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# Discovery of plasma proteome markers associated with clinical outcome and immunological stress after cardiac surgery

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**Background:** Molecular mechanisms underlying perioperative acute phase reactions in cardiac surgery are largely unknown. We aimed to characterise perioperative alterations of the acute phase plasma proteome in a cohort of adult patients undergoing on-pump cardiac surgery using high-throughput mass spectrometry and to identify candidate proteins potentially relevant to postoperative clinical outcome through a novel, multi-step approach.

**Methods:** This study is an analysis of the Bern Perioperative Biobank, a prospective cohort of adults who underwent cardiac surgery with the use of cardiopulmonary bypass (CPB) at Bern University Hospital between January and December 2019. Blood samples were taken before induction of anaesthesia and on postoperative day one. Proteomic analyses were performed by mass spectrometry. Through a multi-step, exploratory approach, hit-proteins were first identified according to their perioperative prevalence and dynamics. The set of hit-proteins were associated with predefined clinical outcome measures (all-cause one-year mortality, length of hospital stay, postoperative myocardial infarction and stroke until hospital discharge).

**Results:** 192 patients [75.5% male, median age 67.0 (IQR 60.0–73.0)] undergoing cardiac surgery with the use of CPB were included in this analysis. In total, we identified and quantified 402 proteins across all samples, whereof 30/402 (7%) proteins were identified as hit-proteins. Three hit-proteins—LDHB, VCAM1 and IGFBP2—demonstrated the strongest associations with clinical outcomes. After adjustment both for age, sex, BMI and for multiple comparisons, the scaled preoperative levels of IGFBP2 were associated with 1-year all-cause mortality (OR 10.63; 95% CI: 2.93–64.00;  $p = 0.046$ ). Additionally, scaled preoperative levels of LDHB (OR 5.58; 95% CI: 2.58–8.57;  $p = 0.009$ ) and VCAM1 (OR 2.32; 95% CI: 0.88–3.77;  $p = 0.05$ ) were found to be associated with length of hospital stay.

**Conclusions:** We identified a subset of promising candidate plasma proteins relevant to outcome after on-pump cardiac surgery. IGFBP2 showed a strong association with clinical outcome measures and a significant association of preoperative levels with 1-year all-cause mortality. Other proteins strongly associated with outcome were LDHB and VCAM1, reflecting the dynamics in the

## Abbreviations

CPB, cardiopulmonary bypass; IGFBP2, insulin-like growth factor binding protein 2; LDHB, lactate dehydrogenase B; SERPIN, serin-protease inhibitor; VCAM1, vascular cellular adhesion molecule-1.

acute phase response, inflammation and myocardial injury. We recommend further investigation of these proteins as potential outcome markers after cardiac surgery.

**Clinical Trial Registration:** [ClinicalTrials.gov](https://clinicaltrials.gov); NCT04767685, data are available via ProteomeXchange with identifier PXD046496.

#### KEYWORDS

cardiopulmonary bypass, acute phase, proteomics, proteome, outcome, cardiovascular surgery, cardiac surgery

## 1. Introduction

Invasive procedures resulting in an altered tissue integrity or contact with foreign materials, such as surgery or the use of cardiopulmonary bypass (CPB), are a source of trauma that induce a variety of inflammatory reactions (1). Such alterations of the immunological homeostasis have a critical impact on patient outcome (1, 2). In cardiac surgery, suspected contributing factors range from effects of the blood contacting the extracorporeal circuit, the sheer stress, the surgical trauma *per se* or lung reperfusion injury upon discontinuation of bypass (3). Nevertheless, despite decades of research, the exact mechanisms behind the systemic inflammatory response, damages of ischemia-reperfusion and subsequent high risk of organ injury and postoperative morbidity in patients undergoing CPB are not well understood (4). Moreover, we are currently unable to reliably identify patients at risk of a pronounced inflammatory response and thus complicated postoperative course.

At least in part, this gap of knowledge can be attributed to the complexity of inflammatory mechanisms, which typically involve a wide array of interrelated pathways, metabolites and interactions. Changes in functional immunity cannot be adequately assessed by routine inflammatory biomarkers such as C-reactive protein, procalcitonin, or numerical analysis of leukocyte (sub)-counts (2). In this context, proteomic analysis includes ideal means to analyse underlying mechanisms of diseases, medical treatments or complex critical conditions such as inflammatory responses to CPB through time-dependent analysis of activated proteins (5). Previous plasma proteomics studies revealed acute phase and inflammatory responses in response to surgical trauma (6), representing a part of complex immunological alterations following surgical stress (7–9), which are highly relevant to clinical outcome: Perioperative hyperinflammation may not only lead to organ dysfunction and death through a massive liberation of pro-inflammatory mediators (10) but can also induce a state of immunosuppression predisposing the individual to post-operative septic complications and infections (11). Identifying those at risk and seeking to attenuate or modulate the response to surgical trauma has the potential to reduce postoperative mortality and morbidity and save billions of dollars in healthcare costs (12).

However, the vast amount of data generated by proteomic analyses presents a challenge to both clinicians and statisticians (13). Tackling this challenge requires innovative methodologic approaches, but might offer the possibility to improve our understanding of

underlying mechanisms in perioperative inflammation and may help to identify new prognostic markers and therapeutic targets, which could ultimately improve perioperative care of the individual patient.

Therefore, we aimed to characterise the perioperative acute phase response through mass-spectrometric analysis of the acute phase proteome in a cohort of adult patients undergoing on-pump cardiac surgery. Through application of a novel, multi-step approach including the association with clinical endpoints, we wanted to identify a subset of hit-proteins among the list of proteins detected by mass-spectrometry, which are likely to play a key role in the perioperative acute phase reaction in cardiac surgery patients and which are potentially relevant to postoperative clinical outcome.

## 2. Materials and methods

### 2.1. Study design and study population

This study is an analysis of the Bern Perioperative Biobank (ClinicalTrials.gov; NCT04767685; Submitted: December 16, 2020; principal investigator: Markus M Luedi), a prospective cohort of 192 adult patients undergoing non-emergency cardiac surgery at the Bern University Hospital between January 2019 and December 2019, described elsewhere (14, 15). In brief, all patients underwent elective cardiac surgery (coronary artery bypass grafting, replacement or repair of aortic, mitral or tricuspid valves, surgery of the ascending aorta or aortic arch) with the use of conventional extracorporeal circulation circuits or minimally invasive extracorporeal circulation circuits (16). Emergency surgery or the presence of (suspected) pregnancy were exclusion criteria. Written informed consent was obtained from all participants and the local ethics committee approved the study (Cantonal Ethics Commission of Bern, Bern, CH—KEK Nr. 2018-01272 for sampling and KEK Nr. 2019–2000 for data analysis). Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines were followed throughout the manuscript.

### 2.2. Blood sampling and acute phase proteome analysis

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE (17) partner repository with the dataset identifier PXD046496. Blood samples (EDTA) were

collected before induction of general anaesthesia (preoperative) and 24 h after surgery (postoperative) and stored at the Bern Liquid Biobank as described before (14, 15). Samples were diluted 10-fold in 5% sodium dodecyl sulphate, 100 mM triethylammonium bicarbonate pH 7.5 then centrifuged at 2,500 rcf for 15 min at ambient temperature. The supernatant was taken and subjected to cysteine reduction and alkylation for 30 min at ambient temperature with 10 mM tris(2-carboxyethyl) phosphine and 50 mM iodoacetamide, respectively. Samples were then processed by S-trap 96-well plate protocol (Protifi) according to the manufacturer's instructions. Digestion was performed overnight at 37°C with 5 µg trypsin (TPCK-treated, Worthington) per 3 µl processed plasma. The resulting peptide samples were dried by vacuum centrifugation then reconstituted in 3% acetonitrile, 0.1% formic acid prior to MS analysis. Peptide samples were loaded onto Evtips (Evosep) according to the manufacturer's instructions and analysed by LC-MS/MS using an Evosep One liquid chromatography system coupled to a TimsTOF Pro mass spectrometer (Bruker). Peptides were chromatographically separated using the 60 samples per day standard Evosep method. The mass spectrometer was operated in diaPASEF mode using 8 diaPASEF scans per TIMS-MS scan. The ion mobility range was set to 0.85–1.3 Vs/cm<sup>2</sup>. Each mass window isolated was 25 m/z wide, ranging from 475 to 1,000 m/z with an ion mobility-dependent collision energy that increased linearly from 20 eV to 59 eV between 0.6–1.6 Vs/cm<sup>2</sup>. Raw MS data were searched in DIA-NN v1.8 against the UniProt human proteome database (UP000000589, downloaded 30th May 2022) plus common contaminants. Identified peptides were permitted a maximum of 1 missed cleavage and cysteine carbamidomethylation was set as a fixed modification, with the MS1 and MS2 mass accuracies both set to 10 ppm, and both match-between-runs and RT-dependent cross-run normalisation enabled.

Conventional CRP was analyzed according to standardized routine laboratory methods (cobas<sup>®</sup> CRP4 by Roche Diagnostics GmbH, Mannheim, Germany). The perioperative CRP measurements featured values below the detection threshold of 3 mg/L. These values were set to a default value of 2.99 mg/L.

## 2.3. Clinical outcome measures and other study variables

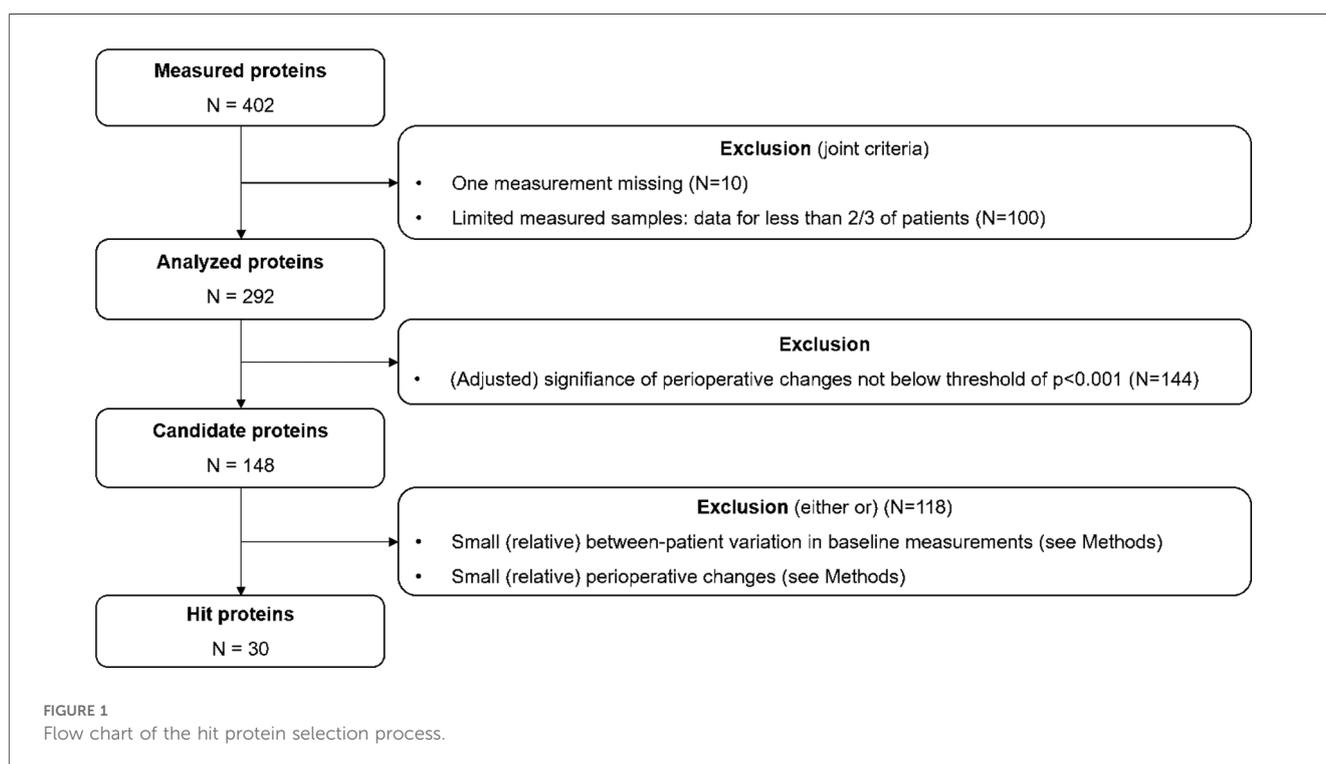
Clinical outcome measures for this analysis were all-cause one-year mortality, length of hospital stay as well as periprocedural myocardial infarction and stroke (recorded until hospital discharge). Myocardial infarction was adjudicated according to the fourth universal definition of myocardial infarction (18) and periprocedural stroke was defined as acute clinically overt neurological deficit with imaging evidence of cerebral ischemia or bleeding.

Pre-, peri-, and postoperative data for each patient were collected from electronic patient charts (Dendrite Clinical Systems Ltd., Henley on-Thames, UK). Information on all-cause mortality was obtained from internal hospital records or from the national records. European System for Cardiac Operative Risk Evaluation (EuroSCORE) II was calculated to assess the presumed risk of 30-day all-cause mortality (19).

## 2.4. Multi-step hit detection procedure

A graphical representation of our hit protein selection process is provided in **Figure 1**.

Initially, we imposed two data availability criteria: First, a measurement of a particular protein should be available both pre-



and post-operatively. Second, a measurement of a particular protein should be available in at least two-thirds of the patients. We refer to the remaining set of proteins as the *analysed proteins*. Note that proteins that are not present at all or only present in a minority of patients preoperatively, but emerge during or after the surgery, are therefore—by definition—excluded from this analysis.

In addition, the analysed acute phase proteins were grouped into Kyoto Encyclopedia of Genes and Genomes (KEGG) PATHWAYS according to the KEGG-PATHWAY database (20), using the following KEGG-PATHWAY-classifications: metabolism, genetic information processing, environmental information processing, cellular processes, organismal systems, multiple categories, unknown/unclassified. This step served as a purely exploratory measure to guide the interpretation of the hit selection procedure.

To be able to compare different proteins, we scaled each protein measurement by first subtracting the median value (pooled across the two time points) and then by dividing the residual by the interquartile range; the scaling is thus similar to the computation of a *z*-score, but accounts for possible skewness in the measured values by considering the median and interquartile range instead of the mean and standard deviation.

As a next step, we assessed the statistical significance of the perioperative change in the analysed proteins by means of a paired samples Wilcoxon test. Given the large number of candidate proteins, the resulting *p*-values were adjusted for multiple comparison by means of the Bonferroni correction. Only proteins with adjusted *p*-values < 0.001 were subsequently considered: we denote this set as the *candidate proteins*.

As final step, a two-dimensional, empirical approach was chosen to determine the final set of hit proteins. The first dimension considers the perioperative change in protein levels among the candidate proteins, which should show either a large decrease or large increase perioperatively. The second dimension requires hit proteins to demonstrate some degree of inter-patient variability: if there is no inter-patient variability, for example all patients feature exactly the same (statistically significant) increase in protein levels, then this change is unlikely to be related to a particular outcome such as length of hospital stay (which does feature inter-patient variability). The joint consideration of these two dimensions defines an area of interest in a two-dimensional space defined by the (scaled) median perioperative change in protein levels (*x*) and the variation (defined by means of the interquartile range) in perioperative protein levels (*y*).

We then defined a distance (*d*) in this space as follows:  $d^2 = 0.66 \cdot x^2 + 0.33 \cdot y^2$ , thus defining an ellipse in the two-dimensional space. The final set of hit proteins is defined as those 20% of proteins with the largest distances: for these proteins, the distance is greater than the 80%-quantile of all distances [denoted as  $d_q(0.8)$ ]. All computations were performed with R version 4.0.2 (21).

## 2.5. Outcome analysis

For the final set of hit proteins, we computed both crude and adjusted (age, gender and BMI) odds ratios (ORs) with the four clinical outcomes of interest by means of univariable and multivariable logistic regression. For each clinical outcome

individually, the *p*-values resulting from this analysis were adjusted for multiple comparison by means of the Bonferroni correction. For exploratory purposes, we further calculated univariable association of preoperative protein levels as well as their perioperative changes with a set of baseline covariates (**Supplementary Material**).

## 3. Results

### 3.1. Patient / study population characteristics

A total of 192 patients [75.5% male, median age 67.0 (IQR 60.0–73.0)] undergoing cardiac surgery with the use of CPB were included in this analysis. Both baseline characteristics as well as procedural and surgical characteristics have been extensively described previously (**Table 1**) (14, 15). Mean CRP

TABLE 1 Patient and surgical characteristics.

	All patients	N
	N = 192	
Age (years)	67 [60;73]	192
Sex (female)	47 (24.5%)	192
BMI (kg/m <sup>2</sup> )	26.1 [23.7;30.4]	192
Diabetes on insulin (Yes)	35 (18.2%)	192
Hypertension (Yes)	130 (68.4%)	190
Dyslipidemia (Yes)	111 (58.1%)	191
Smoker		188
Non-smoker	97 (51.6%)	
Previous / current smoker	91 (48.4%)	
Obesity (Yes)	52 (27.1%)	192
Preoperative renal disease (Yes)	43 (22.4%)	192
Peripheral vascular disease (Yes)	11 (6.2%)	178
Carotid disease (Yes)	6 (3.7%)	162
Myocardial infarction (Yes)	20 (10.5%)	191
COPD (Yes)	23 (12.1%)	190
NYHA (>1)	131 (68.6%)	191
CCS (>0)	71 (37.6%)	189
Ejection Fraction (%)	60 [55;65]	191
EuroSCORE 2	1.73 [0.90;2.93]	184
ECC or MiECC		192
ECC	149 (77.6%)	
MiECC	43 (22.4%)	
Deep hypothermic cardiac arrest (Yes)	19 (10.0%)	191
Aortic valve (Yes)	86 (44.8%)	192
Mitral valve (Yes)	45 (23.4%)	192
Tricuspid valve (Yes)	17 (8.9%)	192
Coronary artery bypass (Yes)	77 (40.1%)	192
Ascending Aortic (Yes)	38 (19.8%)	192
Aortic Arch (Yes)	11 (5.7%)	192
Bypass time (min)	104 [80;132]	192
Aortic cross clamping (min)	68.5 [52.0;91.8]	192
Lowest body temperature (°C)	33.2 [32.1;33.8]	192
Operation duration (min)	234 [195;276]	192

Data expressed as median [IQR] or number (%).

BMI, body mass index; COPD, chronic obstructive pulmonary disease; NYHA, new york heart association; CCS, canadian cardiovascular society.

Both baseline characteristics as well as procedural and surgical characteristics have been extensively described previously (14, 15).

levels measured with conventional routine laboratory methods at baseline were 3.0 mg/dl [3.0; 3.2] and fully reported in **Supplementary Table S2** for the perioperative period.

### 3.2. Multi-step hit detection

In total, we identified 402 proteins in our samples (a list of all detected proteins can be found in the Supplementary: **Supplementary Table S1**). Out of the 402 identified proteins, 292 / 402 (73%) proteins were detectable at both time points of the serum sampling. Of those, 148 / 402 (37%) showed significant perioperative changes at a threshold below  $p < 0.001$ . After exclusion of all proteins with only small (relative) in-between patient variation regarding both, baseline measurements and perioperative changes, 30 / 402 proteins (7%) were identified as hit-proteins (**Figure 2**). An overview of the 30 identified hit-proteins including pre- and postoperative levels, perioperative change and KEGG pathway database category (20) is provided in **Table 2**. Association of unadjusted preoperative protein levels with baseline characteristics is shown in **Supplementary Figures S1, S2**,

and for perioperative change in **Supplementary Figures S3, S4**, respectively.

### 3.3. Association of hit-proteins with clinical outcome measures

After adjusting for age, BMI and gender, several hit-proteins baseline spectral counts ( $n = 9/30$ , 30%) and perioperative changes ( $n = 8/30$ , 27%) were significantly associated with clinical outcomes (**Tables 3, 4**). The three hit-proteins which showed the strongest associations with clinical outcomes were VCAM1, LDHB and IGFBP2, while the SERPINs yielded a large number of associations with outcome measures. After adjustment for multiple comparison, the associations of preoperative levels of IGFBP2 with 1-year all-cause mortality (OR 10.63; 95% CI: 2.93–64.00;  $p = 0.046$ ), and the associations of preoperative levels of LDHB (OR 5.58; 95% CI: 2.58–8.57;  $p = 0.009$ ) and VCAM1 (OR 2.32; 95% CI: 0.88–3.77;  $p = 0.05$ ) with length of hospital stay remained significant (**Table 3**), while no association of perioperative change of any protein with clinical outcome measures could be observed (**Table 4**).

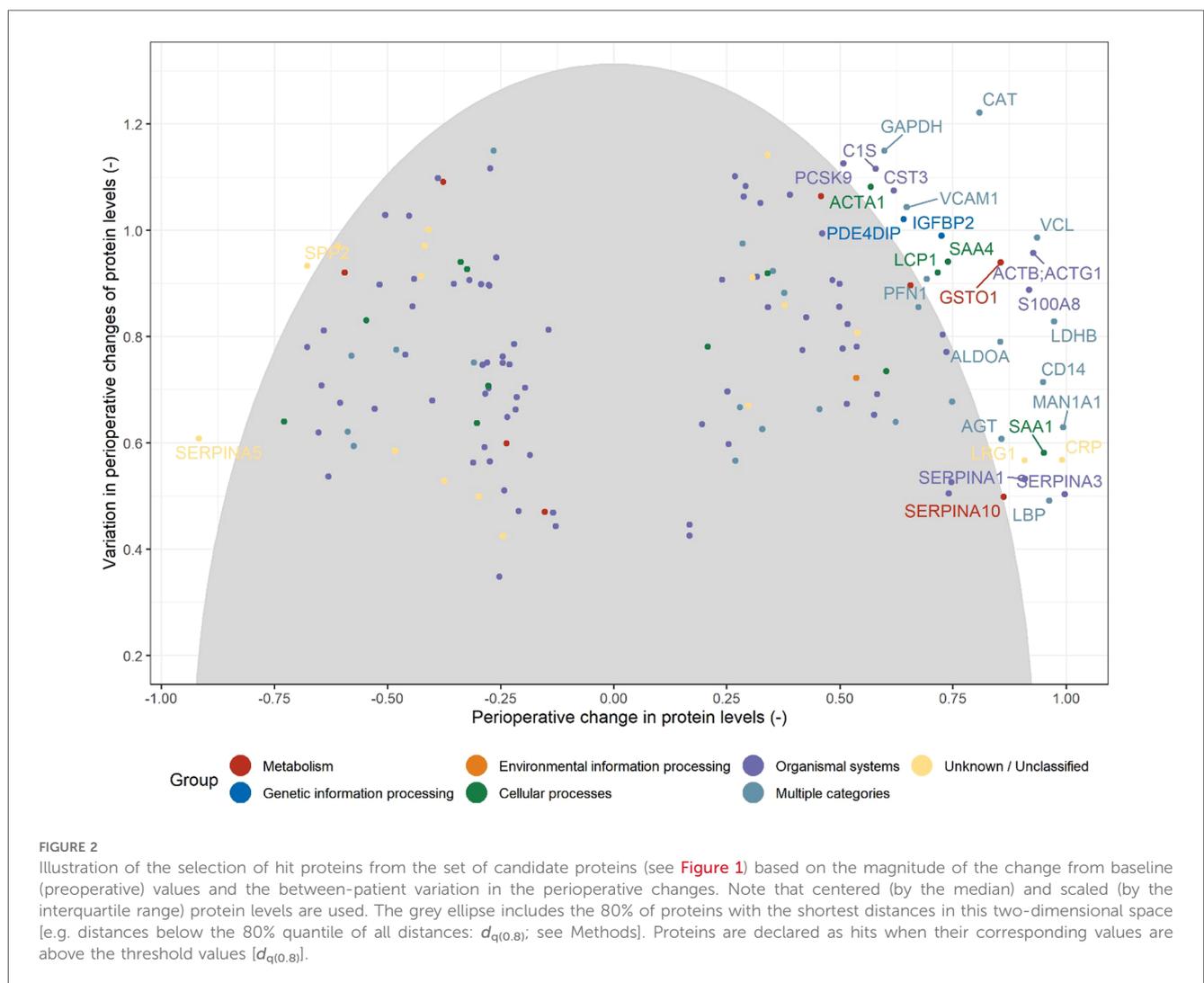


TABLE 2 Characteristics of the 30 proteins selected for further analysis.

Protein	Preoperative levels		Postoperative levels		Perioperative change		p value (adjusted p)	Protein name	Protein description	KEGG pathway database classification	Specification of organismal systems
	Median [IQR]	Median [IQR]	Median [IQR]	Median [IQR]	Median [IQR]	Median [IQR]					
ACTA1	-0.29 [-0.57 to 0.07]	0.47 [-0.03 to 0.89]	0.57 [0.10 to 1.18]	<0.001 (<0.001)	ACTS_HUMAN	Actin, alpha skeletal muscle	4	Immune system, endocrine system, digestive system, environmental adaptation			
ACTB	-0.42 [-0.69 to -0.10]	0.50 [0.12 to 1.04]	0.93 [0.48 to 1.44]	<0.001 (<0.001)	ACTB_HUMAN; ACTG_HUMAN	Actin, cytoplasmic 1	5	Immune system, endocrine system, digestive system, environmental adaptation			
ACTG1								Endocrine, circulatory			
AGT	-0.44 [-0.68 to -0.15]	0.45 [0.10 to 0.78]	0.86 [0.57 to 1.18]	<0.001 (<0.001)	ANGT_HUMAN	Angiotensinogen	3; 5	Endocrine, circulatory			
ALDOA	-0.45 [-0.67 to -0.13]	0.44 [0.09 to 0.85]	0.85 [0.51 to 1.30]	<0.001 (<0.001)	ALDOA_HUMAN	Fructose-bisphosphate aldolase A	1; 3				
C1S	-0.24 [-0.65 to 0.16]	0.28 [-0.19 to 0.74]	0.58 [-0.03 to 1.09]	<0.001 (<0.001)	C1S_HUMAN	Complement C1s subcomponent	5	Immune			
CAT	-0.43 [-0.70 to -0.01]	0.34 [0.00 to 0.92]	0.81 [0.19 to 1.41]	<0.001 (<0.001)	CATA_HUMAN	Catalase	1; 3; 4; 5	Aging			
CD14	-0.41 [-0.64 to -0.15]	0.57 [0.18 to 1.01]	0.95 [0.60 to 1.31]	<0.001 (<0.001)	CD14_HUMAN	Monocyte differentiation antigen CD14	3; 4; 5	Immune			
CRP	-0.39 [-0.40 to -0.36]	0.61 [0.36 to 0.92]	0.99 [0.71 to 1.28]	<0.001 (<0.001)	CRP_HUMAN	C-reactive protein	no KO				
CST3	-0.23 [-0.70 to 0.19]	0.22 [-0.18 to 0.92]	0.62 [0.10 to 1.18]	<0.001 (<0.001)	CYTC_HUMAN	Cystatin-C	5	Digestive			
GAPDH	-0.28 [-0.71 to 0.27]	0.25 [-0.19 to 0.70]	0.60 [-0.09 to 1.06]	<0.001 (<0.001)	G3P_HUMAN	Glyceraldehyde-3-phosphate dehydrogenase	1; 3				
GSTO1	-0.31 [-0.61 to 0.00]	0.54 [0.00 to 1.17]	0.86 [0.35 to 1.29]	<0.001 (<0.001)	GSTO1_HUMAN	Glutathione S-transferase omega-1	1				
IGFBP2	-0.29 [-0.49 to 0.00]	0.50 [0.00 to 1.08]	0.72 [0.38 to 1.37]	<0.001 (<0.001)	IBP2_HUMAN	Insulin-like growth factor-binding protein 2	2				
LBP	-0.35 [-0.43 to -0.27]	0.65 [0.41 to 0.93]	0.96 [0.75 to 1.24]	<0.001 (<0.001)	LBP_HUMAN	Lipopolysaccharide-binding protein	3; 5	Immune			
LCPI1	-0.40 [-0.75 to 0.00]	0.43 [0.00 to 0.73]	0.72 [0.30 to 1.22]	<0.001 (<0.001)	PLSL_HUMAN	Plastin-2	4				
LDHB	-0.33 [-0.49 to -0.15]	0.66 [0.20 to 1.21]	0.97 [0.60 to 1.43]	<0.001 (<0.001)	LDHB_HUMAN	L-lactate dehydrogenase B chain	1; 3; 5	Endocrine			
LRG1	-0.43 [-0.54 to -0.29]	0.52 [0.25 to 0.90]	0.91 [0.66 to 1.23]	<0.001 (<0.001)	A2GL_HUMAN	Leucine-rich alpha-2-glycoprotein	no KO				
MAN1A1	-0.42 [-0.64 to -0.24]	0.56 [0.27 to 0.90]	0.99 [0.68 to 1.31]	<0.001 (<0.001)	MAN1A1_HUMAN	Mannosyl-oligosaccharide 1,2-alpha-mannosidase IA	1; 2				
PCSK9	-0.25 [-0.63 to 0.23]	0.28 [-0.16 to 0.83]	0.51 [-0.09 to 1.03]	<0.001 (<0.001)	PCSK9_HUMAN	Proprotein convertase subtilisin/kexin type 9	5	Digestive			
PDE4DIP	-0.33 [-0.53 to 0.04]	0.41 [-0.03 to 0.91]	0.64 [0.16 to 1.18]	<0.001 (<0.001)	MYOME_HUMAN	Myomegalin	2				
PFN1	-0.40 [-0.67 to 0.07]	0.31 [-0.09 to 0.79]	0.69 [0.28 to 1.19]	<0.001 (<0.001)	PROF1_HUMAN	Profilin-1	3; 4				
S100A8	-0.39 [-0.58 to -0.14]	0.55 [0.17 to 1.16]	0.92 [0.56 to 1.45]	<0.001 (<0.001)	S10A8_HUMAN	Protein S100-A8	5	Immune			
SAA1	-0.27 [-0.27 to -0.25]	0.73 [0.40 to 0.96]	0.95 [0.62 to 1.20]	<0.001 (<0.001)	SAAL_HUMAN	Serum amyloid A-1 protein	4				
SAA4	-0.33 [-0.61 to 0.03]	0.43 [-0.06 to 0.85]	0.74 [0.27 to 1.21]	<0.001 (<0.001)	SAA4_HUMAN	Serum amyloid A-4 protein	4				
SERPINA1	-0.47 [-0.64 to -0.24]	0.52 [0.26 to 0.74]	0.91 [0.65 to 1.18]	<0.001 (<0.001)	AIAT_HUMAN	Alpha-1-antitrypsin	5	Immune			
SERPINA10	-0.47 [-0.66 to -0.23]	0.52 [0.22 to 0.77]	0.86 [0.68 to 1.18]	<0.001 (<0.001)	ZPL_HUMAN	Protein Z-dependent protease inhibitor	1				
SERPINA3	-0.51 [-0.60 to -0.37]	0.49 [0.29 to 0.74]	1.00 [0.74 to 1.25]	<0.001 (<0.001)	AACT_HUMAN	Alpha-1-antichymotrypsin	5	Immune (complement and coagulation)			
SERPINA5	0.58 [0.22 to 0.92]	-0.35 [-0.59 to -0.11]	-0.92 [-1.20 to -0.60]	<0.001 (<0.001)	IPSP_HUMAN	Plasma serine protease inhibitor	5	Immune (complement and coagulation)			
SPP2	0.47 [-0.07 to 0.96]	-0.26 [-0.54 to 0.06]	-0.68 [-1.16 to -0.23]	<0.001 (<0.001)	SPP24_HUMAN	Secreted phosphoprotein 24	no KO				
VCAM1	-0.24 [-0.69 to 0.10]	0.38 [-0.15 to 0.99]	0.65 [0.19 to 1.24]	<0.001 (<0.001)	VCAM1_HUMAN	Vascular cell adhesion protein 1	3; 5	Immune			
VCL	-0.37 [-0.64 to -0.02]	0.51 [0.02 to 1.11]	0.94 [0.47 to 1.46]	<0.001 (<0.001)	VINC_HUMAN	Vinculin	4; 5	Immune			

Note that centered (by the median) and scaled (by the interquartile range) protein levels are used in the computations (see Methods).

TABLE 3 Adjusted associations of preoperative protein levels with postoperative outcome.

Protein	Stroke		Myocardial infarction		1-year mortality		Length of stay	
	Incidence 12/192 (6.3%)		Incidence 6/192 (3.1%)		Incidence 5/192 (2.6%)		Median 7 [IQR: 6–9 ] days	
	OR (95%-CI)	p (adjusted p)	OR (95%-CI)	p (adjusted p)	OR (95%-CI)	p (adjusted p)	Beta (95%-CI)	p (adjusted p)
ACTA1	1.07 (0.24–3.88)	0.92 (>0.99)	0.04 (0.00–1.00)	0.13 (>0.99)	1.56 (0.23–7.67)	0.60 (>0.99)	0.54 (–1.96–3.05)	0.67 (>0.99)
ACTB;ACTG1	0.98 (0.18–4.48)	0.98 (>0.99)	0.10 (0.00–1.24)	0.13 (>0.99)	1.26 (0.13–8.62)	0.82 (>0.99)	1.40 (–0.67–3.46)	0.18 (>0.99)
AGT	1.18 (0.35–3.10)	0.75 (>0.99)	0.05 (0.00–0.67)	0.039 (>0.99)	0.85 (0.08–3.31)	0.86 (>0.99)	2.23 (0.35–4.10)	0.02 (0.61)
ALDOA	1.44 (0.30–5.26)	0.61 (>0.99)	0.21 (0.02–1.50)	0.18 (>0.99)	0.64 (0.04–4.16)	0.70 (>0.99)	1.47 (–0.60–3.53)	0.16 (>0.99)
C1S	0.59 (0.24–1.52)	0.27 (>0.99)	1.84 (0.35–11.78)	0.49 (>0.99)	0.78 (0.24–2.95)	0.70 (>0.99)	0.31 (–0.93–1.56)	0.62 (>0.99)
CAT	1.08 (0.37–2.38)	0.86 (>0.99)	0.03 (0.00–0.70)	0.07 (>0.99)	0.46 (0.03–2.21)	0.49 (>0.99)	0.58 (–0.85–2.00)	0.43 (>0.99)
CD14	1.51 (0.24–9.15)	0.65 (>0.99)	1.19 (0.12–9.17)	0.87 (>0.99)	2.49 (0.20–26.48)	0.46 (>0.99)	–0.90 (–3.43–1.63)	0.48 (>0.99)
CRP	0.35 (0.00–10.49)	0.70 (>0.99)	0.00 (0.00–6.39)	0.29 (>0.99)	2.03 (0.00–40.35)	0.70 (>0.99)	1.27 (–4.84–7.39)	0.68 (>0.99)
CST3	1.56 (0.85–2.73)	0.12 (>0.99)	0.13 (0.01–0.73)	0.049 (>0.99)	2.08 (0.90–4.49)	0.06 (>0.99)	1.02 (–0.09–2.14)	0.07 (>0.99)
GAPDH	0.47 (0.15–1.24)	0.17 (>0.99)	0.47 (0.10–1.57)	0.29 (>0.99)	0.91 (0.22–1.81)	0.87 (>0.99)	–0.45 (–1.31–0.41)	0.30 (>0.99)
GSTO1	0.80 (0.14–3.34)	0.79 (>0.99)	0.07 (0.00–1.63)	0.20 (>0.99)	0.63 (0.03–4.52)	0.72 (>0.99)	0.81 (–1.15–2.78)	0.41 (>0.99)
IGFBP2	1.12 (0.20–3.90)	0.88 (>0.99)	0.24 (0.00–2.73)	0.46 (>0.99)	10.63 (2.93–64.00)	0.002 (0.046)	2.17 (0.25–4.09)	0.027 (0.82)
LBP	2.57 (0.03–67.82)	0.62 (>0.99)	0.50 (0.00–102.71)	0.82 (>0.99)	1.09 (0.00–68.90)	0.97 (>0.99)	1.86 (–3.63–7.35)	0.50 (>0.99)
LCPI	2.08 (0.81–4.80)	0.09 (>0.99)	1.25 (0.20–5.11)	0.78 (>0.99)	0.64 (0.10–2.44)	0.59 (>0.99)	1.48 (0.10–2.86)	0.035 (>0.99)
LDHB	1.60 (0.22–10.29)	0.63 (>0.99)	0.65 (0.02–13.32)	0.80 (>0.99)	0.61 (0.02–10.71)	0.77 (>0.99)	5.58 (2.58–8.57)	0.0003 (0.009)
LRG1	1.09 (0.07–6.77)	0.94 (>0.99)	0.08 (0.00–2.53)	0.34 (>0.99)	4.89 (0.59–28.23)	0.09 (>0.99)	1.29 (–1.77–4.34)	0.41 (>0.99)
MAN1A1	0.28 (0.02–2.41)	0.32 (>0.99)	2.55 (0.09–36.24)	0.55 (>0.99)	0.55 (0.02–5.54)	0.70 (>0.99)	–0.93 (–3.37–1.50)	0.45 (>0.99)
PCSK9	1.25 (0.33–2.78)	0.66 (>0.99)	1.66 (0.54–3.52)	0.24 (>0.99)	0.93 (0.15–2.40)	0.92 (>0.99)	–0.02 (–1.19–1.16)	0.98 (>0.99)
PDE4DIP	0.33 (0.02–1.08)	0.26 (>0.99)	1.29 (0.38–3.15)	0.61 (>0.99)	0.88 (0.19–1.92)	0.81 (>0.99)	0.08 (–1.02–1.19)	0.88 (>0.99)
PFN1	0.49 (0.08–2.16)	0.39 (>0.99)	0.11 (0.00–1.00)	0.15 (>0.99)	1.29 (0.18–7.77)	0.79 (>0.99)	0.50 (–1.53–2.53)	0.63 (>0.99)
S100A8	0.16 (0.01–1.27)	0.17 (>0.99)	0.67 (0.04–2.26)	0.61 (>0.99)	1.25 (0.25–2.87)	0.67 (>0.99)	–0.02 (–1.59–1.56)	0.98 (>0.99)
SAA1	0.07 (0.00–4.93)	0.68 (>0.99)	0.39 (0.00–37.64)	0.81 (>0.99)	1.05 (NA <sup>a</sup> - 10.50)	0.98 (>0.99)	–0.17 (–4.25–3.91)	0.93 (>0.99)
SAA4	0.62 (0.16–1.98)	0.44 (>0.99)	0.45 (0.05–2.38)	0.40 (>0.99)	0.75 (0.11–3.81)	0.75 (>0.99)	0.22 (–1.50–1.94)	0.80 (>0.99)
SERPINA1	0.53 (0.06–3.67)	0.54 (>0.99)	0.01 (0.00–0.78)	0.08 (>0.99)	5.13 (0.51–43.46)	0.14 (>0.99)	3.75 (1.27–6.24)	0.003 (0.1)
SERPINA10	0.67 (0.10–3.79)	0.66 (>0.99)	1.95 (0.09–43.22)	0.66 (>0.99)	1.89 (0.13–22.55)	0.62 (>0.99)	–0.26 (–2.98–2.47)	0.85 (>0.99)
SERPINA3	1.29 (0.06–11.40)	0.85 (>0.99)	0.37 (0.00–5.93)	0.56 (>0.99)	7.76 (0.80–61.44)	0.044 (>0.99)	1.25 (–2.17–4.68)	0.47 (>0.99)
SERPINA5	0.29 (0.09–0.92)	0.038 (>0.99)	1.52 (0.30–9.51)	0.63 (>0.99)	0.24 (0.04–1.17)	0.08 (>0.99)	0.22 (–1.39–1.83)	0.79 (>0.99)
SPP2	0.71 (0.31–1.49)	0.38 (>0.99)	1.18 (0.30–4.40)	0.81 (>0.99)	1.12 (0.34–3.43)	0.85 (>0.99)	–0.84 (–2.08–0.40)	0.18 (>0.99)
VCAM1	1.80 (0.80–4.01)	0.15 (>0.99)	0.50 (0.05–2.94)	0.50 (>0.99)	1.92 (0.54–5.73)	0.26 (>0.99)	2.32 (0.88–3.77)	0.002 (0.05)
VCL	1.60 (0.26–9.58)	0.60 (>0.99)	0.09 (0.00–3.23)	0.26 (>0.99)	1.58 (0.18–12.07)	0.66 (>0.99)	1.98 (–0.59–4.54)	0.13 (>0.99)

<sup>a</sup>Lower bound of profile likelihood confidence interval could not be computed.

Odds ratios (ORs) and 95%-confidence intervals derived from a multivariable linear regression are shown for binary outcomes, whereas regression coefficient and their 95%-confidence intervals computed with a multivariable linear regression are shown for the continuous outcome length of hospital stay. The associations are adjusted for age, gender and BMI. P-values are adjusted for multiple comparison for each outcome separately by means of the Bonferroni correction.

### 3.4. Discussion

Through our innovative multi-step approach, we were able to identify a small subset of proteins within the acute phase proteome, which show relevant associations with clinical outcome measures, and are therefore promising candidates for pre- and perioperative acute phase response monitoring and potentially risk stratification.

As is the case in our patients with CPB, major changes to the serum proteome from the day of induction of anaesthesia to later postoperative stages have been described in cohorts involving individuals undergoing veno-arterial extracorporeal membrane oxygenation (22). In this study, three hit-proteins showed the strongest associations with clinical outcomes, VCAM1, IGFBP2 and LDHB, while the SERPINS yielded a large number of associations with outcome measures.

The SERPINS are known to play an important role in cardiovascular disease (23, 24). They are involved in the finely tuned balance between procoagulant and anticoagulant systems, due to their anticoagulant and antifibrinolytic properties and have even been proposed as a potential therapeutic target (25).

Further, SERPINA1 is involved in the immune response by inhibiting ATP-induced interleukin-β-release (26) and secreted into the bloodstream in response to myocardial infarction (27). Overall, SERPINA1 regulates the expression of chemokines, chemotaxis and cell adhesion and reduces the expression of pro-inflammatory cytokines and up-regulates anti-inflammatory mediators (28). Previous studies have even investigated SERPINA1 augmentation therapy during heart surgery to attenuate postoperative inflammation (26). Both SERPINA3 and SERPINA5 were detected in RNA sequencing of failing right ventricles and proposed as biomarkers for abnormalities in the involved inflammatory processes (29). In another study, SERPINA3 was identified as a potential predictive marker of clinical outcome after myocardial infarction, as the level of this protease inhibitor was found to be directly correlated with other measured inflammatory markers (30). The relevance of the observed association of uncorrected preoperative values with several clinical outcome measures in our study remains to be investigated—speculatively, the SERPINS might serve as a marker of subtle preoperative inflammation and cardiovascular stress.

TABLE 4 Adjusted associations of perioperative change in proteins levels with postoperative outcome.

Protein	Stroke		Myocardial infarction		1-year mortality		Length of stay	
	Incidence 12/192 (6.3%)		Incidence 6/192 (3.1%)		Incidence 5/192 (2.6%)		Median 7 [IQR: 6–9 ] days	
	OR (95%-CI)	<i>p</i> (adjusted <i>p</i> )	OR (95%-CI)	<i>p</i> (adjusted <i>p</i> )	OR (95%-CI)	<i>p</i> (adjusted <i>p</i> )	Beta (95%-CI)	<i>p</i> (adjusted <i>p</i> )
ACTA1	1.72 (0.82–3.70)	0.15 (>0.99)	0.98 (0.24–3.29)	0.97 (>0.99)	2.04 (0.81–5.11)	0.12 (>0.99)	0.25 (–1.14–1.64)	0.72 (>0.99)
ACTB;ACTG1	1.64 (0.82–3.39)	0.16 (>0.99)	0.96 (0.23–3.28)	0.95 (>0.99)	3.31 (1.32–8.93)	0.011 (0.32)	0.76 (–0.37–1.90)	0.19 (>0.99)
AGT	0.47 (0.14–1.48)	0.21 (>0.99)	0.79 (0.13–5.10)	0.80 (>0.99)	1.82 (0.29–12.16)	0.52 (>0.99)	–1.63 (–3.44–0.18)	0.08 (>0.99)
ALDOA	1.24 (0.48–2.55)	0.60 (>0.99)	1.07 (0.43–2.15)	0.86 (>0.99)	2.00 (0.84–5.76)	0.12 (>0.99)	0.98 (–0.31–2.27)	0.14 (>0.99)
CIS	0.89 (0.40–1.98)	0.78 (>0.99)	0.94 (0.34–2.59)	0.91 (>0.99)	0.85 (0.30–2.40)	0.77 (>0.99)	0.14 (–0.87–1.16)	0.78 (>0.99)
CAT	1.33 (0.79–2.32)	0.28 (>0.99)	2.01 (0.63–7.04)	0.24 (>0.99)	1.76 (0.71–4.70)	0.22 (>0.99)	–0.40 (–1.32–0.52)	0.39 (>0.99)
CD14	1.61 (0.53–4.56)	0.38 (>0.99)	0.90 (0.17–3.79)	0.89 (>0.99)	1.03 (0.19–4.28)	0.97 (>0.99)	1.02 (–0.50–2.53)	0.19 (>0.99)
CRP	0.99 (0.22–4.70)	0.99 (>0.99)	0.42 (0.02–4.91)	0.50 (>0.99)	1.00 (0.13–7.79)	>0.99 (>0.99)	0.21 (–1.95–2.37)	0.85 (>0.99)
CST3	0.82 (0.38–1.52)	0.58 (>0.99)	0.76 (0.24–2.09)	0.61 (>0.99)	1.48 (0.73–2.55)	0.17 (>0.99)	0.82 (–0.04–1.67)	0.06 (>0.99)
GAPDH	1.62 (0.84–3.14)	0.15 (>0.99)	0.79 (0.40–1.88)	0.54 (>0.99)	1.39 (0.65–3.40)	0.48 (>0.99)	0.52 (–0.19–1.23)	0.15 (>0.99)
GSTO1	2.24 (1.01–5.18)	0.05 (>0.99)	2.46 (0.53–12.31)	0.24 (>0.99)	3.85 (1.27–14.09)	0.021 (0.62)	0.50 (–0.80–1.81)	0.45 (>0.99)
IGFBP2	1.23 (0.66–1.94)	0.42 (>0.99)	0.90 (0.20–1.93)	0.84 (>0.99)	1.83 (1.16–3.06)	0.009 (0.26)	0.96 (0.18–1.74)	0.016 (0.48)
LBP	0.51 (0.06–3.44)	0.51 (>0.99)	0.38 (0.04–2.66)	0.34 (>0.99)	0.29 (0.01–3.79)	0.38 (>0.99)	0.09 (–2.27–2.44)	0.94 (>0.99)
LCP1	0.84 (0.38–1.85)	0.67 (>0.99)	0.82 (0.18–3.62)	0.80 (>0.99)	2.08 (0.68–5.95)	0.18 (>0.99)	–0.66 (–1.77–0.46)	0.25 (>0.99)
LDHB	1.33 (0.55–2.75)	0.48 (>0.99)	2.23 (0.93–6.13)	0.09 (>0.99)	2.51 (1.07–5.80)	0.024 (0.71)	0.63 (–0.53–1.78)	0.29 (>0.99)
LRG1	0.70 (0.12–3.41)	0.67 (>0.99)	0.21 (0.01–2.12)	0.22 (>0.99)	0.21 (0.02–2.02)	0.21 (>0.99)	1.29 (–0.68–3.27)	0.20 (>0.99)
MAN1A1	1.16 (0.36–3.42)	0.79 (>0.99)	0.74 (0.12–3.88)	0.73 (>0.99)	0.49 (0.09–2.45)	0.40 (>0.99)	0.72 (–0.83–2.27)	0.36 (>0.99)
PCSK9	0.75 (0.29–1.99)	0.56 (>0.99)	0.98 (0.41–2.62)	0.97 (>0.99)	0.77 (0.28–2.37)	0.62 (>0.99)	–0.51 (–1.52–0.50)	0.32 (>0.99)
PDE4DIP	1.53 (1.03–2.43)	0.048 (>0.99)	0.44 (0.16–1.03)	0.07 (>0.99)	0.88 (0.49–1.55)	0.67 (>0.99)	0.66 (–0.08–1.40)	0.08 (>0.99)
PFN1	1.25 (0.46–3.56)	0.67 (>0.99)	1.41 (0.31–7.47)	0.67 (>0.99)	1.84 (0.43–9.32)	0.43 (>0.99)	0.48 (–1.12–2.07)	0.56 (>0.99)
S100A8	1.24 (0.87–2.16)	0.30 (>0.99)	0.90 (0.16–1.75)	0.87 (>0.99)	1.88 (1.16–3.65)	0.022 (0.67)	–0.56 (–1.37–0.26)	0.18 (>0.99)
SAA1	0.63 (0.14–2.48)	0.53 (>0.99)	0.91 (0.12–6.34)	0.92 (>0.99)	0.19 (0.02–1.61)	0.16 (>0.99)	–0.57 (–2.45–1.31)	0.55 (>0.99)
SAA4	0.76 (0.35–1.74)	0.49 (>0.99)	0.75 (0.25–2.27)	0.60 (>0.99)	0.45 (0.16–1.34)	0.13 (>0.99)	–0.15 (–1.32–1.02)	0.8 (>0.99)
SERPINA1	0.23 (0.03–1.30)	0.11 (>0.99)	1.48 (0.17–13.56)	0.72 (>0.99)	0.36 (0.03–3.38)	0.39 (>0.99)	–0.74 (–3.04–1.56)	0.53 (>0.99)
SERPINA10	0.25 (0.04–1.35)	0.12 (>0.99)	0.16 (0.01–2.11)	0.19 (>0.99)	0.57 (0.06–5.44)	0.62 (>0.99)	–0.26 (–2.50–1.98)	0.82 (>0.99)
SERPINA3	0.36 (0.05–2.41)	0.30 (>0.99)	0.74 (0.16–3.62)	0.69 (>0.99)	0.47 (0.04–4.57)	0.54 (>0.99)	–0.04 (–2.24–2.15)	0.97 (>0.99)
SERPINA5	1.97 (0.44–9.04)	0.37 (>0.99)	0.81 (0.09–5.95)	0.84 (>0.99)	10.67 (1.31–115.38)	0.034 (>0.99)	–0.19 (–2.12–1.74)	0.85 (>0.99)
SPP2	1.02 (0.44–2.50)	0.97 (>0.99)	1.10 (0.28–5.56)	0.90 (>0.99)	0.89 (0.27–3.42)	0.86 (>0.99)	–0.05 (–1.42–1.31)	0.94 (>0.99)
VCAM1	0.73 (0.33–1.57)	0.43 (>0.99)	2.74 (0.66–13.11)	0.17 (>0.99)	0.52 (0.17–1.49)	0.24 (>0.99)	–0.33 (–1.41–0.75)	0.55 (>0.99)
VCL	2.54 (1.13–6.10)	0.025 (0.76)	0.70 (0.11–3.68)	0.70 (>0.99)	2.06 (0.86–4.70)	0.08 (>0.99)	0.65 (–0.63–1.94)	0.32 (>0.99)

Odds ratios (ORs) and 95%-confidence intervals derived from a multivariable linear regression are shown for binary outcomes, whereas regression coefficient and their 95%-confidence intervals computed with a multivariable linear regression are shown for the continuous outcome length of hospital stay. The associations are adjusted for age, gender and BMI. *P*-values are adjusted for multiple comparison for each outcome separately by means of the Bonferroni correction.

VCAM1, just as SERPINA1, is also involved in inflammatory processes and is activated by tumor necrosis factor alpha and mediates vascular adhesion and trans-endothelial migration of leukocytes (31). This is also the case in myocardial injury, where VCAM1 has been shown to mediate rapid neutrophil mobilization (32). Therefore, the dynamics in VCAM1 levels observed in our study, might be attributed to perioperative myocardial injury and the consecutive inflammatory response. This is important as it serves as a potential target and has been proposed for the treatment of immune disease including autoimmune myocarditis or cancer (33). Furthermore, considering the association of preoperative levels with clinical outcome, VCAM1 has been suggested as a predictive biomarker for heart failure-related mortality and morbidity, endothelial injury in coronary artery disease and arrhythmias such as atrial fibrillation (33). This is also true for the perioperative setting, where an association of high preoperative VCAM1 levels with long-term (median follow-up of 6.7 years) all-cause mortality could be found in patients suffering from cardiovascular disease undergoing on-pump cardiac surgery. This association was independent of inflammatory markers and

other cardiovascular risk factors (34). Contrarily, preoperative VCAM1 levels were associated with length of hospital stay but not mortality in our cohort.

IGFBP2 has also been suggested for cardiovascular risk monitoring (35). Its plasma concentration can be used to not only detect heart failure but also distinguish healthy individuals from patients with stable chronic disease. Thus, its diagnostic and prognostic value in heart failure especially when compared to natriuretic peptides is highly promising (36). Further, an early activation of IGFBP2 represents a marker of early smooth muscle cell phenotype modulation in patients suffering from thoracic aortic aneurysms (37). After acute MI, higher IGFBP2-levels were prognostic for higher risks of major adverse cardiac events after discharge (33). Of note, inflammation was shown to be a modulator of the insulin-like growth factor binding protein system in several pathological conditions (38, 39). In light of those findings, it is highly likely, that the association of preoperative IGFBP2 with all-cause mortality is a marker of a higher burden of cardiovascular disease at baseline and that the dynamics during cardiac surgery probably represent further myocardial injury.

The body of evidence confirms that our hit approach led to a conclusive selection of proteins, which are relevant to the dynamics of cardiovascular disease and are heavily altered through the exposition to surgical trauma and CPB. Overall, the identified proteins are likely to play an important role in the acute phase response to cardiac surgery due to their known biological functions in vascular function, coagulation, immune system homeostasis, tissue damage, and hypoxic stress. The inflammatory response to CPB is distinctively different from off-pump surgical trauma, resulting in an upregulation of apoptosis and remodeling markers (40). As shown by Ghorbel et al., cytokines and chemokines after CPB were elevated both at the mRNA level in the myocardium and at the protein level in the blood, suggesting the myocardium as likely source for these proteomic changes (40). The link between myocardial injury, ischemia-reperfusion and inflammation has been extensively investigated (41). While most of the identified proteins are not a direct component of the immune system, their exhibition during myocardial injury together with the interactions with the immune system described above, might reflect the interaction of perioperative myocardial injury with the acute phase response—however, this remains speculative. Most importantly, we cannot deduct whether the dynamics in the perioperative levels of these proteins should be attributed to myocardial injury, the exposition to CPB or a combination of the two. As prophylactically addressing inflammation in cardiac surgery has not shown any advantages (42, 43), a better understanding of the involved pathways during perioperative myocardial injury and the interaction with the acute phase response is crucial to potentially identify new therapeutic targets.

The most established and probably most investigated acute phase protein C-reactive protein (CRP) was also included in our selection of hit proteins. Preoperative CRP levels have been repeatedly shown to be an important determinant of short- and long-term postoperative outcome after on-pump cardiac surgery (44, 45). However, this was not the case in our cohort. Overall, baseline values of the hit-proteins identified in our study seemed to have stronger associations with outcome compared to perioperative change, which might suggest that the preoperative state of the investigated proteins is a stronger determinant of outcome than the perioperative response. Similarly, in contrast to the preoperative levels mentioned above, postoperative CRP levels did not seem to be useful for predicting postoperative outcome in several studies (46, 47).

Accordingly, after adjustment for multiple comparison, the associations of preoperative levels of IGFBP2 with 1-year all-cause mortality, and the associations of preoperative levels of LDHB and VCAM1 with length of hospital stay remained significant, while no association of perioperative change of any protein with clinical outcome measures could be observed. As IGFBP2 and VCAM1 have been described above, measuring LDH has traditionally been used as an indicator of myocardial damage or necrosis and has been found to be elevated in patients suffering from valve heart disease, heart failure, and coronary heart disease (48). Levels of preoperative IGFB2 and LDHB were significantly associated with EuroSCORE II (19) at baseline in our cohort, indicating that those patients were probably in a more severe or progressed disease state.

Hence, the identified markers showed potential as prognostic markers before cardiac surgery. Creating combined scores consisting of multiple biomarkers might be a promising new approach for predicting outcome after cardiac surgery and our findings yielded potential candidates for further evaluation. As a most prominent example, the introduction of the cardiac-specific biomarkers natriuretic peptides and cardiac troponins have substantially refined the prognostication of cardiovascular risk in non-cardiac surgery, both independently and complementary to other important indicators of risk (49). Other recent studies have assessed the predictive power of other multimodal scores consisting of hit-proteins and previously identified risk factors such as age, haemoglobin values or serum lactate concentrations to predict neurologic outcomes in emergency patients undergoing cardiac surgery after out-of-hospital cardiac arrest (50). In paediatric patients undergoing on-pump cardiac surgery, a holistic approach to outcome prediction has previously been applied by creating a potential predictor model involving 24 key proteins with significant changes along the perioperative time course (51).

This study has several limitations. The observational design of the study prevents from inferring causal relationship. We did not exclude patients with autoimmune disease or infection. However, a clinically relevant infection is a contraindication for elective heart surgery at our center, which was also represented by the low CRP levels measured at baseline measured by routine laboratory methods, suggesting that the overall preoperative inflammatory status of our patients was low. Further, spectral counting is commonly used for identification and quantitative analysis in proteomics (52). This method has a tendency to mask low-abundance proteins, which are below the detection limit for mass spectrometers (53). Spectral counting allows to indirectly quantify protein levels, but does not represent their biological activity and exact functionality within a complex interrelated system. Further, while our multi-step approach allowed to identify hit-proteins, this methodology might also introduce a selection bias. Most importantly, due to the rigorous predefined selection process, proteins which only emerge during or after surgery or which are only present in a small subset of patients were not included in this analysis. We acknowledge, that those proteins might also play a crucial role in the perioperative acute phase response to cardiac surgery. Further, our analysis protocol led to a very small number of actual hit-proteins. While many of these showed significant associations with clinical outcome measures, an even smaller number of associations remained significant after correcting for multiple testing. However, this correction is essential, as the mass of data generated, might otherwise produce significance by chance. Once again, other important proteins might not have been included in our selection. Overall, despite being clinically motivated, the empirical choices inherent in our multi-step procedures—e.g., the definition of the ellipse when assessing the perioperative changes and the between-patient variation in perioperative protein changes jointly—might be chosen differently.

In conclusion, we were able to identify a subset of promising candidate proteins relevant to outcome after on-pump cardiac

surgery, through applying an innovative multi-step approach. IGFBP2 yielded the most promising results with a strong association with clinical outcome measures and a significant association of preoperative levels with 1-year all-cause mortality. Further proteins with clinically relevant associations were LDHB and VCAM1. We recommend further investigation of these proteins as potential outcome markers after cardiac surgery.

## Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: <https://www.ebi.ac.uk/pride/archive/projects/PXD046496>.

## Ethics statement

The local ethics committee approved the study (Cantonal Ethics Commission of Bern, Bern, Switzerland). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

## Author contributions

CB: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. MF: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. MH: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. SF: Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – review & editing. BK: Data curation, Investigation, Project administration, Visualization, Writing – review & editing. RF: Data curation, Formal analysis, Investigation, Methodology, Resources, Validation, Visualization, Writing – review & editing. BK: Data curation, Formal analysis, Investigation, Methodology, Resources,

Validation, Visualization, Writing – review & editing. LR: Investigation, Validation, Visualization, Writing – review & editing. FS: Methodology, Project administration, Resources, Supervision, Writing – review & editing. ML: Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing.

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## Conflict of interest

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fcvm.2023.1287724/full#supplementary-material>

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