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1. Study Summary

Full title	Epigenetic susceptibility to inflammatory complications after cardiac surgery.
Short title	Epi-SICCS
Study design	Single-centre prospective observational biomarker study.
Study participants	Adults undergoing major cardiac surgery (coronary artery bypass grafting (CABG) with cardiopulmonary bypass).
Study duration	36 months
Study setting	Golden Jubilee National Hospital.
Medical condition under investigation	Post-operative organ dysfunction.
Planned sample size	30
Primary outcome measure	Post-operative organ dysfunction quantified by measurement of mean Sequential Organ Failure Assessment (SOFA) score over the first seven postoperative days.
Exploratory outcome measures	**Secondary outcomes including:** 1. Clinical outcomes: Days alive and at home at 30 days postoperatively, cardiovascular, renal, pulmonary, infective, neurological and major complications, clinical indicators, patient quality of recovery, patient quality of life and functional assessment. 2. Laboratory endpoints indicative of: • Inflammation: C-reactive protein, albumin, differential white cell count, inflammatory gene activation/suppression (transcriptomics), inflammatory protein quantification (proteomics). • Organ dysfunction: Creatinine, estimated glomerular filtration rate, liver function tests, arterial blood gases, troponins.

Follow-up duration	One year.
End of trial definition	The time that the final patient has had their 12 month follow up.

2. Lay Abstract - Epigenetic regulation of inflammation leading to complications after heart surgery

This protocol describes a study which aims to better understand 3 crucial questions:

1. Why do some patients experience more severe complications after heart surgery than others?
2. Are there markers on our genetic makeup (called epigenetic modifications) that can predict complications after major surgery?
3. How do epigenetic modifications lead to overactive inflammation that causes harm to patients after heart surgery?

2.1 Background

Over 270,000 patients undergo major surgery in Scotland each year. Major surgery is associated with increased risk of complications, particularly in older patients with multiple health conditions. Complications are a leading cause of death, delayed recovery, long-term illness and increased healthcare cost to the NHS. Significant complications occur in up to 57% of patients after heart surgery. In response to major surgery, the body stimulates an inflammatory response as part of a complex repair mechanism, however, in some cases inflammation can become exaggerated, becoming harmful and contributing to the development of complications.

We believe that a patient's risk of overactive inflammation, which can lead to complications after surgery, is heavily influenced by a patient-specific system of biological markers, layered upon their DNA; a scientific field known as epigenetics. Where a patient's DNA (their 'genetics') may be considered as an instruction manual for each cell's molecular processes, these 'epigenetic' markers work as if someone has highlighted sections of the manual to be read (and actioned). The pattern of these markers on immune system cells can therefore dictate the extent of the inflammatory response, especially after a major insult such as surgery. While the DNA itself cannot be modified, epigenetic markers can be modified, through strategies including diet, exercise, and medication.

Our aim is to demonstrate that the modifiable epigenetic code of key immune-system cells dictates the extent of post-operative inflammation, contributing to organ damage and complications.

With patients' permission, we will:

1. Obtain blood samples before and after major heart surgery, to test for epigenetic markers and inflammation.

2. Observe patients after surgery and collect routine data from laboratory tests and patient observations which assess for organ dysfunction and inflammation.

3. Conduct patient questionnaires before and after surgery to assess physical fitness and patient-centred quality of life measures.

4. Analyse patterns between patients who have severe organ dysfunction after surgery and those patients who have minimal organ dysfunction after similar surgery, to better understand which patients are at greatest risk, according to their epigenetic makeup.

This study will give us a better understanding of which patients are likely to suffer organ damage and complications after major surgery, and which patients may have a protective epigenetic makeup which reduces complications. With this knowledge, our aim is that future patients could receive personalised treatment plans before surgery, to modify their epigenetic makeup, reduce harm from complications and improve outcomes for patients. We are not testing any new treatments in this study.

3. Introduction

3.1 Hypothesis

1. Expression of the epigenetic markers H3K4me3 and H3K27me3 by CD14+ monocytes increases the incidence and severity of organ dysfunction after major cardiac surgery through an exaggerated postoperative inflammatory response.

To test this hypothesis, a prospective observational study recruiting 30 patients undergoing major cardiac surgery (coronary artery bypass grafting (CABG)) will be conducted to assess for association between expression of 2 key epigenetic markers within CD14+ monocytes (H3K4me3 and H3K27me3) associated with dysregulated innate immune responses and validated clinical and biochemical measures of organ dysfunction and complications. This study will add knowledge to the recruiting multicentre prospective observational study IMPRoVE[1,2] (Incidence, impact and Mechanisms of Perioperative Right VEntricular dysfunction), as a sub-study of IMPRoVE assesses epigenetic susceptibility to organ dysfunction, via the same mechanism, in patients undergoing major thoracic and vascular surgery. Epi-SICCS will therefore provide a novel opportunity to extend investigation of patients undergoing major thoracic and vascular surgery (via IMPRoVE) by studying patients undergoing major cardiac surgery (Epi-SICCS).

3.2 Background and Rationale

Background

Postoperative complications are a major cause of morbidity, mortality and increased health care cost in patients undergoing elective surgery[3] and are particularly prevalent after cardiac (57%) surgery[4]. Further, whilst mortality and significant morbidity are easily recognised sequelae of major surgery, it is increasingly recognised that overt complications are the 'tip of the iceberg' and a significant burden of covert postoperative complications exist and have a significant long-term impact[3]. Seeking to better understand and ameliorate this burden of complications remains a research priority.

The 'stress response' to surgical trauma results from well-choreographed activation of autonomic, neuroendocrine, metabolic, and inflammatory responses which are necessary to maintain host homeostasis and facilitate tissue repair. The hypothesis that dysregulation of this response can lead to a non-specific whole-body response (commonly referred to as systemic inflammatory response syndromes (SIRS)) is not new but its mechanisms and pathogenesis are incompletely understood.

Common to the host response to a wide variety of acute tissue injuries e.g., burns, sepsis and trauma, the concept of a 'genomic storm' occurring within hours of major surgery is increasingly recognised [5,6]. This initial response affects more than 80% of cellular pathways and dynamically alters the leukocyte transcriptome with upregulation of the innate immune system and down regulation of the adaptive immune response. Whilst such a response in evolutionary terms might be considered adaptive, it can have deleterious effects on capillary permeability, immune function and wound healing and predispose to organ dysfunction.

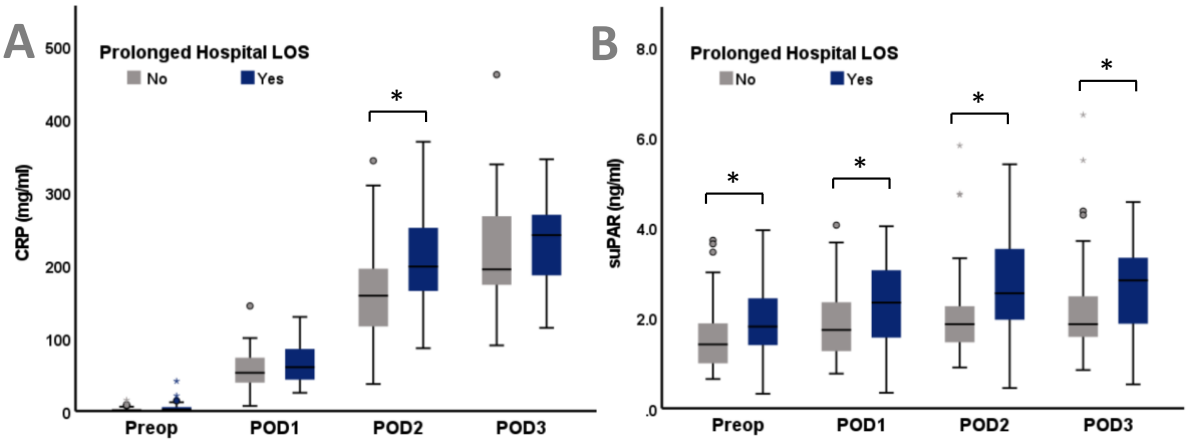


Figure 1. Previous work from our group demonstrating the magnitude of the inflammatory response and outcome after cardiac surgery.
Inflammatory markers A) C-reactive protein and B) Soluble urokinase plasminogen activator receptor (suPAR) verses hospital stay in 90 patients undergoing cardiac surgery with cardiopulmonary bypass. Prolonged hospital stay defined as 12 days or later as a composite for postoperative complications.
Plasma suPAR was also increased in patients experiencing prolonged duration of mechanical ventilation and prolonged ICU stay whilst CRP was elevated in patients experiencing postoperative atrial fibrillation (not shown). From Schultz-Swarthfigure et al[(6)], N=90.
*p<0.05, Mann-Whitney *U*-test. LOS, length of stay; POD, post operative day.

Though simplistic, 'inflammatory markers' have historically been used to describe the magnitude of the postoperative immune response and are consistently associated with

post-operative complications following cardiac (Figure-1[7]), thoracic[8] and vascular surgery[9]. Inflammatory injury can be identified in many organs postoperatively (even those remote from the site of surgery - for example, our work demonstrating cardiac inflammation following thoracic surgery - Figure 2)) and the magnitude of the postoperative inflammatory response has been associated with multi-organ morbidities including myocardial injury[10], lung injury[11], post operative cognitive dysfunction[12] and kidney injury[13].

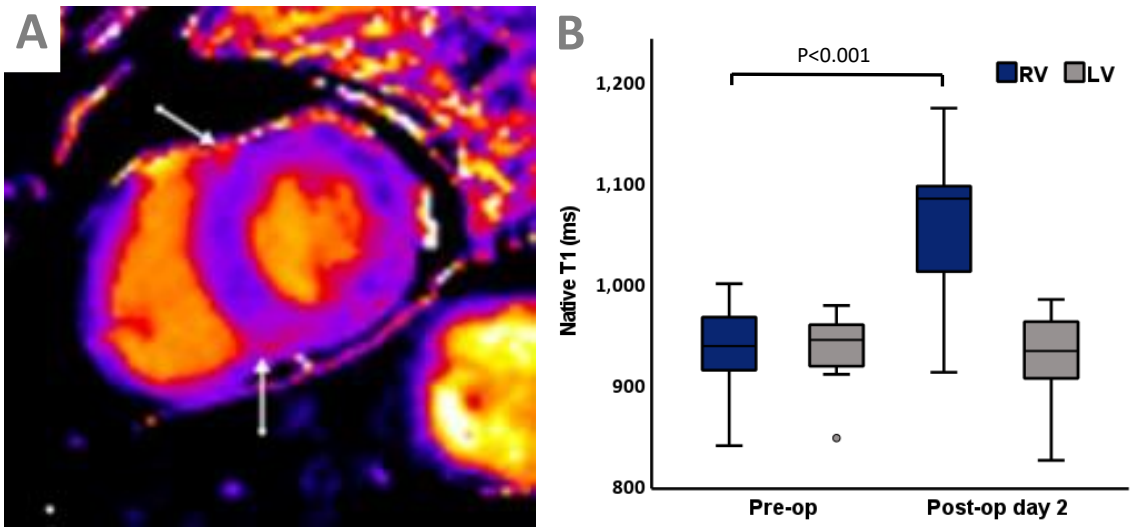


Figure 2. Previous work from our group identifying perioperative myocardial inflammation following thoracic surgery.
RV and LV tissue was characterised by T1 cardiovascular magnetic resonance at three perioperative timepoints in 14 patients undergoing lung resection. A) Pre-contrast T1-map on day 2 postoperatively in a 57-year-old lady who underwent lobectomy. Inflammation is identified by increased T1 (bright signal intensity) at the ventricular insertion points (VIPs - white arrows). B) Marked changes in native T1 and extracellular volume (not shown) indicative of myocardial oedema are evident on post-op day 2 in the RV (at VIPs) but not in the LV. Unpublished data.

Transcriptomic analyses aid understanding of this 'genomic storm'; in patients undergoing major thoracoabdominal surgery Allen et al demonstrated that 522 genes were differentially expressed (in leucocytes) 24-hours after surgery; 248 (48%) were upregulated (reflecting innate immunity and inflammation) and 274 (52%) were downregulated (reflecting adaptive immunity)[6]. In this study, within-patient gene expression change was positively associated with postoperative infection and hospital length of stay; in patients where gene expression was most radically altered postoperatively, outcomes were worse.

Host susceptibility to complications

Prediction of perioperative risk is a cornerstone of perioperative medicine. Whilst individual patient and procedural risk factors for complications and mortality exist, and are widely integrated into risk prediction scores, risk prediction remains an imprecise science. Clinicians recognise the heterogeneity in the clinical response to major surgery; apparently similar patients undergoing the same surgical procedure can behave very differently during the post-operative period. This highlights the importance of differing host susceptibility to post-operative organ dysfunction.

The potential for host genomics to impact perioperative outcome has received some study, and whilst it has been possible to identify single nucleotide polymormphisms which might lead to specific perioperative morbidity syndromes (e.g. butyrylcholinesterase deficiency[14]), it is unlikely that any single or combination of polymorphisms is responsible for the wide heterogeneity seen in patient responses to surgery. The potential value of studying genomic susceptibility to complications is further limited by the static nature of the genome; should predictors of outcome be identified, these changes are fixed with no potential for therapeutic manipulation. Because the epigenome is dynamic and as such, may be amenable to therapeutic manipulation or upstream modification, its study provides the potential to not just risk-predict, but risk-modify.

Epigenomics

Epigenomics concerns the study of how cells control gene activity without changing the DNA sequence (i.e. without altering genomic makeup). Cellular DNA is bound in chromatin as a complex of DNA and proteins. Modification of these proteins (classically by DNA methylation or histone modification) will alter the way these proteins interact with DNA and can regulate gene expression changing the way a cell responds to a stimulus.

Classical descriptions of the immune system describe the innate and adaptive responses with only the adaptive immune response being able to build immunological memory. In recent years, the potential for epigenetic reprogramming of innate immune cells in response to stimulation has been recognised [15,16]. Following an initial challenge (from a

pathogen or inflammatory insult), cells return to a non-activated state, but through modification of the epigenome are primed for response to a subsequent stimulus allowing rapid and enhanced recruitment of transcription factors after a secondary challenge. While much of this study has taken place in-vitro, the in-vivo corollary is that an individual's environment – the accumulated effects of aging, prior toxin ingestion, pathogen exposure and the influence of comorbidities – cumulatively influences the epigenome with the potential to fundamentally alter a cells response to a stimulus (Figure 3).

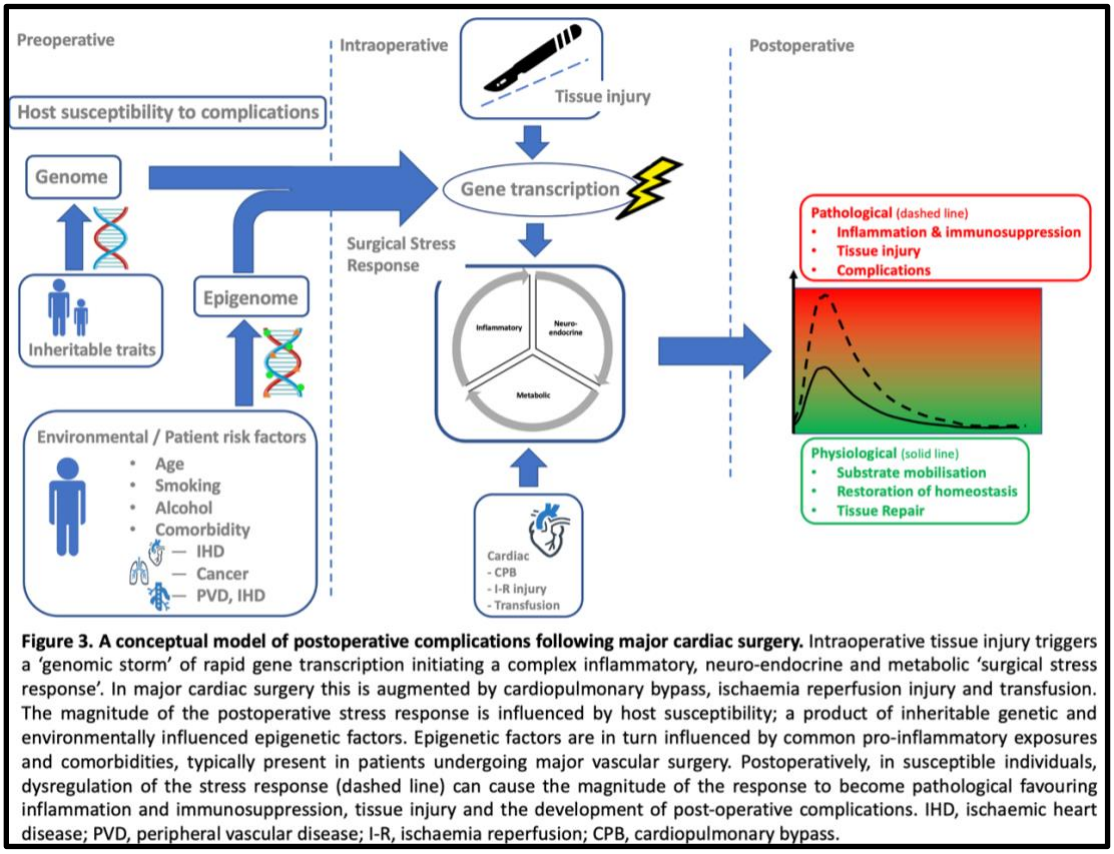


Figure 3. A conceptual model of postoperative complications following major cardiac surgery. Intraoperative tissue injury triggers a 'genomic storm' of rapid gene transcription initiating a complex inflammatory, neuro-endocrine and metabolic 'surgical stress response'. In major cardiac surgery this is augmented by cardiopulmonary bypass, ischaemia reperfusion injury and transfusion. The magnitude of the postoperative stress response is influenced by host susceptibility; a product of inheritable genetic and environmentally influenced epigenetic factors. Epigenetic factors are in turn influenced by common pro-inflammatory exposures and comorbidities, typically present in patients undergoing major vascular surgery. Postoperatively, in susceptible individuals, dysregulation of the stress response (dashed line) can cause the magnitude of the response to become pathological favouring inflammation and immunosuppression, tissue injury and the development of post-operative complications. IHD, ischaemic heart disease; PVD, peripheral vascular disease; I-R, ischaemia reperfusion; CPB, cardiopulmonary bypass.

The $CD14^+$ monocyte (the predominant human monocyte sub-species), whose immunological role is to 'sense and respond' is a key player in the innate immune response, reacting early to a host of stimuli. This fundamental role as trigger of the acute inflammatory response makes it a natural cell population for investigation in this setting. Moreover, due to their innate nature (i.e., immediate and prolonged response to stimuli) $CD14^+$ monocytes are unique circulating sensors of physiological state, and thus their epigenome can provide a surrogate readout of how the wider environment has influenced the cellular state.

H3K4me3 and H3K27me3 are modifications to the DNA-packaging histone H3 which have received wide study as two key epigenetic marks accompanying trained immunity[15,17]. Tri-methylation of H3K4 (the 4th lysine residue of the histone H3 protein) makes the DNA in chromatin more accessible to transcription factors, promoting gene transcription (conferring a so called 'on-state'). Conversely tri-methylation of H3K27 results in downregulation of nearby genes ('off-state'). With simultaneous presence of both H3 marks (known as bivalency), genes are referred to as 'poised'[18]. Such a poised state confers the ability to rapidly become activated in response to a stimulus. Analogously, the engine is revving (H3K4me3), but the hand brake is on (H3K27me3). Release of the 'brake' in response to tissue injury has the potential to result in a rapid and robust immune response. Previous work by our group demonstrates wide variety in the epigenetic signature of circulating $CD14^+$ monocytes in patients with chronic inflammatory diseases (Figure 4).

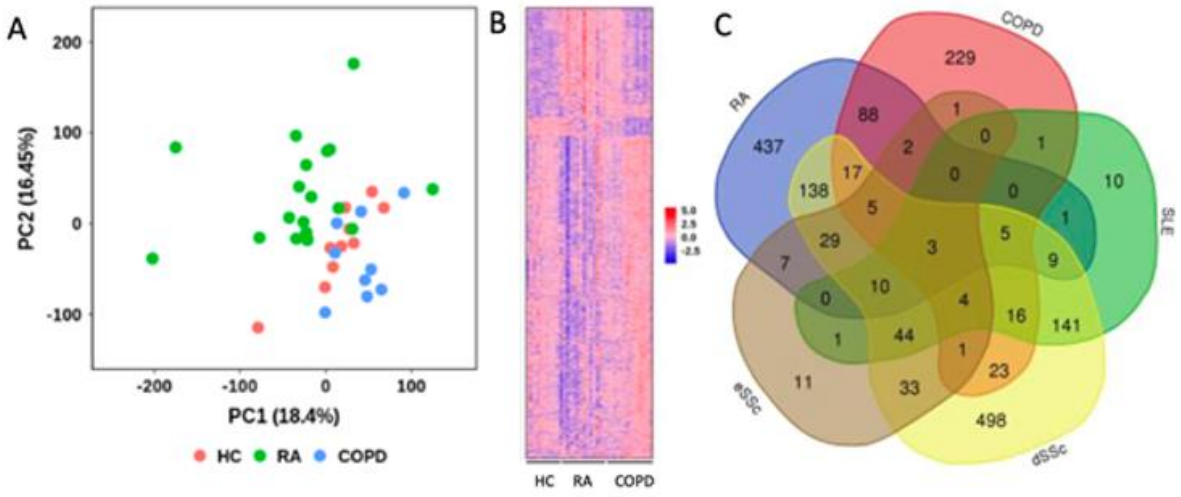


Figure 4. Epigenetic signatures within circulating monocytes in patients with chronic inflammatory conditions.
Circulating monocytes were obtained from patients with Rheumatoid Arthritis (RA, n=18), Chronic Obstructive Pulmonary Disease (COPD, n=9) and healthy controls (HC, n=9). To evaluate the epigenetic profile in the circulating monocyte pool, ChIPseq technology employing H3K4me3 and H3K27me3 antibodies was deployed in CD14+ cells sorted from patients' peripheral blood mononuclear cells. (A) Principal component analysis of ChIPseq data (H3K4me3) demonstrating striking differences in epigenetic signatures between groups. (B) Heatmaps depicting significantly different H3K4me3 peaks. (C) Venn diagram depicting the unique and shared H3K4me3/H3K27me3 profile of monocytes from RA, COPD, systemic lupus erythematosus (SLE) and early- (eSSc) and diffuse- systemic sclerosis (dSSc). Whilst the expression of the H3K4me3/H3K27me3 profile is ubiquitous, the pattern of distribution varies widely radically altering the potential downstream behaviour of these monocyte populations. Unpublished.

Whilst on an evolutionary basis, immune training should confer a survival benefit when faced with a potential pathogen, there are examples where such adaptation is deleterious, particularly where it could trigger enhanced tissue damage[15]. In addition to chronic

inflammatory co-morbidities, common indications for cardiac surgery such as ischemic heart disease, alongside patient factors such as cigarette smoking and alcohol consumption, have the potential to serve as primary stimuli for epigenetic modification altering a patient's susceptibility to a secondary response (i.e. surgery).

The role of H3K4me3/H3K27me3 modification in the acute inflammatory response has seen limited study and the influence of such immune priming on CD14+ monocytes in the perioperative immune system response has not been explored. What data does exist, predominantly examining the effects of prior pathogen exposure (e.g. P.falciparum malaria[19]) or inflammatory comorbidity (e.g. IHD) on the ex-vivo response to experimental sepsis speaks of a fundamental influence. Bekkering et al for example demonstrated monocytes from patients with severe symptomatic atherosclerosis (i.e. surgical candidates) to have an approximately 2-fold increase in production of pro-inflammatory cytokines upon lipo-polysaccharide stimulation compared to healthy controls[20], an effect which appears to be mediated by altered H3K4me3 and H3K27me3 levels on the TNFα, Interleukin-6 and -1ß promotors[20]. Sadahiro and colleagues investigated 30 patients undergoing either major colorectal or orthopaedic surgery, performing genome-wide DNA methylation analysis perioperatively on patient peripheral blood mononuclear cells and identified distinct methylation changes associated with inflammatory gene activation triggered by surgery which persist to the day of discharge, correlating with increased inflammation[21]. The authors discuss a limitation of their study is the diverse populations of leukocytes (monocytes, plasmablasts, CD8+ lymphocytes, CD4+ lymphocytes, Natural Killer lymphocytes) present in their analysis and caution that future studies should analyse specific cell populations (as proposed in the Epi-SCCS study) to accurately determine the inflammatory contribution of distinct leukocyte populations to major surgery. They further acknowledge that they have not performed gene expression analysis and therefore the link between DNA methylation patterns and inflammatory protein abundance is not causally explained. Laudanski et al later investigated the presence of free histones H3 and H3K4me3 and H3K27ac at several time-points (including 3-months post-operatively) in serum of 59 patients undergoing a variety of major cardiac surgeries[22], demonstrating the presence of "smouldering inflammation" present at 3-months after major cardiac surgery.

In summary

Major surgery triggers inflammation as part of the physiological response to tissue injury, however, excessive inflammation in the post-operative period is associated with organ dysfunction, complications and morbidity. Epigenetic markers can dictate the magnitude and duration of powerful inflammatory responses. We aim to study monocyte epigenetic expression, determine subsequent inflammatory gene activation/deactivation patterns (transcriptomics) and quantify inflammation (proteomics) to describe the epigenetic regulation of inflammation and its role in perioperative morbidity. Due to the modifiable nature of the epigenome, future intervention trials can then study existing or new therapies prior to surgery, to recondition the epigenome in 'at risk' patient groups as part of perioperative prehabilitation programmes, to reduce the morbidity associated with major surgery.

4. Study Plan

4.1 Study Design

Single-centre prospective observational study.

4.2 Study Setting

Golden Jubilee National Hospital (GJNH), Clydebank – National Waiting times Centre Board: Recruitment of patients undergoing major cardiac surgery (coronary artery bypass grafting (CABG) with cardiopulmonary bypass).

4.3 Study Summary

Thirty patients undergoing major cardiac surgery (CABG) will be recruited at the Golden Jubilee National Hospital, to a prospective, observational haemodynamic and biomarker study to determine the effect of CD14+ monocyte expression of H3K4me3 and H3K27me3 epigenetic modifications on post-operative inflammatory morbidity measured by validated clinical and biochemical measures of organ dysfunction and complications.

Patients will have blood samples taken for epigenetic, transcriptomic and proteomic analysis pre-operatively on the day of surgery, on post-operative day 1 and post-operative day 7. Routinely collected daily biochemistry will be analysed, including: full blood count, coagulation screen, c-reactive protein, liver function tests, urea and electrolytes, and additional available measures of organ dysfunction such as: troponin, BNP, ESR and procalcitonin (for example).

All patients will have blood samples taken at the same time as the epigenetic blood samples, and biobanked for transcriptomic and proteomic analysis. These tests will investigate how different epigenetic expression profiles variably enable or suppress different inflammatory genes, and the effect of this differential inflammatory gene expression on overall systemic inflammatory protein abundance.

5. Outcome Measures

5.1 Primary Outcome Measures

Primary outcome

Post-operative organ dysfunction quantified by measurement of mean Sequential Organ Failure Assessment (SOFA) score over the first seven postoperative days.

Rationale for primary outcome

The SOFA score is calculated from the degree of cardiovascular, respiratory, renal, hepatic, neurological, and haematological organ dysfunction and was originally validated in patients with sepsis, where higher SOFA scores were associated with higher rates of mortality [23]. The role of SOFA score has expanded beyond patients with sepsis, and it has shown utility in perioperative populations; SOFA scores have been shown to predict mortality and correlate with ICU length of stay in patients following cardiac surgery [24]. As the magnitude of the inflammatory response can lead to multi-system dysfunction, the SOFA score will be used to detect impairment of these organ systems. Where patients are well, and in a non-HDU/ICU environment, the use of the modified SOFA score respiratory domain (where a PaO_2/FiO_2 SOFA score equivalent is calculated from the SpO_2/FiO2 ratio) will permit a SOFA score to be calculated without the need for arterial blood gas (ABG) sampling. The modified SOFA score has shown highly comparable predictive capabilities to the SOFA score [25].

5.2 Secondary outcomes

Secondary outcomes

- Laboratory endpoints – routinely measured peri-operative laboratory data indicative of:
  - Inflammation: C-reactive protein, albumin, differential white cell count.
  - Organ dysfunction: Creatinine, estimated glomerular filtration rate, liver function tests, arterial blood gases, troponins.

- Monocyte inflammatory gene expression measured using transcriptomic analysis (RNA-seq) and systemic inflammatory protein quantification using proteomic analysis (O-link).

- Clinical outcomes informed by the Standardising Endpoints in Perioperative Medicine (StEP) consensus definitions:
  - Cardiovascular[26], respiratory[27] and renal complications[28].
  - Composite major complications – Postoperative Morbidity Survey (POMS)[29].
  - Length of critical care and hospital stay[30].
  - Need for unplanned ICU admission[30].
  - Days alive and at home[31].

5.2.1 Laboratory Methods

Epigenetic and transcriptomic analyses

Peripheral blood mononuclear cells (CD14+) will be isolated and biobanked from all 30 patients pre-operatively (on the day of surgery), on the 1st post-operative day and on the 7th post-operative day. Samples will be drawn contemporaneously in 10ml EDTA vials with other blood samples and transported via taxi to the Institute of Infection, Immunity and Inflammation at the University of Glasgow where CD14+ monocytes will be isolated and stored (in CryoStor® cryopreservation medium) at -80^{o}C for subsequent analysis. Samples must be transported to the Institute of Infection, Immunity and Inflammation in a timely manner to ensure their correct storage. A standard operating procedure (SOP) will be followed to ensure appropriate and consistent handling of study samples.

In batched analyses, CD14+ monocytes will then undergo Chromatin Immunoprecipitation and next generation sequencing (ChIPseq) and transcriptomic analysis using RNA sequencing (RNAseq). For ChIPseq, monocyte cellular lysates will be sonicated to obtain DNA/protein fragments, which will be mixed with either H3K4me3 or H3K27me3 specific antibodies to enable immunoprecipitation. For RNAseq, RNA sample libraries will be prepared using the NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (New England BioLabs) following the manufacturer's instructions and a standard operating procedure. NEBNext Ultra-II DNA Library Prep Kit for Illumina (New-England BioLabs) will be used on the resulting purified DNA (from ChIPseq and RNAseq samples) to generate DNA libraries. These libraries will be sequenced on the NextSeq-500 platform (Illumina), with each sample sequenced to a depth of ~30million reads, with fragments undergoing single-end sequencing of 75 base pair read lengths.

Proteomic analysis

Proteomic test samples will be taken in 3ml EDTA containers for whole blood analysis. Samples will be aliquoted (0.5ml) into 3 cryogenic tubes prior to storage at -80^{o}C. These samples will then be sent in bulk under -80^{o}C conditions via courier to the Olink Immunogenomics Facility, University of Leeds. Following plating to an appropriately randomised sequence, samples will be batch-analysed and proteomic analysis conducted. We will use the Olink Target 48 panel to quantify the abundance of 45 key inflammatory proteins during the perioperative period and to map these findings to CD14+ monocyte epigenetic expression and resulting monocyte inflammatory gene expression.

5.3 Statistical Considerations

1. Data-processing

Epigenetic (ChIP-Seq) and transcriptomic (RNA-Seq) data will undergo quality control using FastP (v2.1.1). Sequencing reads will be aligned to the human reference genome (GRCh38.p13, Ensembl) using Bowtie2 (v2.4.1). Regions of enriched histone modification (peaks) will be identified using MACS3 (v3.2.7). The epigenetic H3K4me3/H3K27me3 peaks, inflammatory gene and protein peaks will be annotated using HOMER (v4.11), and regions of differential abundance between clinical categories determined using DiffBInd (4.1),

under a strict false discovery rate (FDR<0.01) threshold. Where necessary the data will be corrected for clinical confounders or effects of 'Fraction of Reads in Peaks' using DiffBind and SVA (v4.1). Confounders will be identified using variance partition.

2. Data analysis

After data is initially explored, we will use global and unbiased dimensionality reduction methods such as PCA and UMAP, to look for global clustering of patients by postoperative outcome. This will also inform us as to the proportion of variation in the epigenetic landscape explained by clinical outcome. Next, we will use clustering methods such as K-means to classify patients into unbiased data driven groups, which will then be statistically compared to clinical outcome data.

We will then proceed with biased approaches. The data will be split into thirds by each clinical metric of postoperative outcome (e.g. CRP mean SOFA score, hospital stay), and differential binding analysis performed on the extremes. This will identify individual peaks/promoters with differential H3K4me3 and H3K27me3 profiles significantly linked to patient outcome. Similarly, transcriptomic and proteomic analysis will identify abundant inflammatory gene and protein expression. We will visualise these using boxplots, browser traces and heatmaps. Differential peaks will be annotated using HOMER, and a pathway analysis performed using Gene Set Enrichment Analysis, STRING and the GO database. This will inform our interpretation and potential identification of possible therapeutic targets. Differential regions identified between extreme tertile groups will be further validated using the complete dataset, via correlation analysis.

3. Power

Due to the exploratory nature of the study a traditional power calculation is not feasible. To provide some indication we have calculated[A] the power we would have when treating the first principal component as an actual variable instead of a meta-variable. When comparing the extreme tertiles in terms of mean SOFA score, i.e. with 30 per group, we

would have 90% power to detect a difference of 0.73 standard deviations. The actual power however cannot be determined since the preceding step of deriving the principal components is data-driven. RnaSeqSampleSize is a sample size package based on distributions of read counts and dispersions from real data to provide accurate sample size calculation with appropriate control of the FDR[32]. Simulations based within this model (Figure 5) and using data from previous work from our group[33] demonstrate that a sample size of 30 should provide adequate statistical power in most conceivable scenarios (statistical handling of RNA-seq and ChIP-seq experiments is analogous).

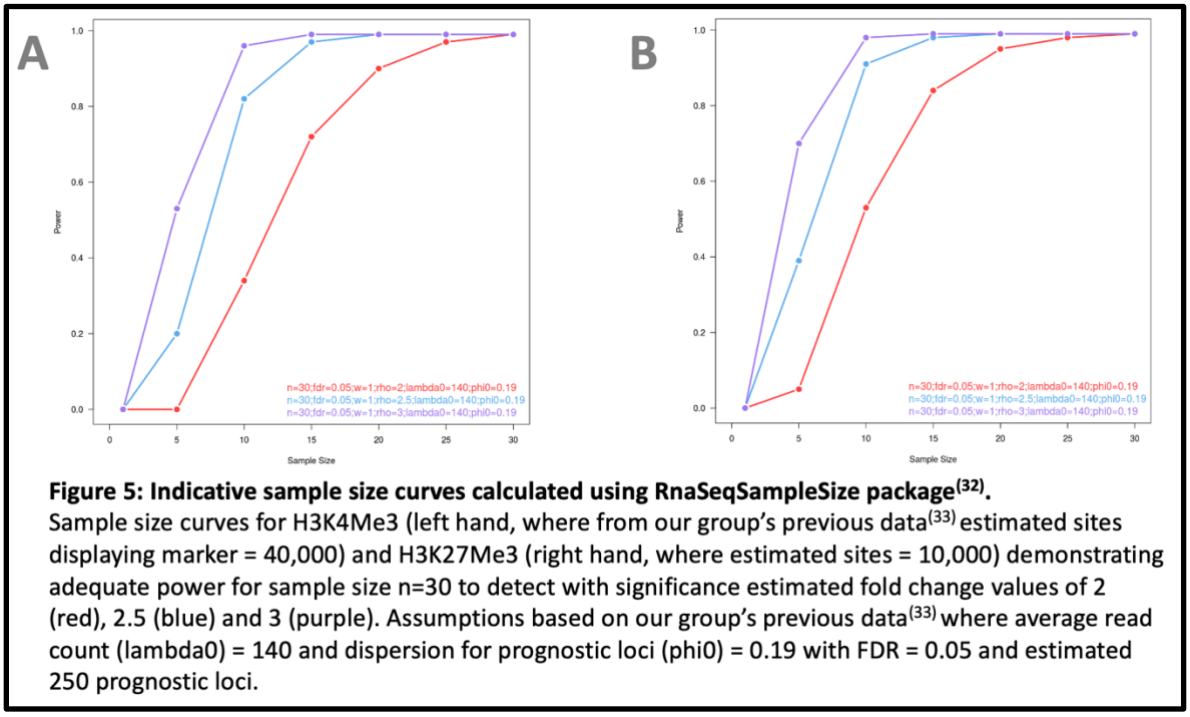


Figure 5: Indicative sample size curves calculated using RnaSeqSampleSize package[32].
Sample size curves for H3K4Me3 (left hand, where from our group's previous data[33] estimated sites displaying marker = 40,000) and H3K27Me3 (right hand, where estimated sites = 10,000) demonstrating adequate power for sample size n=30 to detect with significance estimated fold change values of 2 (red), 2.5 (blue) and 3 (purple). Assumptions based on our group's previous data[33] where average read count (lambda0) = 140 and dispersion for prognostic loci (phi0) = 0.19 with FDR = 0.05 and estimated 250 prognostic loci.

[A]Performed in collaboration with Dr Martina Messow, Consultant Statistician at the Robertson Centre for Biostatistics, University of Glasgow.

6. Selection and Withdrawal of Study Subjects

6.1 Inclusion Criteria

1) Provision of informed consent
2) Age >18 years
3) Planned elective coronary artery bypass grafting (CABG) surgery with cardiopulmonary bypass

6.2 Exclusion Criteria

Subjects fulfilling any of the criteria below will be excluded from the study:

1) Pregnancy.
2) On-going participation in any investigational research which could undermine the scientific basis of the study.
3) Previous major surgery (as per PQIP procedures list) within three months prior to recruitment, or previous midline sternotomy.
4) Patients receiving immunosuppressive therapies for a chronic inflammatory condition or cancer.
5) Patients with a haematological malignancy.
6) Inadequate comprehension of English resulting in inability to comply with instructions.

6.3 Withdrawals and Exclusions

Participants will be made aware from the beginning of their participation in the study that they can freely withdraw (discontinue participation) from the study (or part of) at any time. The patient will not have to give a reason for their decision to withdraw. Withdrawal rates will be monitored throughout the study by the study team.

Types of withdrawal will be defined as:

- **Withdraw but consent to remote follow-up:** The participant would like to withdraw from the study but is willing to be remotely followed up in accordance with the schedule of assessments (i.e., the participant has agreed that data from medical notes can be collected and used in the study analysis but they do not want to be contacted via e-mail/ telephone for questionnaires from that point onwards).

- **Withdraw from follow-up but consent for use of data collected so far:** The participant would like to withdraw from the study and is not willing to be followed up in any way for the purposes of the study and for no further data to be collected, but consents to data collected prior to the withdrawal to be used in the study analyses.

- **Withdraw completely:** The participant wishes to withdraw completely from the study and all study follow up and is not willing to have any of their data, including that already collected, to be used in any study analyses.

The details of withdrawal (date, reason and type of withdrawal) will be clearly documented in the source data and on the Change of Status Form.

All patients will be specifically informed preoperatively at the time of study consent of the potential (in a small minority of participants) for transient loss of capacity postoperatively due to delirium or the need for sedation and intensive care unit management (which is routine in the immediate postoperative period following cardiac surgery). As organ dysfunction from the studied epigenetic mechanisms could plausibly contribute to these complications, it is essential for the scientific validity of the study that data continues to be collected from these patients. As such consent will be sought (at the time of study consent) from patients such that in the event of ICU admission and need for sedation, clinical data will continue to be collected, and blood sampling will be performed in this situation.

7. Study Conduct

7.1 Recruitment

Patients will be identified from the waiting list for coronary artery bypass graft through consultation between the patient's normal care team (operating surgeons and anesthetists) and a member of the local research team. Patients will be informed of the study, offered a patient information sheet (PIS), and invited to participate at the earliest possible opportunity after they have been informed of the decision to operate. This may take place at either the surgical clinic of the operating surgeon, on attendance at the pre-assessment clinic, by posting or e-mailing a cover letter and follow-up phone-call or by approach on admission to hospital for surgery. Patients will always have been informed of the plan to undergo surgery prior to approach by the study team. Following appropriate time to consider participation (as a minimum, participants will receive the PIS and study information from the research team on the day prior to consent for participation), informed consent will be obtained by the site PI or other appropriately trained member of site team.

7.2 Consent

Written informed consent will be obtained, following a face-to-face discussion about the study (where patients are given the opportunity to ask questions) by a member of the study team. Signing of consent form and preoperative blood sampling may take place at any time in the 30 days prior to surgery or on day of surgery.

7.3 Medical Management and Safety Reporting

Medical management will be according to the standard of care and is not changed as part of this research protocol.

7.4 Adverse event management

AEs and SAEs are common in major cardiac surgery. Many AEs and SAEs are therefore considered to be expected postoperative complications and as such will be recorded as study outcomes and do not need to be reported as AEs / SAEs.

Epi-SICCS is an observational study where the risk of AEs and SAEs attributable to study conduct is highly unlikely as the only change to routine clinical care is the collection of blood samples and questionnaires. Where possible, blood samples will be obtained at the same time as blood samples required for clinical monitoring/investigation. Only AEs / SAEs which can be directly attributed to study conduct should be reported.

7.4.1.1 Notification of deaths

Death is included as a trial outcome. No separate reporting of death is required.

8. Data Management

8.1 Data Collection

Patient data will be collected via case report forms (CRFs) by one of the investigators or an appropriately trained research nurse. This data will be submitted electronically to a secure online database (Research Electronic Data Capture (REDCap), Vanderbilt University, Tennessee, USA) in linked-anonymised form with a unique alphanumeric study number allocated.

The study will adhere to all data protection regulations. Patients' identities will be protected and their information held securely.

8.1.1 Schedule of Assessments

Table 2. Schedule of assessments for patients enrolled into Epi-SICCS study							
Visit Window	**Pre-op**	**Day of surgery (Day 0)**	**POD 1-6**	**POD 7**	**Discharge**	**30 days**	**12 months**
- Inclusion/exclusion criteria - Informed consent - Baseline demographics - Baseline 4AT score - EuroSCORE - DASI	x						
Immediate perioperative data		x					
QoR-15	x			x			
SOFA score	x		x	x			
Organ specific complications (Clavien-Dindo ≥ 2)			x	x	x	x	
Length of Hospital stay					x		
- Requirement for new invasive or non-invasive ventilation (including HFNO) - Unplanned ICU Admission - Length of ICU/HDU stay			x	x	x		
Mortality							x
DAH_{30}, Hospital readmission						x	
EQ-5D-5L, WHODAS 2.0	x					x	x

8.1.2 Baseline and Demographic Data

- Age
- Sex
- Height and Weight
- Ethnicity
- Smoking status
- Alcohol consumption
- Rockwood Clinical Frailty Score
- American Society of Anesthetists Score
- 4AT delirium assessment score
- Comorbidities
- Medication history
- COVID infection and vaccination history
- EuroSCORE

Patient data will be collected from clinical history taken at time of enrolment and from electronic patient records such as Clinical Portal.

8.1.3 Clinical Data

8.1.3.1 Pre-operative data

- Routine observations including oxygen saturations, heart rate and blood pressure
- SOFA score
- Pre-operative questionnaires- Duke Activity Status Index (DASI) and baseline QoR-15, EQ-5D-5L and WHODAS 2.0
- EuroSCORE

8.1.3.2 Immediate perioperative data

- Date of operation
- Proposed Operation

- Duration of surgery and anaesthesia
- Regional analgesic technique
- Modality of blood pressure monitoring
- Maintenance of primary anaesthesia
- Duration of invasive ventilation
- Bypass duration
- Cross-clamp duration
- Need for vasopressor or inotrope administered by infusion (inc dose and timing)
- Need for IABP
- Blood transfusion requirement
- Total other fluid input
- Total urine output
- Major intra-operative complications: anaphylaxis; major hemorrhage; other.
- Operation performed
- Postoperative destination and level of care

Where available continuous recording of interoperative haemodynamic and ventilatory data captured electronically and drug administration e.g., via RECALL or CHA-a system at the Golden Jubilee National Hospital, will be obtained for post-hoc analysis.

8.1.4 Laboratory Data

- Laboratory data will include routinely collected data used for clinical care which inform on the degree of inflammation (such as CRP, WCC) and organ dysfunction (such as serum creatinine, troponin), in addition to the experimental data obtained through this study (epigenetic expression of H3K4me3 and H3K27me3, transcriptomic expression of inflammatory genes and proteomic expression of inflammatory proteins).

8.1.5 Follow-up Data

- Patients will be screened for major complications (graded Clavien-Dindo ≥ 2), requirement for new non-invasive or invasive ventilation, or re-admission to HDU or ICU on postoperative days 1-7, on discharge and at 30-days post-operatively. The highest grade of each complication since last screening will be recorded.

- Duration of postoperative invasive ventilation.

- SOFA score will be calculated on each postoperative day up to day seven.

- QoR-15 questionnaires will be completed on postoperative day 7.

- Participants will be contacted by telephone at Day 30 (+ 5 days) by site research staff to collect data on DAH30, hospital readmission and any major complications since discharge.

- EQ-5D-5L and WHODAS questionnaires will be conducted with patients via telephone, post or email (depending on their preference) at 30 days, three months and one year postoperatively.

8.2 Database / Data storage

Anonymized patient data will be stored on password protected university and NHS hospital computers. A patient identifier list will be kept in a secure location within the Research & Development offices at Golden Jubilee National Hospital. As stated previously data from paper CRFs will be anonymised and transcribed into the secure online database REDCap (see 8.1). Trial data downloaded from REDCap will be sent in an anonymised format to either NHS or University computers where statistical analyses will be carried out.

8.3 Archiving

Electronic data will be stored in an encrypted and anonymised format for 10 years following the completion of the trial. At the end of this period, the dataset will be destroyed according to DoD 5220.22-M standards. All data will be held in accordance with The General Data Protection Regulation (2018).

9. Study organisation

9.1 Study Sponsor

The Study will be sponsored by the National Waiting Times Centre Board (Golden Jubilee National Hospital).

9.2 Administration

All routine clinical and non-clinical co-ordination of the study will be the responsibility of the principal investigator (BS) in conjunction with research team staff at the GJNH. The chief investigator (BS) will assume responsibility for the overall management and conduct of the study.

9.3 Indemnity

The NHS research indemnity scheme will apply.

9.4 Monitoring

This study will be sponsored by the National Waiting Times Centre Board (Golden Jubilee National Hospital). As such, the Research and Development department undertake to monitor this research in line with the Research Governance Framework for Health and Community Care (Scotland).

9.5 Funding

This study has been funded by the European Association of Cardiothoracic Anaesthesiologists and Intensive Care (EACTAIC) (RW).

10. Ethics and Regulatory Approval

The study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and to Good Clinical Practice (GCP) Guidelines. The study has been granted approval by the London South East Research Ethics Committee (Ref: 26/LO/0117) and will comply with all applicable UK legislation. All local site Standardised Operating Procedures (SOPs) will be followed.

11. Protocol Amendments

Any substantial amendments to the final protocol will be clearly documented and forwarded to the Research Ethics Committee for approval prior to implementation via the IRAS process of Notification of (Substantial) Amendment.

12. Dissemination

All publications and presentations relating to this study will be authorized by the trial Chief Investigator (BS). Authorship will be determined according to the international committee of medical journal editors' recommendations for the conduct, reporting, editing and publication of scholarly work in medical journals. The results of the study will be first reported to study collaborators. Subsequently, we will communicate our results by reporting them to the funder and presentation at national meetings, with publication in appropriate peer reviewed journals. At the conclusion of the study, all participants will be thanked in writing for their participation in the study and will be provided with a summary of the trial findings.

Further details about the trial results and final report will be available on request to the scientific community in a timely manner.

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14. Appendices

13.1 Appendix 1: Definition of postoperative complications

All complications from the day of surgery will be recorded at day of discharge and 30 days postoperatively (up to 35 days to allow contacting patients at home).

All complications that occur in the interval from last recording will be screened for with the highest Clavien-Dindo score during that interval recorded.

Definitions

1. **Acute Kidney Injury**

According to the KDIGO consensus definition of AKI[1]:

Stage	Serum Creatinine	Urine output
1	1.5-1.9x baseline OR ≥26.5umol/L increase	<0.5ml/kg/hr for 6-12 hours
2	2.0-2.9x baseline	<0.5ml/kg/hr for ≥12 hours
3	3.0x baseline OR ≥353.6umol/L increase OR initiation of renal replacement therapy	<0.3ml/kg/hr for ≥24 hours OR no urine output for ≥12 hours

If urine output is not measured/recorded, incidence of AKI will be solely based on serum creatinine or commencement of renal replacement therapy.

2. **Clavien-Dindo scale**

All reported postoperative complications will be graded on severity by using the Clavien-Dindo scale[1]. Scores ≥2 will be included as a complication.

Grade	Definition
Grade I	Any deviation from the normal postoperative course without the need for pharmacological treatment or surgical, endoscopic, and radiological interventions
	Allowed therapeutic regimens are: drugs as antiemetics, antipyretics, analgetics, diuretics, electrolytes, and physiotherapy. This grade also includes wound infections opened at the bedside
Grade II	Requiring pharmacological treatment with drugs other than such allowed for grade I complications
	Blood transfusions and total parenteral nutrition are also included
Grade III	Requiring surgical, endoscopic or radiological intervention
Grade IIIa	Intervention not under general anesthesia
Grade IIIb	Intervention under general anesthesia
Grade IV	Life-threatening complication (including CNS complications)* requiring IC/ICU management
Grade IVa	Single organ dysfunction (including dialysis)
Grade IVb	Multiorgan dysfunction
Grade V	Death of a patient
Suffix "d"	If the patient suffers from a complication at the time of discharge (see examples in Table 2), the suffix "d" (for "disability") is added to the respective grade of complication. This label indicates the need for a follow-up to fully evaluate the complication.

*Brain hemorrhage, ischemic stroke, subarrachnoidal bleeding, but excluding transient ischemic attacks.
CNS, central nervous system; IC, intermediate care; ICU, intensive care unit.

3. **Myocardial infarction (MI)**

Acute myocardial infarction after cardiac surgery as defined by the European Association of Cardio-Thoracic Surgery (EACTS) expert consensus statement on perioperative myocardial infarction after cardiac surgery. Post-operative high sensitivity troponin (hs-Tn) ≥35-500x URL (upper reference limit) with supportive signs of new myocardial infarction (see below) or hs-Tn ≥500x URL. Supportive signs of new myocardial infarction, include at least one of:

- New ischaemic ECG changes.
- Imaging (such as TTE, TOE, CMR or PET) evidence of new loss of viable myocardium or regional wall motion abnormality consistent with an ischaemic aetiology**.**
- Identification of coronary thrombus by angiography or CTA/CMR.

4. **Myocardial injury**

Perioperative myocardial injury is defined as post-operative high sensitivity troponin (hs-Tn) ≥35-500x URL (upper reference limit) without the above supportive signs of new myocardial ischaemia.

5. **Coronary revascularisation**

Percutaneous coronary intervention or repeat coronary artery bypass graft surgery within 30 days of the index surgery

6. **Cardiac death**

Death with a vascular cause, including deaths after an MI, cardiac arrest and cardiac revascularisation procedures. Does not include death after pulmonary embolism, haemorrhage, multi-organ failure or unknown cause of death.

7. **Non-fatal cardiac arrest**

Successful resuscitation from either documented or presumed ventricular fibrillation, sustained ventricular tachycardia, asystole or pulseless electrical activity requiring cardiopulmonary resuscitation, pharmacological therapy or cardiac defibrillation.

8. **Pulmonary embolism (PE)**

For radiologically confirmed PE requires one of the following:

- High probability ventilation/perfusion lung scan
- Intraluminal filling defect of segmental or larger artery on a helical CT scan
- Intraluminal filling defect on pulmonary angiography
- Positive diagnostic test for deep venous thrombosis (e.g., positive compression ultrasound) plus one of:
  - Non-diagnostic ventilation/perfusion lung scan
  - Non-diagnostic helical CT scan

For clinically suspected PE requires documented suspected diagnosis plus management plan.

9. **Deep venous thrombosis**

Requires one of the following:

- Persistent intraluminal filling defect on contrast venography

- Non-compressibility of one or more venous segments on B-mode compression ultrasonography
- Clearly defined intraluminal filling defect on contrast enhanced CT

10. **Atrial fibrillation**

New onset of irregularly irregular heart rate in the absence of P waves lasting for at least 30 seconds on monitor or for the duration of the ECG recording (if performed).

11. **Major Adverse Cardiac Events (MACE)**

A composite outcome includes:

- Cardiac death (as defined above)
- Myocardial infarction (as defined previously)
- Non-fatal cardiac arrest (as defined previously)
- Coronary revascularisation (as defined previously) within 30 days of the index surgery.

12. **Infective complications**

Fever

Core body temperature over 38.5$^{\circ C}$ more than 24 hours following surgery with two readings in a 12-hour period.

Clinical suspicion of infection

Use of non-prophylactic antibiotics PLUS documentation of suspected site (respiratory/urinary/blood/wound/other).

13. **Delirium**

Instigation of new anti-delirium medication (including antipsychotics, benzodiazepines or other sedatives)**.** Do not record if the patient is usually on regular anti-psychotics or benzodiazepine**.**

Or

A 4AT score of ≥ 4

14. **Stroke**

New neurological signs (e.g., weakness, expressive/receptive difficulties) lasting over 24 hours or cerebral infarction or intracerebral haemorrhage on computed tomography or magnetic resonance imaging scan.

15. **Atelectasis**

Reported on chest radiograph or computed tomography.

16. **Pneumonia**

Diagnosed using the US Centre for Disease control criteria[3]:

Two or more serial chest radiographs with at least one following feature:

- New or progressive and persistent infiltrate

- Consolidation
- Cavitation

(One radiograph is sufficient for patients with no underlying pulmonary/cardiac disease and at least one of the above features)
AND at least one of:

- Fever (>38.0°C) with no other recognised cause
- Leukopaenia (<4x10^9/L) or leukocytosis (>12x10^9/L)
- Altered mental state with no other cause in adults >70 years old
- AND at least two of:
- New onset of purulent sputum/change in character of sputum/increased respiratory secretions/increased suctioning requirements
- New onset/worsening cough, dyspnoea or tachypnoea
- Rales or bronchial breath sounds
- Worsening gas exchange (hypoxia/increased oxygen requirement/increased ventilator demand)

17. **Acute Respiratory Distress Syndrome**

As defined by the Berlin Consensus criteria (2012)[4]:

- Within one week of a known clinical insult or new worsening respiratory symptoms
- AND bilateral infiltrates on chest imaging, not fully explained by effusions, lobar/lung collapse or nodules
- AND respiratory failure not explained by cardiac fluid/fluid overload (requires objective assessment)
- AND supplemental oxygenation:
  - Mild = PaO_2:FiO_2 26.7-40.0kPa with PEEP or CPAP ≥5cmH_2O
  - Moderate = PaO_2:FiO_2 13.3-26.6kPa with PEEP ≥5cmH_2O

Severe = PaO_2:FiO_2 ≤13.3kPa with PEEP ≥5cmH_2O

18. **Pulmonary Aspiration**

Diagnosed by clear clinical history AND clear radiological evidence

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