergoing liver transplantation and to prolong the albumin mass balance part of the study primarily until POD7, but if applicable to POD21 or hospital discharge.

Isotopic analysis of albumin synthesis rate will be performed in the above mentioned patients that are in the ICU at POD4 and might be repeated after 3-10 days. Although albumin synthesis increases in connection with inflammation, liver transplantation represents a special case in several ways. Some degree of ischemia and liver cell injury is always present after

surgery, possibly affecting synthesis capacity for some time. Bleeding and fluid shifts are sometimes great, and losses in drains after surgery might be profound. The use of immunosuppressive drugs bound to albumin makes interpretation of drug concentrations difficult if plasma albumin varies too much over time. In our hospital a plasma albumin of 25 g/L is routinely used as target post-operatively.

5.3 ALBUMIN MASS BALANCE CALCULATIONS

By rigorous measurement of losses and gains of albumin during and after surgery, i.e. assessing bleeding, losses in drains, urine etc and measuring hemoglobin and albumin in plasma samples and in all blood products given, it is possible to calculate the loss of albumin from plasma that is not accounted for by other means. This is the cumulative perioperative albumin shift (CPAS) that represents albumin lost from the blood by capillary leakage or catabolism.

In major gynecological cancer surgery protein loss was reported as 49 g [Rehm 1998], and after major abdominal surgery cumulative perioperative albumin shift was 19 g (range 2-61) [Norberg 2015]. In liver transplantation the perioperative albumin loss was 37 ± 17 g (EPN D-nr 2015-1048-31/2, preliminary results) at end of surgery.

Blood sampling outside clinical routine for albumin mass balance calculations will not exceed 40 ml during a 21 day period. For children there is no study specific blood sampling at all for the mass balance analysis.

5.4 ALBUMIN DE NOVO SYNTHESIS

Albumin is synthesized in the liver at a rate of 150 mg/kg/day, or 13 g/24 h [Barle 2002]. This corresponds to a fractional synthesis rate of 6-8 %/24 h in a healthy subject with a plasma volume of 3 liters and 120 g of albumin in the intravascular space [Kaysen 1998]. In addition there is another 180 g of albumin in the interstitial space. These two compartments are in equilibrium by capillary leakage of 5 % per hour that is returned to the vascular space through the lymph flow. In states of an increased capillary leakage or circulatory instability, these figures may be dramatically altered [Margaron 2002].

Albumin synthesis rate is determined by incorporation of isotopically labeled amino acids into albumin [Ballmer 1990]. Today the most common technique is to use deuterated phenylalanine given as a flooding dose. De novo synthesis of albumin is stimulated by food intake, while it is decreased in states of liver insufficiency [Caso 2007, Ballmer 1996].

The blood sampling volume for one measurement of albumin synthesis will be 20 ml.

5.5 MARKERS OF ENDOTHELIAL INJURY AND INFLAMMATION

The mechanism of albumin loss is not well uncovered but increased capillary leakage in connection with fluid overload and inflammation are likely to contribute. Several substances have been suggested as markers of endothelial injury. Commercial enzyme-linked immunoabsorbant assay (ELISA) kits are available for syndecan-1, hyaluron, heparansulphate etc. They represent components of the glycocalyx that are suggested to be flaking in inflammation or injury. Heparin binding protein has been suggested as a mediator of capillary leakage and is also measured by ELISA. Interleukins 6, 8 and 10 are early markers of inflammation and are likewise measured by ELISA. CRP is a too slow parameter to be of relevance in this context, but will be routinely measured and presented.

The field is rapidly developing and possible new ELISAs in the field of glycocalyx function, injury or inflammation might be considered for this investigation.

The blood sampling volume for one measurement by ELISA will be 4 mL for adults and 2 ml for children. This is the only blood sampling in children.

6 STUDY AIMS

6.1 MAIN STUDY

The primary aim of the main study is to measure if the cumulative perioperative albumin shift is normalized on POD 7 in patients undergoing liver transplantation at Karolinska Huddinge.

Secondary aims are to compare the loss at POD 7 with that at EOS and POD 3, the albumin loss at hospital discharge, and to explore predictors of increased albumin shift from plasma.

6.2 PLANNED SUB STUDIES

Within this heterogeneous group of patients we plan to do subgroup analyses in some pre-defined groups. In addition we plan to study synthesis rate of albumin, the capillary leakage and lymphatic return as secondary outcomes.

Sub study A, Children < 18 years

The aim is to find if cumulative perioperative loss of albumin from plasma is positive at EOS, POD3 and POD7 respectively, after liver transplantation.

Sub study B, Adults in need of prolonged ICU stay

The aim is to investigate if cumulative perioperative loss of albumin from plasma is different at EOS, POD3 and POD7, respectively, in adults in need of prolonged ICU stay compared to adults not in need of prolonged ICU stay after liver transplantation.

7 RESEARCH QUESTION

7.1 PRIMARY QUESTION

Is cumulative perioperative loss of albumin from plasma positive at post-operative day 7 (4-10) after liver transplantation?

7.2 SECONDARY QUESTIONS

1. Does perioperative loss on POD7 (4-10) correlate to losses at EOS and POD3?
2. Is cumulative perioperative loss of albumin from plasma still positive at hospital discharge (or POD 21) following liver transplantation?
3. Dichotomize patients in two equally sized groups depending on the amount of cumulative loss of albumin from plasma on EOS, POD3 and POD7 respectively, i.e. to a "leaky" and a "less leaky" group. Which factors predict "leakiness" (demographics, bleeding, fluid balance, ascites, ELISAs etc)?

4. Is cumulative perioperative loss of albumin from plasma positive at EOS, POD3 and POD7 (4-10), respectively, after liver transplantation in children? This is the primary question in the children substudy .
5. Does perioperative loss on POD7 (4-10) correlate to losses at EOS and POD3 in children after liver transplantation?
6. Which factors characterize children with large postoperative drain losses after liver transplantation?
7. Does cumulative perioperative loss of albumin from plasma at EOS, POD3 and POD7 (4-10), respectively, differ between adults in need or not in need of prolonged ICU stay, respectively, after liver transplantation? This is the primary question in the prolonged ICU substudy.
8. Which factors predict “need of prolonged ICU stay” after liver transplantation (demographics, bleeding, fluid balance, ascites etc)?
9. How does synthesis rate of albumin develop during prolonged ICU stay?

8 ENDPOINTS

8.1 PRIMARY ENDPOINT

Cumulative perioperative albumin shift (CPAS) by mass balance at POD 7 (4-10).

8.2 SECONDARY ENDPOINTS

- A. Cumulated albumin mass balance at EOS, POD 3, and at hospital discharge (POD21)
- B. Indices of inflammation and endothelial dysfunction (such as IL6, syndecan, HBP)
- C. Albumin synthesis rate on POD 4-6 and on POD 7-14 after liver transplantation in adult patients (>18 years) in need of a prolonged ICU stay.

9 STUDY DESIGN

The study is a prospective exploratory study of patient physiologic parameters during and after liver transplantation.

Albumin mass balance is measured during liver transplantation and up to hospital discharge or 21 days after end of surgery, by keeping close track of gains and losses (all subjects). Albumin synthesis is measured by incorporation of ⁵D-phenylalanine during 90 minutes (adults).

Samples for ELISA will be obtained from all patient groups.

All other factors are routinely measured and calculated within the liver transplantation protocol.

10 STUDY PROCEDURES

10.1 SCREENING, INFORMATION AND CONSENT

All patients accepted for liver transplantation will be approached when accepted for the operation. They will then be informed orally and in writing about the study and the study-specific procedures by the investigator or co-investigator. Preliminary oral informed consent will be noted in the patient record. The written informed consent will be obtained from the patient (or parent) in connection with the liver transplantation. Preliminary recruitment will likely outnumber the anticipated number of patients that are actually studied due to the emergency nature of the procedure, and the very variable time from acceptance to the operation. Albumin mass balance will start in the operating room and continue until hospital discharge or POD21.

Three different versions of the study information will be used.

Adults can participate in all measurements

Children can only participate in albumin mass balance and ELISAs, and parents and children over 12 years will have two different study informations. Children below the age of twelve will only be represented by their parents.

10.2 FIRST STUDY DAY

The procedure will be an emergency, except for living donors which occurs mainly in children. The patients arrive at the operating room at Perioperative Medicine and Intensive Care at Karolinska University Hospital Huddinge. Venous and arterial lines will be routinely inserted in connection with anesthesia and used for blood sampling. Mass balance of albumin will be achieved by keeping track of all losses (bleeding and drains) and infusions containing albumin or hemoglobin during and after the surgical procedure and repeated sampling of P-albumin. Mass balance sampling and calculation will continue after the surgical procedure.

In children there is no study specific blood sampling during the surgical procedure. Samples will only be taken from suction bottles, drains, urine, and administered blood products.

10.3 POSTOPERATIVE STUDY DAY 1-21 OR TO HOSPITAL DISCHARGE

Mass balance sampling and calculation will continue up to hospital discharge or at most for 21 days after the surgical procedure. POD 1 ELISAs will be taken in all study patients. POD 4 also in adults not in the ICU.

10.4 POSTOPERATIVE STUDY DAY 4 -21 IN ICU

If an adult patient is in the ICU after POD4 measurement of albumin synthesis rate will be performed by the flooding method. The exact time point will be to the discretion of an investigator. In the semi-recumbent position measurements of albumin synthesis rate (11.3) is performed over 90 minutes. Also a blood sample for ELISAs will be taken. The procedure can be repeated once or twice for measurement of albumin synthesis rate but only within the permitted total sampling blood volume (100 ml).

Children in ICU after POD 4 can be subject to ELISAs (2+2 ml).

11 METHODS AND ANALYSIS

11.1 ALBUMIN MASS BALANCE

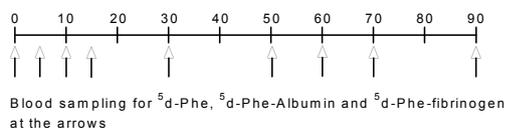
During preparations before surgery and during the surgical procedure blood samples for albumin concentration determination will be taken at the same time intervals as routine blood gases. Thereafter sampling interval will be 1-2 times per day. In parallel all fluid losses will be collected, measured and documented, sampled and saved for analysis. Also, infusions containing albumin or hemoglobin will be monitored.

11.2 ALBUMIN CONCENTRATION

Albumin concentrations in plasma will be measured employing nephelometry at Studieceter at Karolinska University Hospital. Albumin in other fluids (X-alb) will be analyzed at Karolinska Stable Isotope Core Facility at KFC Novum.

11.3 ALBUMIN SYNTHESIS RATE

To measure albumin synthesis rate an infusion of D5-phenylalanine 20 mg/mL 10 MPE, 0.45 g/kg will be given over 10 minutes. A 90-minute blood sampling protocol for determination of the ratio D5-phenylalanine/phenylalanine and the incorporation of D5-phenylalanine into albumin will be applied as illustrated in Figure 1. Analysis is performed by gaschromatography-massspectrometry (GC-MS) at Karolinska Stable Isotope Core Facility at KFC Novum. The fractional synthesis rate (FSR) is expressed as %/24 hours of albumin in plasma, while the absolute synthesis rate (ASR) expressed as mg/kg/24 hours, can be calculated if also plasma volume (from anthropometry) and plasma albumin are known.



11.4 SAMPLE HANDLING

Samples for GC-MS and ELISA analysis are anonymized and kept in Stockholms Medicinska Biobank reg nr 914 at Perioperative Medicine and Intensive Care at Karolinska University Hospital Huddinge, at -80°C pending finalization of study to be analyzed in one sequence to optimize precision in accord with our local routines. Samples for albumin analysis will likewise be marked in a non-identifiable way and kept in the biobank at -20°C pending finalization of study to be analyzed in one sequence to optimize precision in accord with our local routines.

Dr Åke Norberg will be the responsible person for sample handling, anonymization and destruction after publication. The responsible organization for personal data is Stockholm City Council, Box 22550, 104 22 Stockholm.

12 SUBJECTS

12.1 INCLUSION CRITERIA

Patients undergoing liver transplantation: adult males and females, and children 0-17 years.

Written informed consent. In children below 12 years consent is only obtained from the parents.

- A. Adults (mass balance, albumin synthesis and ELISAs)
- B. Children (only assessment of losses and gains, no blood sampling except for ELISAs)

12.2 EXCLUSION CRITERIA

Circumstance that makes the investigator judge patient participation unsuitable, mainly inability to understand study information (language difficulties or encephalopathy)

13 CRITERIA FOR DISCONTINUATION

13.1 PATIENT RELATED CRITERIA

- The patient may, at any time during the study, chose to end his/hers further participation in the study, without giving any cause.

If possible, the reason for discontinuation shall be stated in the CRF with date and time.

13.2 INVESTIGATORS CRITERIA AND EVALUABILITY

The primary investigator can determine that the patient cannot stay in the study. This can be founded on lack of evaluability or the reasons stated below:

- Errors in technical equipment
- Staff shortage that makes safe and standardized methods impossible.

The reason for discontinuation shall be stated in the CRF with date and time.

13.3 SUBJECT LOG

The investigator will keep a record of all eligible patients on a log list. The log list is stored in the investigator brochure available Perioperative Medicine and Intensive Care at Karolinska University Hospital Huddinge, in a locked room. It will show all screened subjects, included and not included.

14 TREATMENT

There is no study specific treatment. Study tracers are only used to monitor body physiology, not to treat the patient.

15 ASSESSMENT OF SAFETY AND EFFICACY

The patients are monitored according to local routines at Karolinska University Hospital Huddinge. No external monitoring related to this study is planned.

15.1 ASSESSMENT OF ANALYSIS RESULTS

Laboratory will be analyzed at the ISO certified Karolinska University Laboratory, Huddinge. Blood gases are analyzed at the Department of Anesthesiology. P-albumin will be analyzed by immunochemical method through the Studiecenter at the Karolinska University Laboratory, Solna. X-albumin and ELISAs will be performed at Karolinska Stable Isotope Core Facility at KFC Novum.

15.2 ASSESSMENT OF PATIENT SAFETY

Patients will be monitored in the operating room, at the intensive care unit and at the transplant surgical ward, respectively, for the mass balance sampling. Stable isotopes will be administered in the ICU or in the transplant surgical ward by a staff that is familiar with emergency incidents. Monitoring of the patient's heart rate, ECG, respiratory rate, oxygen saturation (oximetry), and blood pressure are taken before and during the first 90 min after administration.

15.3 ASSESSMENT OF ANALYSIS RESULTS

Laboratory samples, taken during and after surgery, are analyzed at the ISO certified Karolinska University Laboratory, Huddinge. Blood gases are analyzed by analyzers at the Department of Anesthesiology and Intensive Care Unit, respectively. P-albumin will be analyzed by immunochemical method through the Studiecenter at the Karolinska University Laboratory, Solna. ELISAs and X-albumin (blood products, drains etc) will be analyzed at Karolinska Stable Isotope Core Facility at KFC Novum. Other samples are analyzed as described in paragraph 11.

16 MANAGEMENT AND REPORTING OF ADVERSE EVENTS

All patients undergoing a liver transplantation will in the perioperative course experience incidents that would under other circumstances be described as AE/SAE. Major surgery in itself is associated with hospitalization. Therefore expected effects of anesthesia and surgery will not be reported, even if they may be considered undesirable. Such effects do not relate to the planned measurement procedures and they are not controlled by the investigator. Given the observational character of the study However, events that may influence the results of the study or whether subjects are evaluable or not will be noted in the CRF.

17 STATISTICS AND DATA MANAGEMENT

17.1 DATA MANAGEMENT

All laboratory values will be printed from the patient record (Take Care, TC) de-identified and attached to the CRF. All values of vital parameters during in connection with tracer administration will be documented directly on a coded anesthesia paper record by the research nurse or the investigator. This journal is a part of the CRF. Other documents that are stored in the CRF for coding and de-identification are: medical record from the Department of Transplant Surgery, medication list from TC, medication list from CliniSoft (the medical record system at the Intensive Care Unit).

Data from the mass spectrometer measurements of phenylalanine will be exported as a data sheets via a USB stick.

All the data regarding end-points will be added in an Access database that is locked when data collection is complete.

De-identified and coded patient demographics will be prepared and included in the Access database.

17.2 STATISTICAL ANALYSIS

In a previous study on selected patients undergoing liver transplantation at our department, we found a net leakage of albumin of 37 ± 17 g at end of surgery and of 48 ± 33 g at the third postoperative day. In our hospital, post-operative albumin infusions to keep plasma albumin at 25 g/l are very common, suggestive of ongoing losses in this patient group.

The effect and time course of ischemic liver injury on albumin synthesis has not been investigated, but in the previous pilot study albumin synthesis was higher than normal values on post-operative day 3. All data will be presented as mean with standard deviation (SD) or as median and range of data if not normally distributed.

Furthermore, the mutual correlations between the mass balance albumin parameters and albumin synthesis and P-albumin will be calculated and presented with correlation coefficients. Patients will be dichotomized to “leaky” and “not so leaky”, and possible predictors analyzed by univariate and multivariate logistic regression.

With 103 subjects there is a power of 80% to detect an anticipated effect size of 0.11 with 3 predictors and a 2-sided significance level of 5%. This equals the situation where the 3rd predictor gives an increase in the squared correlation coefficient r^2 from 0.30 to 0.37 or from 0.50 to 0.54. Although this is rather hypothetical it seems likely that such inferences might occur, and we will find them if 2 years production of liver transplants are investigated. If only 2 predictors are used an effect size of 0.095 can be found with 80% power.

Correlations will be reported as Pearson's correlation coefficient r or Spearman's rank correlation r_s depending on the data's nature.

Demographic preoperative data such as age, height, weight, BMI, gender, routine lab, history of weight loss, diagnoses, and current medications will be presented as numbers, averages and standard deviation, or as median range as applicable.

17.3 DETERMINATION OF THE NUMBER OF SUBJECTS

On page 4 we state that the number of subjects is 240 in the main study. This refers to an overestimation of the total number of liver transplantations during a approximately 2 year period at our hospital and the primary outcome of albumin mass balance.

The primary endpoint of the study (cumulative perioperative albumin shift at POD7) has never been investigated before. On POD3 48 ± 33 g, $p=0.0017$ versus baseline was found in our previous study on selected patients undergoing liver transplantation [Amouzandeh 2017]. This corresponds to a effect size of 1.4. Although data in consecutive patients are likely to be even more scattered a power of 90% is reached with $n=100$ for an effect size of 0.33 with a one sample 2-sided t-test and a significance level of 5%.

We plan to do linear regression with the thus detected “leakiness” as dependent variable and look at possible predictors. We will also dichotomize the patients in “leaky” and “not so leaky” (cut off to be estimated by ROC-curve analysis) and explore possible predictors by logistic regression and multiple logistic regression. The size of the data base becomes important to the quality of the multiple regression procedure, but data quantity will be governed by our patient flow and research resources. Data collection over much more than 2 years is likely to be confounded by changes in routines over time.

The large number of correlations mentioned above must be seen against the background that this is an exploratory pilot study of albumin mass balance and albumin kinetic parameters in liver transplantation. Because this is an unselected patient group for this kind of measurements, there is an intention that the study also should be hypothesis generating.

Sub study A (children, n=20) is included in the above mentioned number.

Sub study B (prolonged ICU stay, n=20) is a cohort included in the main study above.

The expected numbers in substudies A and B are the main reason to extend the whole study to approximately 2 years, and are based on historical records. Although data are likely to be scattered, with n=10-20 there is a reasonable chance to perform statistical inference analysis. The groups will also be compared with the complete data set.

18 ACCESS TO SOURCE DATA

The primary investigator is responsible that there is a confidentiality agreement between a monitor or any other independent reviewer (*i.e.* auditor) given permission to verify the data in the patient record and the patient record responsible party at Perioperative Medicine and Intensive Care, Karolinska Huddinge. This requires the patient's written consent.

19 ETHICS

19.1 ETHICAL REVIEW BOARD

The investigator is responsible for the application for approval from the Ethics Review Board.

19.2 ETHICAL CONDUCT OF THE STUDY

The study will be conducted according to the study protocol, GCP, regulatory requirements and the Helsinki Declaration.

Our practice is to not suggest a blood sampling protocol to patients that exceeds 100 mL unless the sampling period is prolonged.

20 DATA MANAGEMENT AND ARCHIVE

20.1 CASE REPORT FORMS

Data collected in the study will be recorded in Case Report Forms (CRF) in which the patient is only identified with a code number and initials. The code key kept by the investigator in such a way that unauthorized persons cannot take part of it. Anonymous and coded copies of medical records such as anesthesia records, monitoring sheet for post-operative care, journal text (Take Care) and laboratory lists (Take Care) will also be included in the CRF.

Data collected in the study and registered in the CRF will be transferred to a database that the sponsor is responsible of.

20.2 ARCHIVE

Study documents will be filed at least 10 years after the study report is submitted to the Swedish MPA. The documents should be archived in a legible condition for eventual future audit or inspection by authorities.

21 FINANCIAL SUPPORT AND INSURANCE

The study is funded by research grants. The patients are insured through medical insurance and patient injury insurance.

22 PUBLICATION OF RESULTS

The intention is to publish the results in a scientific journal. The main study and the two planned sub studies will be published separately.

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Final 1.01

24 SIGNATURE

This page should be signed by the sponsor and the primary investigator.

Primary Investigator and sponsor

Åke Norberg, MD, PhD, Associate Professor
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Signature

Date

