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Dexamethasone vs. placebo modulation of the perioperative blood immune proteome in patients undergoing total knee arthroplasty

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Summary

Background Pre- and post-operative immune status has gained interest in recent years, as it has been shown to be related to postoperative complications and recovery. The change in immune status has also been known to constitute a large part of the surgical stress response, and it has been speculated that immunomodulatory treatment by glucocorticoids may impact it. Profiling of the impact of specific surgeries and medications on immune status are therefore needed.

Methods We characterized the postoperative blood immune proteome in 83 patients receiving either placebo ($n=20$) or IV 24 mg dexamethasone ($n=60$) preoperative before total knee arthroplasty (TKA). The primary outcome was the effect of dexamethasone on total knee arthroplasty surgical stress by comparing postoperative immune proteome in the dexamethasone group and the placebo group. Secondary outcomes were the surgical stress by total knee arthroplasty by comparing pre- to postoperative immune proteome in the placebo group, and the combined effect of surgical stress and dexamethasone by comparing pre- to postoperative immune proteome in the dexamethasone group. Characterization was performed with the Olink Explorer Inflammation panel on blood samples from the biobank for future research collected during the randomized, clinical DEX-2-TKA Trial. Protein change was reported as log2-fold-change and *p*-values were corrected a.m. Benjamini-Hochberg.

Results The surgical stress (placebo) was characterized by a 4.7 log2-fold-change of IL6 (adjusted p-value < 0.01) and up-regulation of central immune signaling pathways and bone marrow mobilization. The combined effect of surgery and dexamethasone showed a less pro-inflammatory profile: IL6 2.5 log2-fold-change (adjusted p-value < 0.01), with decreased signaling for osteoclast activity and innate, immune cell reaction. The effect of dexamethasone showed upregulation of CSF3 (1.55 log2-fold-change, adjusted p-value < 0.01) and an inhibitory effect on both innate and adaptive immune response, immune cell reactivity and formation of extracellular matrix.

Conclusions Preoperative dexamethasone indicated anti-inflammatory properties on both innate and adaptive immune response, while surgery was pro-inflammatory. the combination of total knee arthroplasty and dexamethasone inhibited pathways for osteoclast-activity, indicating possible implications on aseptic prosthesis loosening. Dexamethasone showed strong modulation of the surgical stress response following total knee arthroplasty and future studies must explore the clinical associations of these findings.

Trial registration NCT03506789.

Keywords Knee arthroplasty, Dexamethasone, Proteome, Surgical stress, Olink

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Background

The surgical stress response elicited by surgery increases demand on all organ functions [1, 2]. Especially the strain by inflammation and surgical stress on pre- and post-operative immunological status has gained interest in recent years, as it has been found as a predictor of clinical recovery [3–7]. Modulation of the surgical stress response with a classic immune-modulatory medication such as a glucocorticoid (GCC), could therefore be a potential target for improving recovery, reducing short term complications, and preventing postoperative pain after surgery [8–10].

Until recently the evidence indicated that excessive innate immune response with concurrent suppression of the adaptive immunity was causally linked to complications after surgery. Recent studies though are indicating that complications more likely stem from simultaneous dysregulated responses in both "branches" of the immune system [11]. Descriptive studies on the physiological response to specific surgeries (the surgical stress response) and the effect of dexamethasone hereon, are therefore needed as they may serve as hypothesis-generating studies for future studies into specific immunologic trajectories in risk of complications. Results from such studies are important for exploring and identifying biomarkers which could serve as future targets to improve clinical outcome after surgery.

Previously, hydrocortisone has been examined in healthy volunteers and found to exert different effects on B and T lymphocytes and natural killer cells and down-regulated circulating mRNA related to innate immune signaling [12]. Methylprednisolone was found to alter adaptive immune cell signaling pathways, while sparing innate pathways related to pain and functional outcome following total hip arthroplasty [13]. Also in classical biomarkers on inflammation, glucocorticoids have been found to attenuate the perioperative acute phase response [9, 14, 15]. Recent developments in proteomics, such as Olink, may provide depth in characterizing immune-related protein changes and their physiological effects enabling, efficient profiling of a broad range of biomarkers, including those associated with acute-phase responses. Our primary objective in the present study, was to describe the changes in blood proteome induced by dexamethasone, when administered perioperative to patients undergoing total knee arthroplasty.

Methods

This study investigated the effect of dexamethasone on the surgical stress following total knee arthroplasty, the surgical stress itself and the combined effect of surgical stress and dexamethasone. The study was conducted on material from a biobank for future research collected at a selected site in the multi-center, randomized, blinded, placebo-controlled DEX-2-TKA Trial (ClinicalTrials: NCT03506789, 24/04/2018; Ethics Committee in Region Zealand, Denmark identifier: SJ-695; EudraCT-number 2018–001099) [16–18]. The present, secondary analysis study of the prior RCT and biobank was approved by the Ethics Committee in Region Zealand, Denmark (SJ-887) and the Danish Data Protection Agency (REG-174–2020). Due to the retrospective nature of the study, a renewed informed consent was waived off.

The DEX-2-TKA Trial – the study population

Patients were randomly allocated to one of three interventions as an adjunct to a multi-modal analgesic regime of paracetamol, NSAID, and local infiltration analgesia following total knee arthroplasty: IV dexamethasone 24mg after induction of anesthesia and after 24 h, IV dexamethasone 24mg after induction of anesthesia and IV placebo after 24 h, or IV placebo after induction of anesthesia and after 24 h (Fig. 1). Placebo was isotonic saline. The trial found a significant and patient-relevant reduction in 48 h-opioid-use in the dexamethasone-twice-group compared to placebo [16].

Outcomes

Our primary outcome was the modulation of the blood proteome induced by a single dose of IV 24mg dexamethasone given perioperative in patients undergoing total knee arthroplasty. Secondary outcomes were the proteome changes induced by surgery (the surgical stress response) and by the combination of surgery and dexamethasone.

Sampling and storing of samples

Twenty mL whole blood was sampled with a standard phlebotomy kit from a peripheral vein before surgery and at 8 AM on the first and second postoperative day (POD) after surgery. If the patient had an earlier discharge than 48 h after surgery, no more samples were collected. Samples were centrifuged and stored as aliquots of plasma in a −80 degree Celsius freezer. Study flow diagram in Fig. 1.

Olink analysis

One aliquot from 176 samples were transferred from the Region Zealand Biobank at Næstved Hospital to the private laboratory BioXpedia A/S (Incuba Science Park; Aarhus N; Denmark) on dry ice in Styrofoam-boxes. Although only one aliquot from each sample was analyzed, the term aliquot and sample will be used interchangeably hereafter.

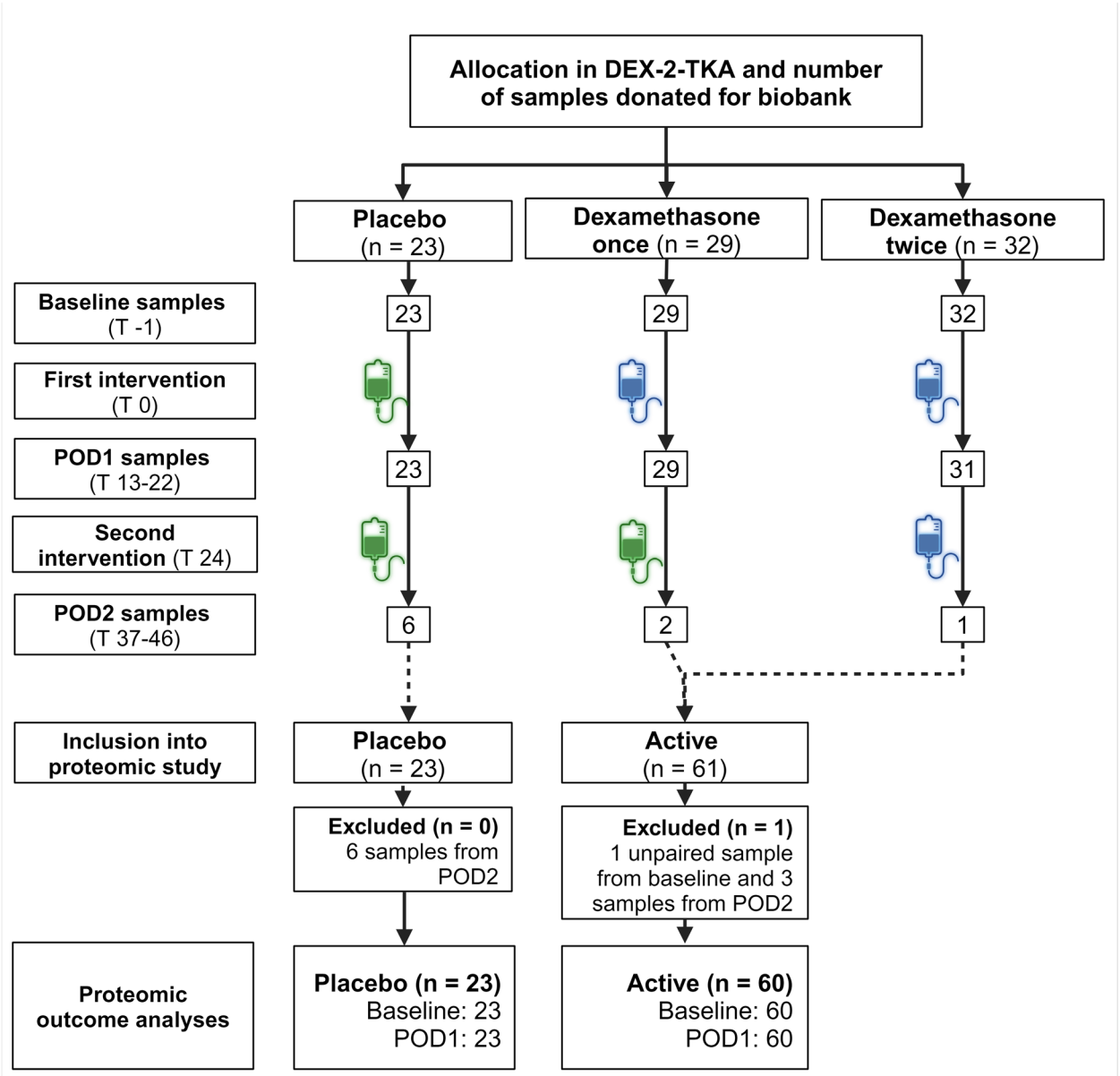

Fig. 1 Study flow diagram with timeline of interventions, sampling of blood and inclusion of samples to the present trial. Colors are placebo (green) and 24mg dexamethasone (blue). POD, Post-Operative Day. Created with BioRender.com

One-hundred-and-seventy-six was chosen as 88 samples can be analyzed per Olink-plate. Samples were picked to match 135 samples from the 64 patients, which were earlier analyzed with a transcriptomic analysis within the same project approvals (unpublished data). The 41 samples which needed to be chosen in addition, were chosen from randomly selected patients in the biobank.

Samples were analyzed with the Olink Explorer 384 Inflammation panel measuring 368 different proteins related to systemic immune response. Proteins in the panel are listed in the appendix along with excluded proteins as written in the results section.

The Olink Explorer platform uses Proximity Extension Assay (PEA) technology, which generates a readout [19], which is a relative protein quantification on an arbitrary unit in log2 called NPX (normalized Protein eXpression) [20]. Normalization was performed with an intensity normalization before readout of NPX counts [21].

Quality control of data

Any proteins not meeting Olink's batch release quality control were excluded. In addition, a quality control warning could be issued for each sample as three internal controls (incubation, extension and amplification control) were added. Lastly, any proteins with more than 90% of measurements being less than limit-of-detection (LOD) would be excluded. Proteins below LOD were not missing – there were just a larger degree of uncertainty about their NPX count. Ninety percent was chosen, in order to retain as many proteins as possible in the study [22].

Distance- and correlation-plots for all samples and principal-component-analyses (PCA) plots for clinical variables of interest were made and eye-balled for any samples or clinical variables skewing the dataset.

Statistical analyses

Patients were allocated to one of three interventions, however, we aimed to describe only the effects within the first day. Therefore, the dexamethasone groups (24mg either twice or once) were statistically merged to one active group. This was justified, as both groups had only received one dose on the morning of the first postoperative day and the second intervention were performed after sampling of blood on the first postoperative day. All samples from day 2 were excluded from the statistical analyses for the same reason in this study.

A biostatistician at BioXpedia A/S made analyses for differentially expressed proteins while AKM analyzed data for differentially expressed proteins and exploratory Gene Set Enrichment Analyses (GSEA).

BioXpedia A/S used R version 4.2.2 (R Core Team, 2023, R Foundation for Statistical Computing, Vienna, Austria) and the EnvStats-package [23]. AKM used R version 4.3.0 (R Core Team, 2023, R Foundation for Statistical Computing, Vienna, Austria) and the packages Tidyverse [24], EnvStats [23], OlinkAnalyze, ggplot2 [25] and ComplexHeatmap [26].

The primary objective was investigated by contrasting the difference in protein expression in the active group by difference in protein expression in the placebo group from baseline to day 1 [27]. The secondary outcomes were investigated by contrasting day 1 protein expression by baseline expression. Shapiro-Wilkes test was used for assessment of normal distribution. If a protein was normally distributed at both baseline and day 1, a paired t-test was performed. Otherwise, a Wilcoxon Signed Rank Test was performed. For the isolated dexamethasone effect, a Students t-test was used for normally distributed differences; otherwise a Mann–Whitney-U test was performed.

Log2-Fold-Change (log2FC) was calculated on geometric mean on NPX counts exponentiated to linear-scale and transformed back to log2-scale for reporting. A positive log2FC signifies an increase in protein expression from baseline to day 1 and vice versa for a negative log2FC.

P-values below 0.05 was deemed significant and adjusting was performed a.m. Benjamin-Hochberg [28].

Gene Set Enrichment Analysis (GSEA) [29] was performed with the olink_pathway_enrichment function from the R package OlinkAnalyze with genesets from the Molecular Signatures Database (MSigDb), KEGG, GO and REACTOME based on the human organism. As pathway enrichment was non-discriminatory regarding whether the pathways were relevant for our study, they were manually reviewed for relevance for inflammation, surgery and glucocorticoids or excluded. We considered a pathway significant if adjusted p-value < 0.05.

There were no missing data. A post-hoc power calculation was estimated for 0.91 for an effect of 1 log2FC, based on 365 proteins per sample, 23 samples and 0.6 variance of means of NPX counts.

Results

One-hundred-and-seventy-six samples from 84 patients were analyzed. Surgeries were performed between November 12th 2018 and March 9th 2020 and samples were analyzed on March 6th 2023. Intervention groups were comparable in regard to clinical variables (Patient characteristics in Table 1). Ten samples were excluded: 9 from the second postoperative day (POD2) and 1 from baseline as it was unpaired by a sample from POD1. Thus, 166 samples from 23 patients receiving placebo and 60 patients receiving IV 24mg dexamethasone were eligible for statistical analyses (Fig. 1).

The proteins BCL2L11, BID and MGLL did not pass Olink quality control (QC) test. IL4 and IL24 had more than 90% of measurements below LOD. With 5 proteins excluded, 363 proteins were eligible for statistical analyses. Gene Set Enrichment Analysis (GSEA) could not map 2 proteins for MSigDb, 168 for KEGG, 3 for GO and 73 for REACTOME (see Table 2). GSEA are therefore not based on 363 proteins, but are limited to only mappable genes.

Secondary outcome 1: the surgical stress response after TKA

We characterized the surgical stress response after total knee arthroplasty by analyzing for differentially expressed proteins on the first postoperative day compared to baseline in the group of 23 patients receiving placebo. One-hundred-and-thirty-four proteins were significantly, differentially expressed (Log2FC $\neq 0$ and p-value < 0.05)

Table 1 Patient characteristics. Values are mean (SD), median [range] or number (proportions). *P*-values are t-test (mean), Mann-Whitney U test (median) or Fisher exact test (proportion)

	Placebo *n* = 23	Active *n* = 60
Age; y	73 [57, 86]	72 [48, 87]
Sex; female	15 (65.2%)	32 (55.0%)
ASA physical status		
1	4 (17.4%)	9 (15.0%)
2	15 (65.2%)	40 (66.7%)
3	4 (17.4%)	11 (18.3%)
Height; cm	169.4 (9.8)	171.6 (8.3)
Weight; kg	81.4 (13.3)	87.7 (16.7)
BMI; kg.$(cm^2)^{-1}$	28.3 (3.3)	29.6 (4.9)
Diabetes type 2; yes	2 (8.7%)	3 (5.0%)
Use of paracetamol in the month prior to TKA		
no	1 (4.3%)	8 (13.3%)
Occasionally	3 (13.0%)	13 (21.7%)
everyday	19 (82.6%)	39 (65.0%)
Use of NSAID in the month prior to TKA		
no	13 (56.5%)	30 (50.0%)
Occasionally	2 (8.7%)	11 (18.3%)
everyday	8 (34.8%)	19 (31.7%)
Use of any opioid in the month prior to TKA		
no	20 (87.0)	55 (91.7)
Occasionally	2 (8.7)	3 (5.0)
everyday	1 (4.3)	2 (3.3)
VAS at rest before surgery (0-100mm)	19 (19.4)	22 (19.4)
VAS during active flexion before surgery (0-100mm)	52 (23.7)	60 (22.0)
Smoking		
no	13 (56.5%)	27 (45.8%)
yes	3 (13.0%)	10 (16.9%)
Former (minimum 6months abstinence)	7 (30.4%)	22 (37.3%)
Alcohol consumption per week; units	3 [0, 7]	3 [0, 7]
Anesthesia type		
Spinal	19 (82.6%)	49 (81.7%)
General anesthesia	2 (8.7%)	9 (15.0%)
Spinal converted to general anesthesia	2 (8.7%)	2 (3.3%)
TKA type; Cemented	22 (95.7%)	56 (93.3%)
Length of surgery; minutes	60 [47, 130]	58 [39, 99]
Blood loss during surgery; mL	197 (134.8)	191 (146.3)

and 87 of these proteins were significant after adjusting the *p*-value (p-adj < 0.05; see Appendix). Five proteins were up-regulated by more than 1.0 log2FC and 3 were downregulated by more than −1.0 log2FC (Fig. 2 and Table 3) and the response was deemed uniform between patients (Fig. 3). The inflammatory cytokine IL6 was the most upregulated protein with a log2FC of 4.7 (adjusted *p*-value $2.16*10^{-5}$). IFN-gamma (log2FC −1.47; adjusted *p*-value: $3.11*10^{-4}$) was the most downregulated but had 25.6% of measurements below LOD. All differentially expressed proteins with > 1.0 change in log2FC either up- or down were associated to immune processes, with the exception of EPO (1.65 log2FC; adjusted *p*-value 0.002) which was mainly associated to ischemia induced hematopoietic processes.

After manual review 4 significant pathways (p-adj < 0.05) were found relevant through GSEA (Fig. 4A and Appendix). Pathways indicated that surgery upregulated central immune regulation (JAK-STAT-pathways) along with hematopoietic cell lines of the bone marrow. In addition, a pathway associated to hypoxia induced genes was activated.

The surgical stress response following TKA, in this group of 23 patients, could therefore be characterized by a marked increase in IL6 (4.7 log2FC equaling a 26 times increase on linear scale) with upregulation of central immune pathways and activation of hematopoietic cell lines of the bone marrow (pathway for e.g. leukocyte and red blood cell mobilization).

Secondary outcome 2: the combined response to surgery and dexamethasone

We characterized the combined effect of total knee arthroplasty and dexamethasone by analyzing for differentially expressed proteins on the first postoperative day compared to baseline in 60 patients receiving IV 24mg dexamethasone after induction of anesthesia for total knee arthroplasty. A total of 250 proteins were significantly, differentially expressed (log2FC ≠ 0 and *p*-value < 0.05) with 229 proteins being significant after adjusting the *p*-value (p-adj < 0.05, list of results in Appendix). IFN-gamma showed a log2FC of −4.2 (adjusted *p*-value $2.64*10^{-10}$) as the most affected protein by surgery and dexamethasone. Six proteins were significantly up-regulated by more than 1.0 log2FC, while 13 were downregulated by more than 1.0 log2FC (Fig. 5 and Table 4). Up- and down-regulation was deemed uniform and therefore not outlier driven (Fig. 6). IFN-gamma had 25.6% of measurements below LOD and GZMB had 64.8%, meaning that there may be technical uncertainty about the differential expression of GZMB. All other proteins had no measurements below LOD.

Based on differentially expressed genes it seems that surgery combined with dexamethasone was less pro-inflammatory than surgery, as IL6 was less up-regulated while IFN-gamma was more down-regulated. Interestingly, CSF3 was the most up-regulated protein (2.68 log2FC, p-adj $1.54*10^{-27}$) pointing to a stimulation of granulocyte production by surgery and dexamethasone in combination.

Table 2 Proteins that could not map and number of pathways with *p*-value <0.05 (number of pathways with adjusted *p*-value < 0.05)

Database	Proteins that could not map of 363 proteins	Secondary outcome 1: the surgical stress response	Secondary outcome 2: the combined effect	Primary outcome: effect of dexamethasone
MSigDb	2	275 (7)	244 (11)	458 (71)
KEGG	168	6 (3)	7 (4)	12 (7)
GO	3	174 (0)	154 (7)	321 (103)
REACTOME	73	12 (3)	9 (1)	7 (0)
After review		211 (4)	175 (9)	320 (59)

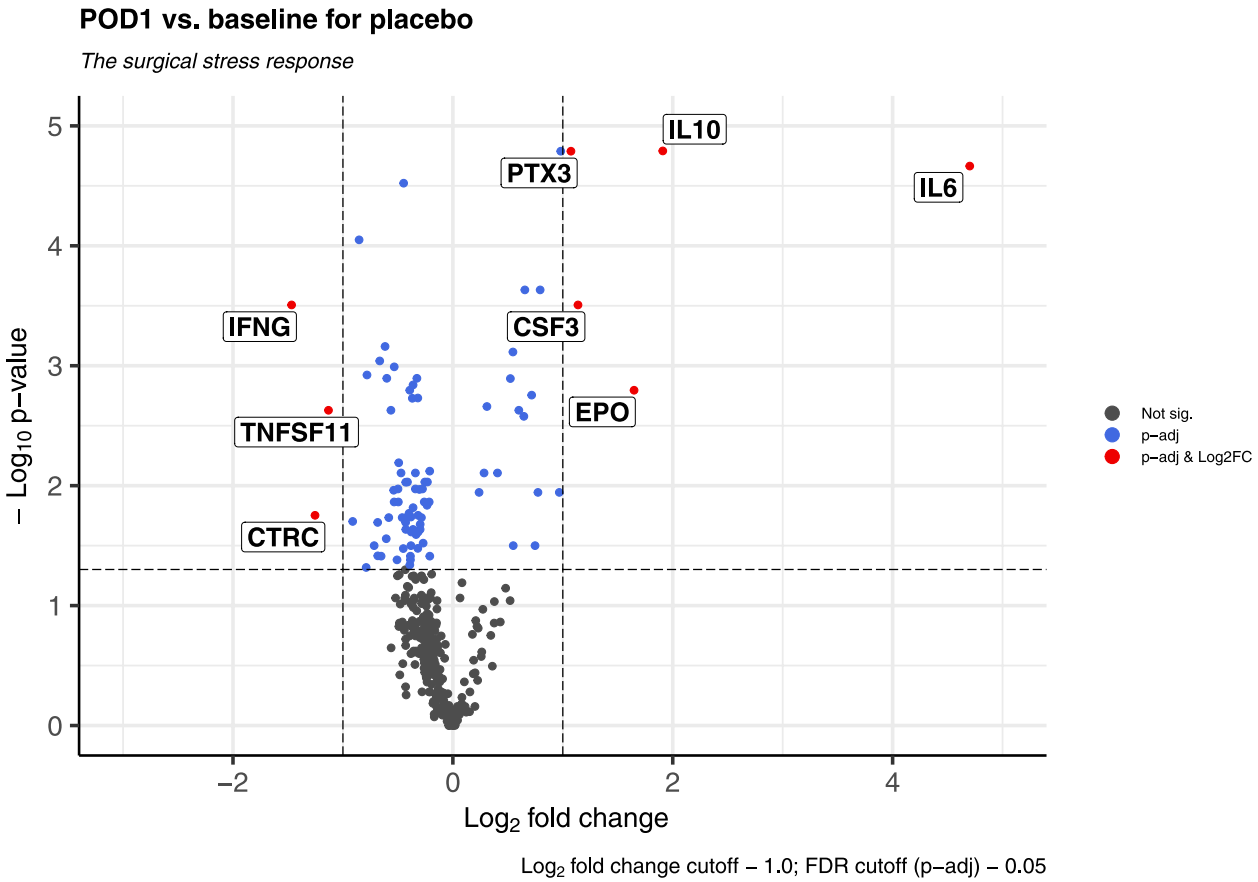

Fig. 2 Vulcano-plot of differential expression of proteins induced by surgery (the surgical stress response). Log2-Fold-Change (log2FC), False Discovery Rate (FDR), adjusted *p*-value (p-adj). Proteins showing more than 1 log2FC and p-adj < 0.05 has been labelled

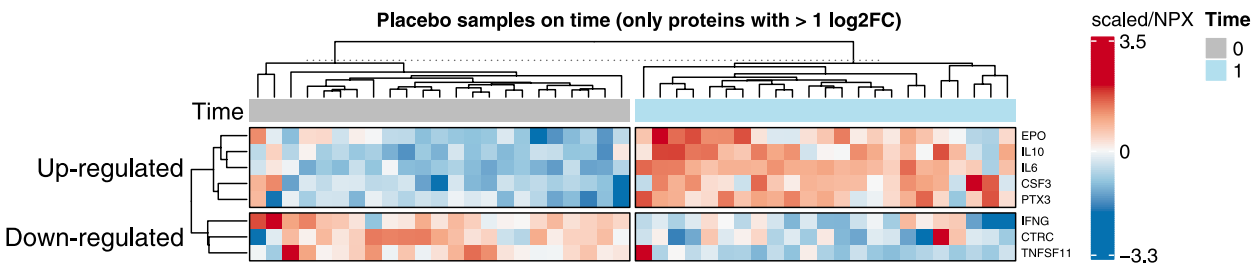

Fig. 3 Heatmap of proteins which showed more than 1 log2FC up- or down-regulation in the placebo group (the surgical stress response)

Table 3 All proteins either up- or down-regulated by more than 1.0 log2FC by the surgical stress induced by total knee arthroplasty

Assay	log2FC	*p*-value	Adjusted *p*-value
IL6	4.70	$2.38*10^{-7}$	$2.16*10^{-5}$
IL10	1.91	$4.46*10^{-8}$	$1.62*10^{-5}$
EPO	1.65	$9.26*10^{-5}$	0.002
CSF3	1.14	$8.58*10^{-6}$	$3.11*10^{-4}$
PTX3	1.07	$1.05*10^{-7}$	$1.63*10^{-5}$
TNFSF11	−1.13	$1.81*10^{-4}$	0.002
CTRC	−1.25	0.003	0.018
IFNG	−1.47	$7.87*10^{-6}$	$3.11*10^{-4}$

After review for relevance, 9 pathways were found through GSEA to be significant and relevant (Fig. 4B and Appendix). All pathways were negatively affected (negative normalized estimation score). Two pathways were related to differentiation of osteoclast differentiation, two pathways to myeloid cell differentiation, two pathways to chemokine signaling, one pathway to cell-to-cell-killing, one to recognition of potential pathogens in innate immune response and one pathway related to allograft rejection. In total, GSEA indicates that the surgical stress response elicited by total knee arthroplasty in combination with dexamethasone decreased differentiation of macrophages to osteoclasts and differentiation of myeloid leukocytes while decreasing innate immune cell reaction postoperatively.

Primary outcome: the isolated effect of dexamethasone

The effect of dexamethasone on the surgical stress response following total knee arthroplasty, was characterized by comparing the per patient change in NPX count from baseline to the first postoperative day in the group of patients receiving IV 24mg dexamethasone by the change of NPX count in the group of patients receiving placebo for each protein. One-hundred-and-thirty-four proteins were significantly, differentially expressed (log2FC ≠ 0 and *p*-value < 0.05) between the placebo and the active group, with 99 proteins being significant after adjusting the *p*-value (p-adj < 0.05; list of results in Appendix).

CSF3 was the only protein up-regulated by more than 1.0 log2FC (1.55 log2FC, p-adj. $1.21*10^{-8}$), while 8 proteins showed a down-regulation of more than −1.0 log2FC (Fig. 7 and Table 5). Two of the 8 proteins exhibiting down-regulation (IFN-gamma and GZMB) had measurements below LOD (25.6% respectively 64.8%). Heatmap showed a uniform down-regulation among down-regulated proteins, while up-regulation of CSF3 seemed more heterogenic among the patients. The up-regulation could therefore be outlier driven (Fig. 8). In addition, one placebo patient seems to have a lower expression of proteins compared the other patients in the group.

Through GSEA analysis 59 pathways were found to be both relevant and significant with an adjusted *p*-value < 0.05 (Table 2 and Appendix). All pathways, which our GSEA encompassed, were negatively affected by the isolated dexamethasone response.

The pathways could be grouped into the following groups/responses (number of pathways in parenthesis) in order to provide an overview:

Actions in immune system: general immune response (11), innate acute phase response (2), sepsis and inflammatory response (2), adaptive (1) and humoral response (1).

Cellular actions and processes: Leukocyte activation, migration and adherence (6), T-cell activation and

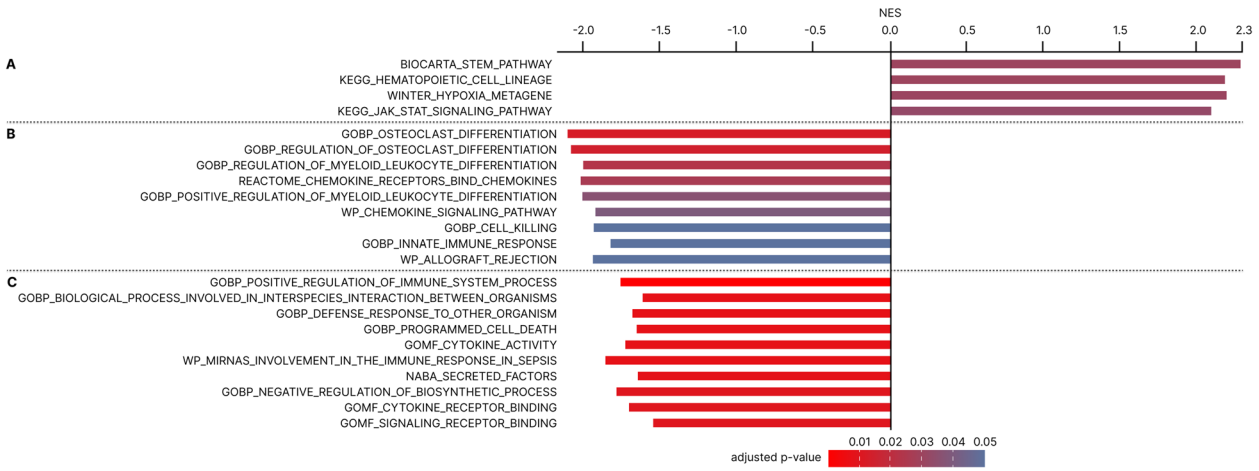

Fig. 4 **A** Bargraph of the significantly affected pathways by surgery. **B** Bargraph of the significantly affected pathways by surgery combined with dexamethasone. **C** Bargraph of top 10 affected pathways by dexamethasone based on adjusted *p*-value

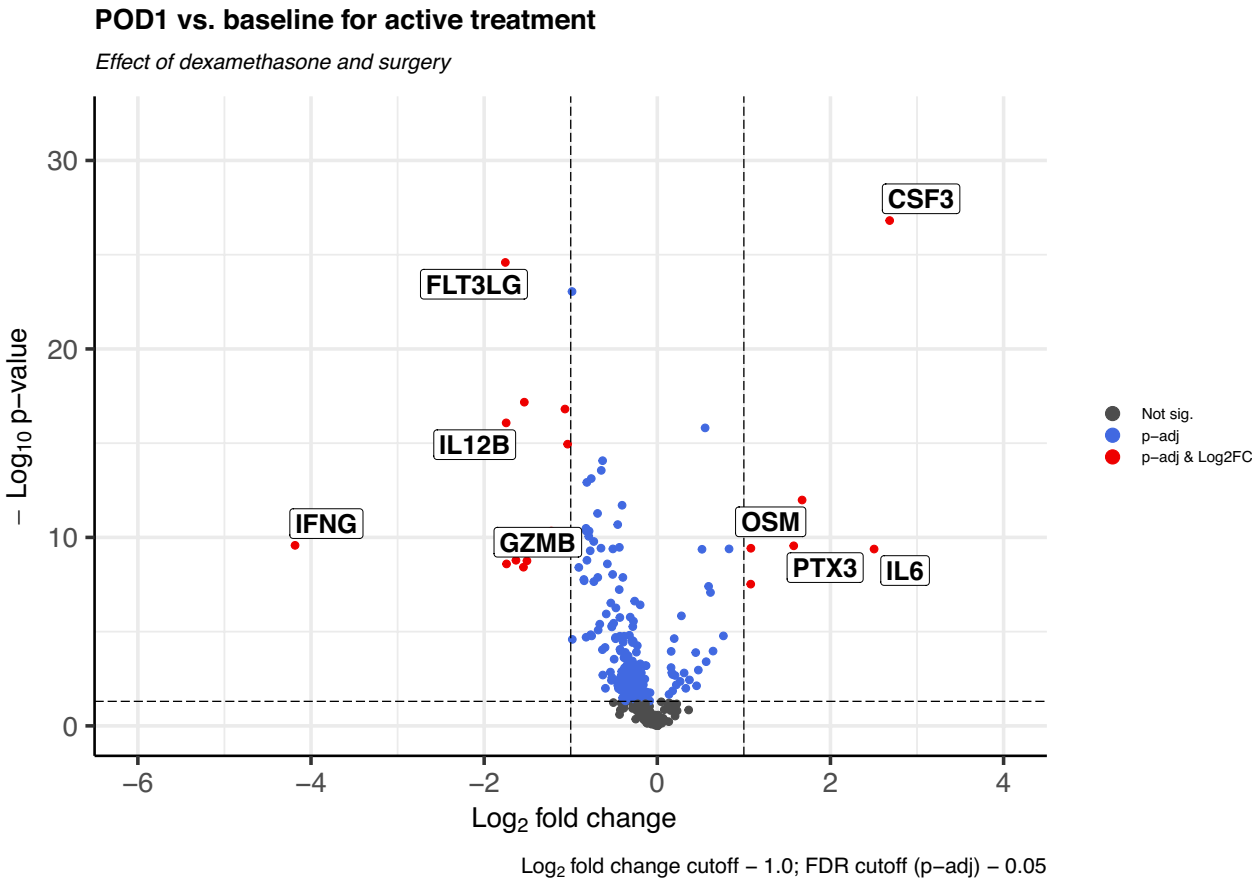

Fig. 5 Vulcano-plot of differential expression of proteins induced by the combined effect of surgery and dexamethasone

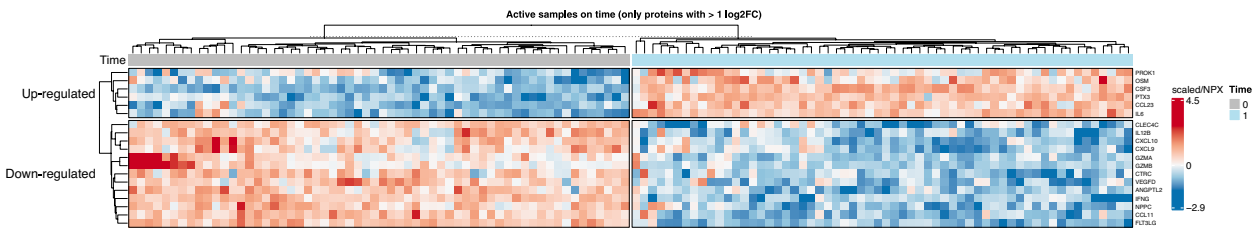

Fig. 6 Heatmap of proteins which showed more than 1 log2FC up- or down-regulation by the combined effect of surgery and dexamethasone

differentiation (3), lymphocyte activation (2), differentiation of macrophages (1), cell–cell adherence (4), general cell migration (2) and programmed cell death (2).

Signaling and cytokines: General cytokine expression, activity and receptor activity (8), JAK-STAT-pathway (2), Toll-like-receptor activity (1), prostaglandin expression (1), IL1-response (1) and IL27-response (1).

Other effects: Remodulation and formation of extracellular matrix (5), allograft rejection (2) and change in gene-expression (1).

The isolated effect of dexamethasone, when performing GSEA on differentially expressed proteins found with the Olink Explorer 384 inflammation following total knee arthroplasty, seems to have had an overall inhibitory effect on the immune system (both innate and adaptive), immune cell reactivity and formation of extracellular matrix.

Figure 4C presents top-10 pathways based on adjusted *p*-values.

Discussion

This study examined the effect of dexamethasone on the surgical stress elicited by total knee arthroplasty as characterized by changes in the blood proteome.

Table 4 All proteins that are either up- or down-regulated by more than 1 log2FC by the surgical stress induced by TKA in combination with perioperative dexamethasone

Assay	log2FC	*p*-value	Adjusted *p*-value
CSF3	2.68	$4.25*10^{-30}$	$1.54*10^{-27}$
IL6	2.50	$3.55*10^{-11}$	$4.16*10^{-10}$
OSM	1.67	$3.72*10^{-14}$	$1.04*10^{-12}$
PTX3	1.58	$1.85*10^{-11}$	$2.80*10^{-10}$
CCL23	1.08	$2.91*10^{-11}$	$3.77*10^{-10}$
PROK1	1.08	$3.99*10^{-9}$	$3.02*10^{-8}$
VEGFD	−1.04	$2.49*10^{-17}$	$1.13*10^{-15}$
ANGPTL2	−1.07	$2.13*10^{-19}$	$1.55*10^{-17}$
CCL11	−1.17	$2.77*10^{-11}$	$3.74*10^{-10}$
NPPC	−1.19	$4.33*10^{-11}$	$4.76*10^{-10}$
CXCL9	−1.22	$2.54*10^{-12}$	$4.60*10^{-11}$
GZMA	−1.51	$1.78*10^{-10}$	$1.75*10^{-9}$
CXCL10	−1.54	$7.37*10^{-20}$	$6.69*10^{-18}$
CTRC	−1.55	$4.20*10^{-10}$	$3.81*10^{-9}$
CLEC4C	−1.63	$1.62*10^{-10}$	$1.63*10^{-9}$
GZMB	−1.74	$2.74*10^{-10}$	$2.55*10^{-9}$
IL12B	−1.75	$1.39*10^{-18}$	$8.38*10^{-17}$
FLT3LG	−1.76	$1.42*10^{-27}$	$2.57*10^{-25}$
IFNG	−4.18	$1.67*10^{-11}$	$2.64*10^{-10}$

Secondary outcomes were to characterize the surgical stress itself and the combined effect of surgical stress and dexamethasone.

We found that dexamethasone down-regulated a range of cytokines (most notable IL6 and IFN-gamma) and only up-regulated CSF3 (Colony Stimulating Factor 3/Granulocyte Colony Stimulating factor). The effects of these changes were characterized by Gene Set Enrichment Analyses (GSEA) and we found inhibition of a range of signaling pathways within both innate and adaptive immune response, immune cell reactivity and formation of extracellular matrix. The surgical stress by total knee arthroplasty elicited a marked up-regulation of IL6 and pathways for hematopoiesis and JAK-STAT-pathway. The combined effect of dexamethasone administered perioperative to total knee arthroplasty elicited an upregulation of CSF3 and IL6, and down-regulation of IFN-gamma. GSEA found signaling pathways for differentiation of osteoclasts and osteoclast activity along with pathways for innate and leukocyte-immune-response to be down-regulated.

In a study on primary, total hip arthroplasty with a similar scope as the present, it was found that surgery engages both innate and adaptive immune response and that methylprednisolone alters primarily adaptive immune cell signaling [13]. An example is that JAK-STAT-pathways were activated by surgery and attenuated by methylprednisolone [13]. This is in line with our results, as we have found that surgery upregulates the pro-inflammatory cytokine IL-6 and GSEA finds that JAK-STAT-pathway along with pathways for mobilization of bone marrow stem cells. Our results though, differ whether a glucocorticoid has a differentiated effect on innate and adaptive immune response. Our results indicated that dexamethasone had an attenuating effect on both innate and adaptive immune results, while Ganio et al. [13] found an effect on selected pathways within the adaptive immune response by methylprednisolone. This difference might have been due to technique, as Ganio et al. [13] used mass spectrometry for immune cell subsets and characterization of intracellular cell signaling, while we used a targeted proteomic approach for a limited range of circulating cytokines. Our pathway enrichment might therefore be regarded as an extrapolation of measured biomarkers as opposed to the direct measurement of reactivity within known pathways. A broader panel could have had the potential to allow our study to in-depth analyze affected pathways more specifically. In addition, it was possible that the surgical stress arising from hip arthroplasty would not be equal to the surgical stress arising from knee arthroplasty, which could be further aggravated by difference in anesthetic technique (primarily general or general with spinal [4, 13] vs. primarily spinal in the present study) and study sizes; 30 placebo, 28 active treatment [13] vs. 26 [4] vs. 20 placebo and 60 active.

A last difference was that Ganio et al. [13] concluded that signaling pathways affected by methylprednisolone were not previously found among pathways important for clinical recovery [4, 5], while dexamethasone had a significant and patient relevant reduction in opioid-use and pain in the cohort on which this study was based.

The implications of the strong adaptive immune response suppression need to be further evaluated in other populations as these data could indicate a possible negative effect of glucocorticoids in patients with cancer undergoing surgery. In these patients perioperative immune suppression may have long term negative oncological consequences [30].

The main limitation of this study was, that we only examined plasma samples and therefore the systemic response (more specifically the response in the circulation). Sampling of synovial fluid could have provided valuable knowledge on local immune status in the knee, which may be the most important for e.g. aseptic prosthesis loosening. Post-operative sampling of synovial fluid on the other hand could have increased the risk of prosthesis infection and hence be an unacceptable risk for studying a physiological response. In addition, if the systemic response is determining clinical outcome, using

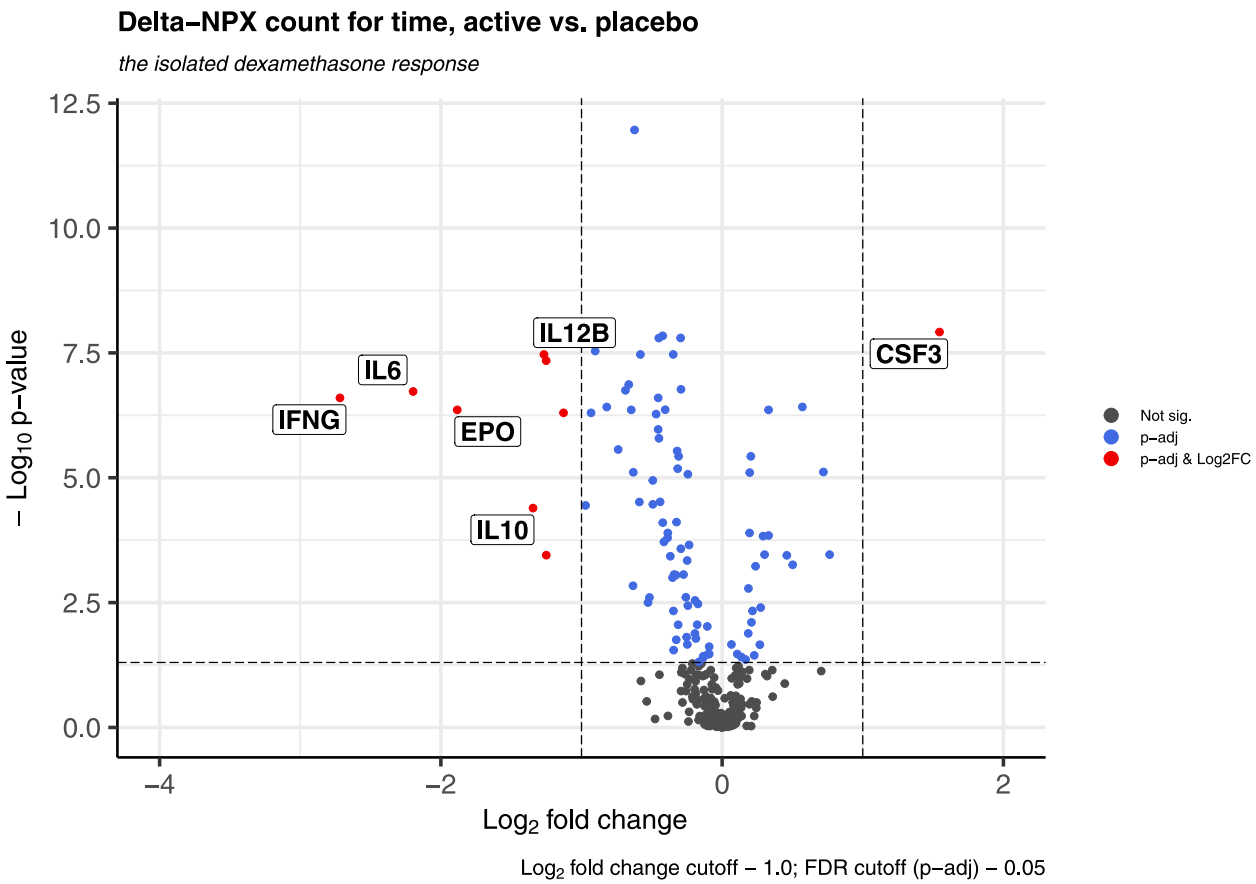

Fig. 7 Vulcano-plot of differential expression of proteins induced by the isolated effect of dexamethasone

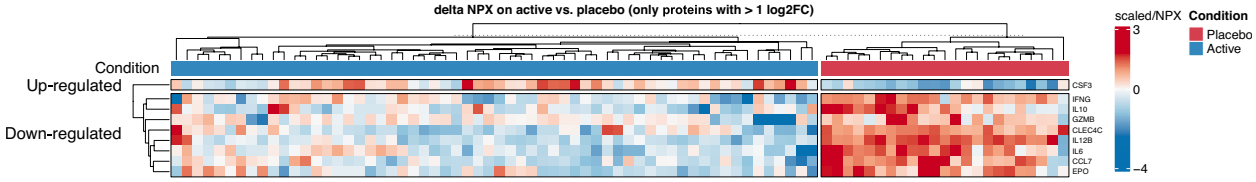

Fig. 8 Heatmap of proteins which showed more than 1 log2FC up- or down-regulation, when isolating for the effect of dexamethasone

plasma samples were the most sensible approach with minimum risk for the patients. Measurement of intracellular signaling within known pathways or changes in cell fractions of circulating cell subsets could furthermore have added an extra dimension to our data. With these data we would not only have been able to characterize change in blood proteome, but also the effect hereof on e.g. circulating fraction of granulocytes and other cells of the adaptive immunity.

A second limitation was that we used a targeted panel for inflammatory biomarkers. Thus, a different pathway enrichment could have been made if more biomarkers were added to the panel or a different panel was chosen. In addition, although we expect that our findings may be universal to TKA in spinal anesthesia, many variables may impact perioperative stress and influence the external validity in e.g., different cohorts from a different health care system or patients of other ethnic origin.

A study using e.g. shotgun-proteomics for full proteome characteristics, could add to the knowledge gained from this study, however, it was practically and economically out of our range. A different approach could have been to use traditional, PCR-based analysis

Table 5 All proteins either up- or downregulated with more than one fold change on a log2-scale (log2FC), when isolating for the effect of dexamethasone

Assay	log2FC	*p*-value	Adjusted *p*-value
CSF3	1.55	$9.99*10^{-11}$	$1.21*10^{-8}$
CCL7	−1.13	$3.45*10^{-8}$	$5.02*10^{-7}$
GZMB	−1.25	$5.48*10^{-5}$	$3.55*10^{-4}$
CLEC4C	−1.25	$1.37*10^{-9}$	$4.52*10^{-8}$
IL12B	−1.27	$8.78*10^{-10}$	$3.40*10^{-8}$
IL10	−1.34	$4.80*10^{-6}$	$4.05*10^{-5}$
EPO	−1.88	$2.69*10^{-8}$	$4.37*10^{-7}$
IL6	−2.20	$7.74*10^{-9}$	$1.87*10^{-7}$
IFNG	−2.72	$1.18*10^{-8}$	$2.52*10^{-7}$

for specific proteins, however, that would have inferred the risk that we chose proteins in the blind and thus found proteins unaffected by glucocorticoids or chose proteins already well-established within the field.

A major strength of this study, was that the biobank was sampled during a randomized, clinical trial, thus increasing internal validity, while using the commercially accessible Olink technology. As more studies are emerging that pre-operative immune state can predict postoperative complications and recovery [5, 6, 31] accessibility of proteomic techniques and interpretation of their results will likely be important – especially if the results of these studies would be implemented as phenotype-specific-panels in everyday practice for patients at risk of chronic pain. We believe that it was a strength of this study that we used an easy to access and interpret technique, although limited by the number of proteins in the panel.

Clinically, this study supports the notation that perioperative dexamethasone attenuates the surgical stress response (especially seem by the marked effect on IL6). On top of that, the surprising finding, that dexamethasone combined with surgery had an effect on osteoclast activity – at least seen in the systemic circulating immune proteome – would warrant further investigations into perioperative dexamethasone's possible effect on incidence of aseptic prosthesis loosening.

Conclusion

In conclusion, we found that preoperative dexamethasone showed anti-inflammatory properties on both innate and adaptive immune response, while surgery alone was pro-inflammatory in patients undergoing total knee arthroplasty. The combination of total knee arthroplasty and dexamethasone inhibited pathways for osteoclast-activity, indicating possible implications on aseptic prosthesis loosening.

Dexamethasone showed strong modulation of the surgical stress response following total knee arthroplasty, and further studies exploring the clinical significance of these findings should be conducted.

Appendix

The following can be read in the appendix:

- List of proteins in the Olink Explore Inflammation Panel
- Table of proteins percent of measurements less than limit of detection
- Table of significantly, differentially expressed proteins by the surgical stress response
- Table of significant and relevant pathways by the surgical stress response
- Table of significantly, differentially expressed proteins by the combination of surgical stress and dexamethasone
- Table of significant and relevant pathways by the combination of surgical stress response and dexamethasone
- Table of significantly, differentially expressed proteins by the isolated effect of dexamethasone
- Table of significant and relevant pathways by the isolated effect of dexamethasone
- Table of post-hoc power analysis for all outcomes and 1.0 and 0.5 log2-fold-change of proteins.

Abbreviations

TKA	Total Knee Arthroplasty
GCC	Glucocorticoid
POD	Post Operative Day
NGS	Next Generation Sequencing
NPX	Normalised Protein eXpression
LOD	Limit of Detection
PCA	Principal Component Analysis
Log2FC	Log-2-Fold-Change
GSEA	Gene Set Entichment Analysis
QC	Quality Control
p-adj	Adjusted *p*-value

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12871-025-03003-3.

Supplementary Material 1

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Not applicable.

Authors' contributions
KSG, OM, DH-G and IG were responsible for design, data acquisition, critical review and final version approval of the manuscript. ASG were responsible for design, data acquisition, analyses, critical review and final version approval of the manuscript.

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Data availability
Dataset are available upon reasonable request to the corresponding author.

Declarations

Ethics approval and consent to participate
The study was conducted on material from a biobank for future research collected at a selected site in the multi-center, randomized, blinded, placebo-controlled DEX-2-TKA Trial after signed informed consent (ClinicalTrials: NCT03506789, 24/04/2018; Ethics Committee in Region Zealand, Denmark identifier: SJ-695; EudraCT-number 2018–001099, 08/06/2018 with amendments on 30/08/2018 and 21/9/2018). The study was approved by the Ethics Committee in Region Zealand, Denmark (SJ-887, 21/12/2020 with amendment 21/09/2022) and the Danish Data Protection Agency (REG-174–2020, 21/01/2021 with amendment 16/01/2023). Due to the retrospective nature of the study and the analyses were describing a physiological process without potential to either identify specific patients or future or current, grave disease, a renewed informed consent was waived off.

Consent for publication
Consent for publication was covered by Regional Ethics Committee approval of the study.

Competing interests
The authors declare no competing interests.

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