STUDY PROTOCOL

Evaluation And Risk Assessment For Persistent Postsurgical Pain After Breast Surgery: A Prospective Cohort Study (Breast CAncer surgery Postsurgical Pain (B-CAPP))

CIRB PROTOCOL NUMBER:
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PRINCIPAL INVESTIGATORS:
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*Also refer to page 14 on the statistical and analytical plan.

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PROTOCOL SIGNATURE PAGE

Protocol Title: Evaluation And Risk Assessment For Persistent Postsurgical Pain After Breast Surgery: A Prospective Cohort Study (Breast CAncer surgery Postsurgical Pain (B-CAPP))

Protocol Number: BCAPP01

Protocol Version/ Date: 1.0, dated 18 Jan 2018

Sponsor Name: Duke-NUS/SingHealth Collaborative Funding

Declaration of Investigator

I confirm that I have read the above-mentioned protocol and its attachments. I agree to conduct the described trial in compliance with all stipulations of the protocol, regulations and ICH E6 Guideline for Good Clinical Practice (GCP).

Principal Investigator Name: A/Prof Sng Ban Leong

Principal Investigator Signature: _____

Date: _____

Principal Investigator Name: Prof Ashraf Habib

Principal Investigator Signature: _____

Date: _____

1. BACKGROUND AND RATIONALE

Impact of Persistent Pain after Breast Cancer Surgery

Breast cancer is a leading cancer diagnosis among women worldwide, with more than 1 million new cases per year. It costs more than $100 billion annually in direct medical costs and lost productivity in the United States. Persistent pain following breast cancer surgery has been recognized as a major humanitarian and socioeconomic burden, affecting about 50% of patients after lumpectomy and mastectomy leading to chronic physical disability and psychological distress (1, 2).

The pathogenesis of breast cancer surgery-related pain is likely multifactorial, and risk factors such as age, body mass index and genetic predisposition have been demonstrated to affect persistent pain development (3-5). Despite a number of studies investigating risk factors, almost all information originated from single centre studies and often focus on only few elements, rather than the full continuum of perioperative care. Additionally, surgical approaches, adjuvant therapy and analgesic regimens have changed in recent years therefore limiting the interpretation of previous studies, yet tools to identify those at high-risk and preventive interventions are still lacking. Here we propose to investigate the risk factors related to persistent postsurgical pain in breast cancer patients, and to develop a prediction model that could serve as a screening tool for patients at high-risk of developing persistent postsurgical pain.

Pain Vulnerability and Psychological Vulnerability May Affect the Risk of Postsurgical Pain

Psychological Vulnerability: Psychological vulnerability, including depression, stress and anxiety are found to be associated with greater psychological or psychiatric morbidity in the presence of persistent pain following breast cancer surgery (6-8). Similarly, the clustering of persistent pain with depression overall occurs in high prevalence ranging from 30% to 60% (9, 10). The association of depression and persistent pain after breast cancer surgery is, however not clear. The knowledge of this relationship could change psychiatric and pain physicians practice, e.g. by leading to screening for depression and persistent pain during patient contact, as well as, stimulate further research in elucidating common pathways in these two distinct disease processes.

Pain Vulnerability: Pain catastrophizing refers to the negative thought processes patients have when they are exposed to pain or painful experiences. There has been increasing evidence suggesting that catastrophizing is related to increased pain and reduced activities of daily living in patients with chronic pain (11, 12). Identifying patients with pain vulnerability may lead to further work in cognitive behaviour or mindfulness therapies that are successful methods of treatment for catastrophizing, leading to better adjustment to pain (11, 13, 14).

Anxiety and Stress

Perceived Stress Scale (PSS) is developed by Cohen et al (15). This instrument assesses the individual's perception of stress over the past month and asks questions about thoughts and feelings in that time period. The PSS aims to extract information about the degree to which the individual values their life as 'unpredictable, uncontrollable, and overloaded'. Each item is rated on a 5-point Likert scale ranging from never (0) to almost always (4). Positive questions are scored in reverse order from 4 to 0. Total scores will range from 0 to 40 with the highest scores indicating the highest perceived stress. Brief Symptom Inventory 18 (BSI-18) has been used as a tool for psychological distress and psychiatric disorders especially in oncology populations. It comprises three components: Somatization, depression and anxiety. The respondent's overall level of psychological distress is measured by Global Severity Index (GSI), which is simply the

total score of BSI-18. Wells et al. showed that in breast cancer patients, those having implant removed had the highest levels of psychological distress (all three components) post-operatively as compared to those without implant removal (16). Similar results were also shown in Roberts et al., by which those having implant removal had higher BSI scores even during short term (4-9 months) and long-term (>10 months) follow-up (17).

Central Sensitisation

Central sensitisation is characterised by allodynia (painful sensation to a normally non painful stimulus, such as touch), hyperalgesia (excessive sensitivity to a normally painful stimulus, such as pressure), expansion of the pain field and prolonged pain after stimulus has been removed (18). The neurobiological mechanism may involve dysregulation in both ascending and descending pain pathways as a result of physical trauma and prolonged persistent pain impulses. In addition, depression and anxiety are often associated with central sensitisation (19, 20).

Central sensitisation inventory (CSI) is a screening instrument designed to help clinicians identify patients with central sensitisation measuring a full array of central sensitisation syndrome related symptoms (18). The CSI has demonstrated good psychometric properties (test retest reliability = 0.0817; the Cronbach alpha = 0.879) (18). Importantly, the CSI scores have been found to be highly associated with the presence of central sensitisation in persistent pain patients referred to an orthopaedic rehabilitation practice specialising in persistent pain and psychological disorders (21).

Pain Catastrophizing

Pain catastrophizing refers to the negative thoughts processes patients have when they are exposed to pain or painful experiences. It consists of three components: (1) rumination, (2) magnification, and (3) helplessness. Sullivan et al developed the 13 item **Pain Catastrophizing Scale (PCS)**, which assesses the three components on a 5 point scale (22). A number of studies have replicated these factor structures using confirmatory factor analytic methods in healthy, pain-free adults (23, 24), chronic pain patients (24) and across diverse cultural groups and in non-English languages (25). Catastrophizing was also found related to increased pain and reduced activities of daily living in chronic pain patients (26, 27). Developed by Rosenstiel and Keefe, **Coping Strategies Questionnaire–Revised (CSQ-R))** uses a pool of items reflecting coping strategies frequently reported by patients and deemed to be important by clinicians involved in pain management (28). In those with chronic pain, this questionnaire is proven to be able to help in determining the patients' cognitive characteristics and predict treatment outcomes (29).

Health assessment

EQ-5D-3L is a generic preference-based instrument that is widely used to measure health-related quality of life and can be used to assign preference values to various health states. Several studies have utilized EQ-5D-3L to discriminate breast cancer patients with different health conditions cross-sectionally (30, 31). **Eysenck Personality Questionnaire, revised (EPQ-R)** indicates the existence of three major traits: neuroticism, extraversion, and psychoticism as well as a lie scale to measure the dissimulation. García-Torres et al. demonstrated that breast cancer survivors had higher levels of psychoticism compared with a control group, however its link to depression remains unclear (32).

Pain Genetics after Breast Cancer Surgery

Catechol-O-methyltransferase (COMT) opioid polymorphisms have been associated with both experimental and clinical pain phenotypes. Studies on fibromyalgia syndrome and low back pain have found significant associations with COMT polymorphisms Val15Met, rs4680, rs4633 (33-35), however there is little data after breast cancer surgery. We will investigate specific candidate COMT polymorphisms including COMT val158Met, rs4680, rs4633, rs4818 polymorphisms.
Mutations in sodium SCN9A gene, which encodes the type IX alpha subunit of the voltage-gated sodium channel, Nav1.7, were found to be linked to pain disorders as primary erythermalgia and small fibre neuropathy (36, 37). Duan et al. demonstrated an association between SCN9A SNPs (rs4286289, rs4387806, rs16851799) and post-operative pain parameters in the gynaecological setting, and further postulated that this polymorphism can be used to identify patients at risk of developing severe postoperative pain (38).

The polymorphisms of candidate genes at CACNG2 calcium channels, in particular those located near exon 3 and exon 4 of the gene, are associated with persistent pain in breast cancer surgery patients (39, 40). Here we propose to look into the role of these SNPs (rs4820242, rs2284015, rs2284017, rs2284018, rs1883988) in the persistent pain development after breast cancer surgery.

Pain Modelling May Predict Postsurgical Pain

Previous studies demonstrated good reliability on predicting postoperative pain with the use of preoperative indicators (41). We shall construct a model with risk factors including psychological vulnerability, pain vulnerability and perioperative factors that influence persistent pain development. The interplay of psychological and pain pathways will also be investigated through targeted candidate gene polymorphism approach. The relationship between the physiological, psychological and genetic factors will guide us in future studies on model validation, potential novel risk stratification and identify therapeutic targets.

2. HYPOTHESIS AND OBJECTIVES

Our long-term goal is to improve the outcome of women after breast cancer surgery by reducing persistent pain and severe acute postsurgical pain. Our objective here is to identify the role of perioperative factors, pain vulnerability, psychological vulnerability, quantitative sensory testing, and genetics in the development of persistent postsurgical pain. Our central hypothesis is that certain risk factors would be associated with the development of persistent pain after breast cancer surgery. To investigate this hypothesis, we will evaluate the association of multiple risk factors with the development of persistent postsurgical pain. We plan the following specific aims:

Primary Aim: To determine if a multivariable model for incidence of persistent postsurgical pain after breast cancer surgery may be built by using previously identified and novel measures of perioperative risk factors, and subsequently be used as a risk predication tool.
Primary Hypothesis: Using a subset of perioperative risk factors and measures of pain and psychological vulnerability, a model may be created for persistent postsurgical pain that attains high discrimination (AUC in ROC analysis >0.70) and calibration.

Secondary Aim: To determine if mechanical temporal summation (MTS) or pressure pain threshold are associated with significant acute and persistent postsurgical pain after breast

cancer surgery.
Secondary Hypothesis (1): Evoked MTS would be associated with significant acute postsurgical pain and persistent postsurgical pain in women after breast cancer surgery.
Secondary Hypothesis (2): Lower pressure pain threshold would be associated with significant acute postsurgical pain and persistent postsurgical pain in women after breast cancer surgery.

Exploratory Aims: To examine the association of personality traits (42), quality of life measures and opioid, sodium channel and calcium channel polymorphisms with persistent postsurgical pain in women after breast cancer surgery (43, 44).

3. EXPECTED RISKS AND BENEFITS

Risks: Universal precautions will be used during intravenous cannulation per routine to obtain 5mls of EDTA blood. During the MTS prick assessment, a test would involve the use of a thin plastic tube using repeated sensation of pinprick testing for increased sensitivity of pain to pinprick in some individuals. The assessment will be done on the inner side of the patient's forearm.
Analgesic choices will be made upon patient request and routine hospital protocol, monitoring and recording will apply. If subjects are screened to have T- score ≥ 63 in global severity index (as measured by BSI-18 questionnaire) or persistent pain, they would be advised to seek clinical psychiatric or pain assessment at their respective Hospital. The assessment would be done as clinical work and not research funded. No new research medications are involved by joining the study. The drugs being delivered are the same as what the patient shall receive as standard care.

Benefits: As this will be an observational study, there will be no direct benefits or harms to patients who choose to participate in this study. On a population level, the data collected in this study may help to improve pain management for women undergoing breast cancer surgery in the future.

4. STUDY POPULATION

4.1. List the number and nature of subjects to be enrolled.

We propose to recruit 300 patients this prospective cohort study, by which women scheduled for mastectomy or lumpectomy surgery for breast cancer in KKH and Duke University Hospital will be invited to participate in the study to identify patients who are at high-risk of developing persistent post-surgical pain. Subjects will be managed according to standard of care at both institutions.

4.2. Criteria for Recruitment and Recruitment Process

This study will be advertised on study posters and brochures. These will be placed in the pre-operative assessment clinics and pre-admission areas. Patients who are undergoing breast cancer surgery will receive study information either at pre-operative assessment clinic or upon admission for surgery if they have not attended the pre-operative assessment clinic. They will be screened for eligibility using the inclusion and exclusion criteria. If eligible for recruitment, the patients will be approached by the investigators for recruitment. Recruitment will be performed in the pre-operative assessment clinic or on the same day of surgery if they have not attended

pre-operative assessment clinic. Research personnel will conduct all discussions about the study and answer any questions in a private manner in the consultation rooms. They will be counselled regarding the alternatives and given an opportunity to ask questions and clarify doubts. Ample time will be given to the potential patients for consent taking. Consent will be obtained in writing upon their willingness and agreement to participate in the study.

4.3. Inclusion Criteria

The subject will only be recruited into this study if she has met all the below criteria:

a. Aged between 21 – 80 year old;
b. Healthy and/or have systemic medical conditions as reviewed by anaesthetist for surgery (ASA 1-3);
c. Undergoing breast cancer surgery.

4.4. Exclusion Criteria

Any subject meeting one and/or above exclusion criteria as listed below will be excluded from participating in the study:

a. History of intravenous drug or opioid abuse;
b. Current chronic daily treatment with corticosteroids (excluding inhaled steroids);
c. Previous history of chronic pain syndrome;
d. Obstetric patients.

5. STUDY DESIGN AND PROCEDURES/METHODOLOGY

We propose to recruit 300 patients this prospective cohort study, by which women scheduled for mastectomy or lumpectomy surgery for breast cancer in KKH and Duke University Hospital will be invited to participate in the study to identify patients who are at high-risk of developing persistent post-surgical pain. Subjects will be managed according to standard of care at their respective institution.

a. Pre-operative assessments

A **pre-operative** evaluation will involve:

(i) Comprehensive preoperative demographic, surgical, anaesthetic and pain evaluation, need for axillary clearance, preoperative/ postoperative chemotherapy/ radiation therapy;

(ii) Pain vulnerability and coping assessment (Pain catastrophizing scale (PCS), Central Sensitisation Inventory (CSI), Coping Strategies Questionnaire–Revised (CSQ-R), postsurgical pain scores);

(iii) Psychological vulnerability assessment (Distress (Brief Symptom Inventory-18 (BSI-18)), and stress (perceived stress scale (PSS)));

(iv) Experimental pain testing (mechanical temporal summation (MTS), pressure pain threshold);

(v) Health assessment (quality of life (EQ-5D-3L) and personality (Eysenck Personality Questionnaire (EPQ));

(vi) Genetic profiling via blood samples.

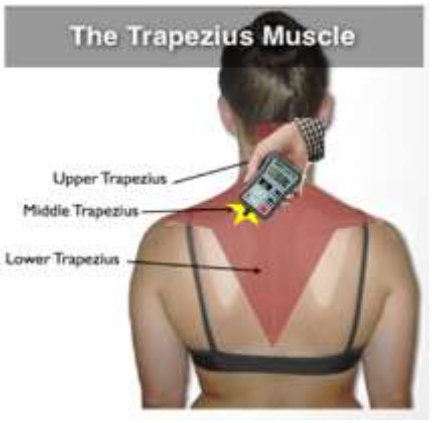
The above activities will be performed in pre-operative assessment clinics and/or pre-admission areas. The questionnaires may also be completed post-operatively in the patients ward, before their discharge.

Mechanical temporal summation (MTS)

A pre-test will be performed on the subject's forearm, by which a 180 gram von Frey filament is applied once or twice to familiarize the patient with the pinprick sensation. Patient will then be asked to rate the pinprick pain score on a verbal rating scale, 0-100.

The actual test will firstly involve the one-time application of the filament. Patient will be asked to rate the pain score (0-100). Following this, 10 consecutive touches at random locations are applied with a 1 second interstimulus interval and within a 1 cm diameter circle. The patient will then be asked to rate the 10^{th} pain score (0-100).

Pressure pain threshold assessment



A pre-test will be performed on the subject's right centre of the trapezius muscle, by which pressure is applied 90 degree straight down force using an algometer. The speed of pressure will be about 1kgf per second (to be estimated by investigator prior to pre-test using pressure on own's hand to estimate speed).

The above procedure is then repeated 2-3 times at the same site which is indicated on the pressure mark with each time having the readings recorded. When patient shows no response ≥ 6kgf, the threshold will be recorded as 6kgf. The patient upon feeling pain will say stop or raise the hand to terminate the test. The number of obtaining readings is determined by the below conditions: i) 2 values are recorded within 0.2kgf of one another. The mean value is obtained by averaging 2 readings; or ii) (If applicable) If the 2 values from i) have a difference of >0.2kgf, a third test will be performed. The mean value is obtained by averaging 3 readings. The final mean value will then be recorded as the threshold estimate.

The above procedures will be repeated again on the patient's left center of the trapezius muscle.

Questionnaire administration

Questionnaires will be administered either via paper format or online survey, whichever the patient prefers and feels comfortable with. Chinese-translated questionnaires are made available provided patients prefer answering the questionnaires using Chinese. The designated study team members shall ensure the completeness of the answers upon questionnaire submission.

Blood taking

Five mls of blood will be obtained during routine intravenous cannulation or routine blood taking before the start of the surgery as per routine, which allow study team to collect the blood in EDTA tube to reduce additional injections. If this is not possible, a separate injection will be done with patient's consent. The blood taking will be performed by accredited nurses and doctors, and no protocol-specific training is required to perform the intravenous cannulation as this is part of the routine practice.

DNA will be extracted from venous blood using the Puregene method. Known functional polymorphisms in the candidate genes, SCN9A, COMT and CACNG2 with evidence of associations with depression, pain, stress and anxiety would be determined by the Taqman allelic discrimination assay method using the ABI Step-One Plus Real-time PCR System (Applied

Biosystems, Foster City, California, USA). Alleles will be called using the software. Genetic data will be analysed for association with post-operative pain and psychological vulnerability. Blood obtained may also be analysed for metabolomics and proteomics for biomarkers. The results obtained from these tests will not be communicated to the subjects as these information would not affect the clinical decisions about the individual's care and have no effect on the participants.

b. Post-operative assessments

Perioperative anaesthetic management will be recorded and outcomes including analgesic consumption, pain scores at surgery site, arm and armpit (at rest/ with movement) as well as adverse events (if any) will be collected in the post anaesthesia recovery unit and at 1-, 2- and 3-days after surgery. Study team members will also follow up with patients to ensure completion of questionnaires administered in section a.

Follow-up evaluation at 4 and 6 months, respectively will involve:

(i) Pain assessment;
(ii) Health assessment via EQ-5D-3L questionnaire.

The above assessment may be done via phone survey or online survey, whichever the patient prefers and feels comfortable with.

Patients will be involved in the study before their admission to the hospital for surgery, which may take up to 4 weeks. Blood sample taking and questionnaires will be conducted during their hospitalization stay, i.e. from the time just before surgery until the patient is discharged from the post-operative observation area. This will take about 3-4 days. Phone or online surveys will be conducted at 4 and 6 months, respectively, after surgery. The total amount of time by which participants are involved in the study will therefore take about 7 months.

Discontinuation/ Withdrawal

Patients are free to withdraw/discontinue the study at any time (including timing after their surgery), should they decide not to participate. Patient will be withdrawn /discontinued from the study if :

- Severe adverse effects and unforeseen reactions that affect the wellbeing of the subjects (with reporting to relevant regulatory authority and CIRB);
- Study closure due to CIRB review.

If the patient withdraws from the study, she will be given routine standard care based on the current hospital procedures. However, the data that have been collected until the time of her withdrawal will be kept and analysed. The reason is to enable a complete and comprehensive evaluation of the study.

Subject who miss any of the follow up, visit or assessment will be excluded from data analysis for that assessment. Subjects who have their assessment outside of window period will be regarded as protocol deviation.

6. SAFETY MEASUREMENTS

6.1. Definitions

An adverse event (AE) is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment.

In this study, the following conditions will be documented as adverse events (AE):

- Unplanned intubation after surgery
- Pulmonary embolism
- Other unexpected adverse event which are related events

A serious adverse event (SAE) or reaction is any untoward medical occurrence that at any dose:

- results in death
- is life-threatening
- requires inpatient hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability/incapacity or
- is a congenital anomaly/birth defect
- is a medical event that may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed above.

A serious adverse event (SAE) or reaction is any untoward medical occurrence that at any dose:

- results in or contributes to death
- is life-threatening
- requires inpatient hospitalisation or prolongation of existing hospitalisation
- results in or contributes to persistent or significant disability/incapacity or
- results in or contributes to a congenital anomaly/birth defect
- results in such other events as may be prescribed

6.2. Collecting, Recording and Reporting of Adverse Events and Serious Adverse Events to CIRB

Reporting of adverse events involves the PI submitting to the approving CIRB the completed SAE Reporting Form within the stipulated timeframe. PI is responsible for informing the institution representative (local SAE resulting in death), sponsor or regulatory bodies as required and appropriate.

Reporting timeline to CIRB:

- SAE that result in death, regardless of causality, should be reported immediately - within 24 hours of the PI becoming aware of the event.
- Local life-threatening (unexpected/ expected) SAE should be reported no later than 7 calendar days after the Investigator is aware of the event, followed by a complete report within 8 additional calendar days.
- Local unexpected SAE that are related events, but not life-threatening, should be reported no later than 15 calendar days after the investigator is aware of the event.
- An increase in the rate of occurrence of local expected SAE, which is judged to be

clinically important, should be reported within 15 calendar days after the PI is aware of the event.

- Local expected SAE should be reported annually (together with Study Status Report for annual review).
- Local unexpected and unlikely related SAE that are not life-threatening should also be reported annually (together with Study Status Report for annual review).
- Local unexpected AE that are related events should be reported at least annually (together with Study Status Report for annual review).
- Non-local unexpected SAE that are fatal or life threatening and definitely/probably/possibly related should be reported not later than 30 calendar days after the PI is aware of the event.

6.3. Safety Monitoring Plan

Patients involved in the study will be monitored for adverse events in the same manner that safety is monitored for standard-of care. The patient's vital signs, i.e. pulse rate, heart rate, blood pressure, respiratory rate will be recorded at regular standard routine intervals during and after the administration of the analgesia medications. The vital signs monitoring will continue throughout the surgery according to routine practice. The incidence and severity of side effects like nausea and/or vomiting and pain will be closely monitored after the procedure. Any serious adverse events will be immediately reported to the principal investigator. The patients will be treated for side effects according to hospital protocols.

Safety Recording:
All adverse events and serious adverse events will be recorded on adverse event form and serious adverse event forms will be recorded on the subject case report form. All unexpected adverse events and serious adverse events (whether expected or unexpected) will be reported to the principal investigator reviewed at each team meeting.

6.4. Complaint Handling

During the informed consent process, subjects will be advised to discuss with the investigator if they have any concerns during the study. The contact information of the principal investigator is provided in the consent form. Complaints arising from the conduct of the study will be discussed at the next available study team meeting.

Subjects will be provided with the contact details of the Principal Investigator and the CIRB. The Principal Investigator will speak directly to the complainant and resolve it as soon as possible. Any serious or potentially litigious complaints will be reported to the respective regulatory authorities and Institutional Review Board.

If the subject is unwilling to continue with the study, she will be assured that she can withdraw without any repercussions. If a subject wants her data excluded from the study, then her records will be removed from the study documents and database.

7. DATA ANALYSIS

7.1. Data Quality Assurance

To ensure data quality, entered data will be systematically checked and validated against the rules, including range and logic checks, which are built into the REDCap database system. Any discrepancies will be channelled to the relevant parties for resolution. All discrepancies will be resolved prior to data analysis. Quality control audits of all key safety and efficacy data in the database will be made before database lock.

Several procedures will be implemented to ensure accurate, complete, and reliable data for the study:

- The roles and responsibilities for the study team will be segregated. The study will be monitored by designated monitors. The study monitors will follow the study monitoring plan and verify that:

  - the rights and well-being of human subjects are protected;
  - the reported trial data are accurate, complete, and verifiable from source documents;
  - the conduct of the trial is in compliance with the currently approved protocol /amendment(s), with GCP, and with the applicable regulatory requirement(s).

- A start up meeting will be held with the whole study team; study personnel who will handle data collection and data entry will be appropriately trained during the meeting before the study start.

- The investigator will keep records of all original source data, in anticipation or audits by respective agencies/institutes. This might include laboratory tests, medical records, and clinical notes. If requested, the investigator will provide them with direct access to the original source documents.

7.2. Data Entry and Storage

Data will be collected from the source documents including the hospital medical records as well as the study specific questionnaires. A source documentation checklist will be used at the site to identify the source data for all data points collected. All source documents will be handled with strict confidentiality. It will be stored in locked cupboards which are only accessible by designated staff only. All source documents can only be reviewed by external personnel after confirmation of the identity check.

An electronic database REDCap will be used for data entry into case report form (CRF). Data reported on the eCRF that are derived from source documents should be consistent with the source documents. If there are discrepancies, these should be explained. Password protected accounts will be created for relevant study personnel and the degree of database access granted to the each relevant study personnel account will correspond to their trial responsibilities. The data will be kept under lock using the computer security of respective institution and will be destroyed upon keeping for up to 6-years after completion of the study.

8. SAMPLE SIZE AND STATISTICAL METHODS

8.1. Determination of Sample Size

Based on the Haneley and McNeil variance formulas (45), a number of 152 patients, with a 50% incidence of persistent pain, will produce a 95% lower confidence bound for area under the ROC curve (AUC) >0.70 if the observed AUC is ≥ 0.77. We anticipate a 30% drop-out rate and will therefore enrol 218 patients to ensure adequate sample to assess our primary hypothesis. If the drop-out rate was lowered to 20% we would be able to produce a 95% lower confidence bound for AUC >0.70 if the observed AUC ≥ 0.76. Since this is a collaborative project between KKH and Duke, both KKH and Duke will therefore recruit 109 patients, respectively in their research sites. To further take into account uncertain circumstances, a number of 150 patients will be recruited in KKH and Duke University Hospital site, respectively.

8.2. Statistical and Analytical Plans

We will study risk factor distributions to determine the appropriate summary statistics [(mean (SD), median (Q1, Q3), or N (%)] and modelling strategies (transformations, functional form, collinearity, interaction potentials). As this study will combine data across two sites we will investigate differences between patients and outcomes between sites to determine how site should be accounted for in the analysis.

We will use generalized estimating equations (GEE) to generate univariate association statistics with a random effect for site (46). A combination of variable selection criteria including statistical significance, improvement of QIC (GEE equivalent of AIC), Mallow's Cp, Brier score, ROC AUC, and integrated discrimination index will be used to build our model (47-52). ROC analysis will be performed by outputting the estimated probabilities of persistent pain from GEE analysis, followed by simple logistic regression of persistent pain on those predicted values. After a final model is selected its performance will be internally validated via bootstrap resampling, and externally validated with prior data from Duke. By performing these validations we can evaluate the model performance for future cohorts and avoid a cohort specific formulation or over-optimization.

Of particular interest in this study is the effects of MTS, pressure pain threshold, minor allele frequencies (MAF) and their associations with the incidence of persistent pain. We will investigate the association of these factors via univariate analysis and by forcing their inclusion in the final multivariable model established during analysis for the primary aim. Hardy-weinberg equilibrium test will be conducted to assess whether MAF in our cohort is representative of the population. GEEs will be used to account for potential cluster effects, and to explore interactions of these factors with patient characteristics to identify possible subgroup effect. All analyses will be conducted in SAS or R. Statistical significance will be set at 0.05 level, and all model parameters will be presented with point estimate and 95% confidence intervals.

We will study risk factor distributions to determine the appropriate summary statistics [(mean (SD), median (Q1, Q3), or N (%)] and modelling strategies (transformations, functional form, collinearity, interaction potentials). As this study will combine data across two sites we will investigate differences between patients and outcomes between sites to determine how site should be accounted for in the analysis.

9. DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

The investigator will keep records of all original source data, in anticipation of study-related monitoring, review and/or audits by respective agencies/institutes. This might include laboratory tests, medical records, clinical notes, and questionnaires. If requested, the investigator will provide them with direct access to the original source documents.

The subject has the right to request access to her personal data and the right to request rectification of any data that is not correct and/or complete. Reasonable steps should be taken to respond to such request, taking into consideration the nature of the request, the conditions of the study, and the applicable laws and regulations.

If subjects are screened to have high depression/anxiety or persistent pain, they would be advised to seek clinical psychiatric or pain assessment at their respective hospital. In this case the subject's data may be assessed by respective personnel upon request.

10. QUALITY CONTROL AND QUALITY ASSURANCE

During and/ or after completion of the study, quality assurance officers named by the study Principal Investigator or regulatory authorities may wish to perform on-site audits. The Investigator and site personnel will be expected to cooperate with any audit and to provide assistance and documentation (including source data) as requested. The Investigator and site personnel will immediately inform the study Principal Investigator of any audit to be performed by a regulatory authority.

11. ETHICAL CONSIDERATIONS

This study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and that are consistent with the Good Clinical Practice and the applicable regulatory requirements.

This final study protocol, including the final version of the Patient Information and Informed Consent Form, must be approved in writing by the Centralised Institutional Review Board (CIRB), prior to enrolment of any patient into the study.

The principle investigator is responsible for informing the CIRB of any amendments to the protocol or other study-related documents, as per local requirement.

11.1. Informed Consent

Informed Consent will be taken for all study subjects. The informed consent process will be conducted by a trained study team member as shown in the delegation log. The informed consent process will be conducted in compliance with ICH-GCP.

11.2. Confidentiality of Data and Patient Records

Names and other identification numbers of all subjects will be de-identified. The subjects are only identified by study numbers, by which these numbers would be communicated through in electronic softcopy format. The data will be kept locked by the principal investigator and by the use of computer security of respective instituition. The data will be accessible only to the investigators and for analysis purposes only.

12. PUBLICATIONS

The publication policy would follow the guidelines that are stated in both KKH and Duke University as well as Singhealth Office of Research for authorship, acknowledgement and review procedures.

13. RETENTION OF STUDY DOCUMENTS

Data entry will be in softcopy format and will be kept under lock using the computer security of respective institution. The data will be destroyed after keeping for 6 years upon completion of the study.

14. FUNDING and INSURANCE

This study is funded by Duke-NUS/SingHealth Collaborative Funding, with an amount of $100,000 respectively for each site, approved in year 2017 (Ref no. Duke/Duke-NUS/RECA(Pilot)/2017/0031).

The Hospital does not make any provisions to compensate study participants for research related injury. However, compensation may be considered on a case-by-case basis for unexpected injuries due to non-negligent causes, and shall be covered by respective institution.

List of Attachments

Appendix 1 Study Schedule

Procedures	Admission to Pre-operative wards/rooms	During surgery	Post-surgical period	
			0 to 72 hours after delivery	4, 6 months after surgery
Informed consent				
Baseline Demographic data collection				
EDTA Blood Collection				
Survey (paper/online) and data collection (pain scores, EQ-5D-3L, MTS, pressure pain threshold, PSS, PCS, CSI, BSI-18, CSQ, EPQ)				
Analgesia administration & surgery				
Pain scores, vital signs monitoring				
Data collection (pain scores, analgesia usage,adverse events if any); ensure questionnaire completion in Visit 1 before patient discharge				
Phone/online survey and data collection (EQ-5D-3L, pain scores)				

Version 3, dated 2 Jan 2017

Appendix 2 References

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