# Transcriptomic and Proteomic Characterization of the Immune Response to Elective Spinal Reconstructive Surgery: Impact of Aging and Comparison with Traumatic Injury Response

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**BACKGROUND:** Major surgery triggers trauma-like stress responses linked to age, surgery duration, and blood loss, resembling polytrauma. This similarity suggests elective surgery as a surrogate model for studying polytrauma immune responses. We investigated stress responses across age groups and compared them with those of polytrauma patients.

**STUDY DESIGN:** Patients undergoing major spinal reconstruction surgery were divided into older (age >65 years, n = 5) and young (age 18 to 39 years, n = 6) groups. A comparison group consisted of matched trauma patients (n = 8). Blood samples were collected before, during, and after surgery. Bone marrow mononuclear cells and peripheral blood mononuclear cells were analyzed using cellular indexing of transcriptomes and epitopes sequencing or single-cell RNA sequencing. Plasma was subjected to dual-platform proteomic analysis (SomaLogic and O-link).

**RESULTS:** Response to polytrauma was highest within 4 hours. By comparison, the response to surgery was highest at 24 hours. Both insults triggered significant changes in cluster of differentiation 14 monocytes, with increased inflammation and lower major histocompatibility complex-class 2 expression. Older patient's cluster of differentiation 14 monocytes displayed higher inflammation and less major histocompatibility complex-class 2 suppression; a trend was also seen in bone marrow mononuclear cells. Although natural killer cells were markedly activated after polytrauma, they were suppressed after surgery, especially in older patients. In plasma, innate immunity proteins dominated at 24 hours, shifting to adaptive immunity proteins by 6 weeks with heightened inflammation in older patients. Senescence-associated secretory phenotype proteins were higher in older patients at baseline and further elevated during and after surgery.

**CONCLUSIONS:** Although both major surgery and polytrauma initiate immune and stress responses, substantial differences exist in timing and cellular profiles, suggesting major elective surgery is not a suitable surrogate for the polytrauma response. Nonetheless, distinct responses in young vs older patients highlight the utility of elective spinal in studying patient-specific factors affecting outcomes after major elective surgery. (J Am Coll Surg 2024;238:924–941. © 2024 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American College of Surgeons. This is an open-access article distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License 4.0 [CCBY-NC-ND], where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.)

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Drs Bonaroti and Ozel contributed equally to this work.

**Disclosure Information: Nothing to disclose.**

**Disclosures outside the scope of this work: Dr Das is a paid consultant to and holds stock options in Seromyx. Other authors have nothing to disclose.**

Support: This work was supported by the NIH (R35-GM-127027 and T32-GM-008516 to Dr Billiar; and R38-HL-150207 to Dr Bonaroti). Dr Reitz's institute is supported by NIH Grant #5T32HL0098036. The University of Pittsburgh holds a Physician-Scientist Institutional Award from the Burroughs Wellcome Fund to Dr Bonaroti. This work was internally supported by the UPMC Immune Transplant and Therapy Center.

Presented at the American College of Surgeons 109th Annual Clinical Congress, Scientific Forum, Boston, MA, October 2023.

Received August 17, 2023; Revised October 15, 2023; Accepted November 28, 2023.

https://doi.org/10.1097/XCS.0000000000000922
ISSN 1879-1190/22

**Abbreviations and Acronyms**

BL = baseline
BMMC = bone marrow mononuclear cell
CITEseq = cellular indexing of transcriptomes and epitopes sequencing
DEG = differentially expressed genes
GOBP = Gene Ontology Biological Process
GSEA = gene set enrichment analysis
HC = healthy controls
HSC = hematopoietic stem cell
IO_4h = 4 hours into the intraoperative period
ISS = Injury Severity Score
NK = natural killer
PBMC = peripheral blood mononuclear cell
PO_24h = 24 hours after the operation
PO_6w = 6 weeks after the operation
SASP = senescence-associated secretory phenotype
scRNAseq = single-cell RNA sequencing
UMAP = Uniform Manifold Approximation and Projection

The systemic response to major open surgery is thought to resemble the response seen in severely injured trauma patients.[1-4] In both clinical conditions, the magnitude of the systemic inflammatory response correlates with major complications and mortality.[5-7] Also in both instances, the extent of the tissue injury and blood loss are known to amplify the host immune and other systemic stress responses. Another significant modifier of the magnitude and characteristics of the systemic response to elective surgery and trauma is age.[8-10] Older patients experience greater complications after surgical procedures or after any severity of trauma than young patients.[11-13] We have previously shown that the circulating immune mediator profiles are markedly different between young and older trauma patients after moderate injury.[10] These differences are likely due to the process of inflammaging, which may encompass higher cellular senescence and the effect of aging on circulating immune cells and bone marrow progenitors.[14-17]

These similarities between major open surgical procedures and trauma have led to the suggestion that elective operations could be used as a surrogate for the study of the human response to trauma.[1-4] Although the human response to severe trauma has been characterized using contemporary analysis of the circulating compartment such as whole blood and single-cell transcriptomics[18,19] and plasma-based multiomics,[5,6] most studies on the response to elective surgery were performed before the introduction of high-dimensional analysis platforms and focus on limited sets of soluble plasma mediators,[20-22] with a few notable exceptions. One is a recent study by Moris and colleagues[23] that characterized lymphocyte subsets from patients undergoing surgery using multiparameter flow cytometry. These authors found that advancing age was correlated with decreased naïve T-cell number, and increased maturation and exhaustion of CD4+ and CD8+ T cells. They were also able to correlate these changes in T cells with postoperative complications after elective surgery. Others have used high-dimensional proteomics to evaluate elective surgery and identified perturbations in pro-inflammatory pathways with significant fold changes in chitinase-3-like protein, C-reactive protein, and interleukin-6 associated with postoperative complications.[24] However, a comprehensive study of the cellular and systemic response to major elective surgery as a function of age has not been performed, nor has this response been compared with the response observed in polytrauma.

To provide a landscape of the systemic response to major elective surgery, we carried out a pilot study using cellular indexing of transcriptomes and epitopes sequencing (CITEseq) and plasma proteomics on patients undergoing an elective spinal reconstructive surgery with sampling at baseline, intraoperative, and postoperative timepoints. We chose major adult spinal reconstructive procedures for the following reasons: (1) this procedure requires significant tissue disruption to access and reconstruct the spine and is typically associated with moderate blood loss; (2) the procedure is commonly performed on both young and older adults electively; and (3) vertebral bodies are instrumented during the procedure, allowing bone marrow sampling from a bone actively involved in hematopoiesis. To compare the early cellular responses between elective surgical procedures and traumatic injury, we reanalyzed a published peripheral blood mononuclear cell (PBMC) single-cell RNA sequencing (scRNAseq) dataset[18] using trauma patients matched in tissue injury severity to the elective surgery patients. This strategy provided insights into the impact of age on the host response to major surgical procedures and on the unique differences in the

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Supplemental digital content is available for this article.

response of circulating immune cells to major surgery vs traumatic injury. This work represents the first published CITEseq dataset and the largest proteomic dataset in elective surgery, and the first comparative analysis between elective surgery and traumatic injury.

# METHODS

## Major spinal reconstructive surgery patient enrollment

Patients undergoing elective surgery for adult spinal deformity requiring spinal fusion greater than 2 spinal segments were eligible for enrollment in a clinical protocol. The study was reviewed and approved by the University of Pittsburgh Human Research Protection Office (STUDY19060214) after the completion of informed consent. Two cohorts were recruited: patients older than 65 years in the older group and those 18 to 39 years old in the young group. Clinical characteristics of the young and older patient cohorts are presented in Table 1; p value calculations from *t*-test reveal no significant differences between cohorts. Blood samples for PBMC isolation were obtained at baseline (BL; ie in the operating room, after induction of anesthesia, before incision), 4 hours into the intraoperative period (IO_4h), 24 hours after the operation (PO_24h), and 6 weeks after the operation (PO_6w). Bone marrow samples for bone marrow mononuclear cell (BMMC) isolation were obtained during surgery between 2 and 4 hours after the incision (Fig. 1A). To estimate the extent of tissue injury after major spinal surgery, we calculated the Injury Severity Score (ISS) for each surgical patient. Incisions that extended from the skin to bony structures were assigned an Abbreviated Injury Scale score of 2, whereas bone disruption and open manipulation in the thoracic, lumbar, and pelvic axial skeleton were each assigned an Abbreviated Injury Scale score of 3 (Table 2). For CITEseq, PBMC and BMMC samples from 3 older and 3 young patients were selected by matching for operative stress including operative time, estimated blood loss, and transfusion requirement. In a separate 10× run, PBMCs from two additional patients undergoing elective surgery were subjected to scRNAseq. Upon analysis, batch effects were observed in the cell clustering between the 10× runs. However, the major shifts induced by surgery were preserved. Therefore, only the representative results from 3 young and 3 older patients included in the CITEseq analysis are shown in this report. Plasma samples taken from all enrolled patients at all 4 timepoints were used for plasma proteomics analysis. PO_6w samples from 1 older and 1 young patient were missing due to remote follow-up visits.

## Trauma patient enrollment

The trauma dataset was derived from a previously published dataset.[18] Patients with blunt or penetrating trauma who were admitted to the ICU and experiencing hypotension (systolic blood pressure <90 mmHg) or tachycardia (heart rate >108 bpm) on admission were eligible for enrollment. For the present analysis, a subset of 8 patients with ISS of 17 to 27 was selected and reanalyzed. This strategy aimed to match the degree of tissue injury, age, and sex between trauma and elective surgery groups. The clinical characteristics of trauma patients are presented in **Supplemental Digital Content 1** (http://links.lww.com/JACS/A325). Blood samples for PBMC isolation were obtained at admission, which corresponded to less than 4

**Table 1.** Demographic Characteristics and Hospital Course of Elective Spinal Reconstruction Surgery Patients

| Characteristic | Older patients | Young patients | p Value |
|---|---|---|---|
| Sex, f/m, n | 2/3 | 5/1 | 0.136 |
| Age, y, mean (range) | 72.6 (71–74) | 27.3 (18–39) | 0.007 |
| Preoperative albumin, g/dL, mean (range) | 4.7 (4.5–5.0) | 4.5 (4.0–4.9) | 0.314 |
| Operative time, min, mean (range) | 397.2 (255–473) | 341.6 (193–467) | 0.324 |
| Estimated blood loss, mL, mean (range) | 2,530 (1,500–4,750) | 1862.5 (575–4,000) | 0.428 |
| Packed RBCs, U, mean (range) | 5.4 (8–3) | 3.5 (0–8) | 0.284 |
| Plasma, U, mean (range) | 3.8 (8–1) | 2.6 (0–8) | 0.568 |
| Cryoprecipitate, U, mean (range) | 1 (0–2) | 0.83 (0–2) | 0.843 |
| Platelets, U, mean (range) | 0.8 (0–2) | 0.66 (0–2) | 0.818 |
| LOS, d, mean (range) | 9.02 (4.6–12.5) | 4.96 (4–8.2) | 0.072 |
| ICU LOS, mean (range) | 1.44 (5.1–0) | 0.26 (0.8–0) | 0.287 |
| Intubated time after the operation, h, mean (range) | 4.8 (12–0) | 0 | 0.177 |

The right column shows the p values after the Bonferroni adjustment between older and young patients. Age was the only significant variable between the cohorts.
LOS, length of stay.

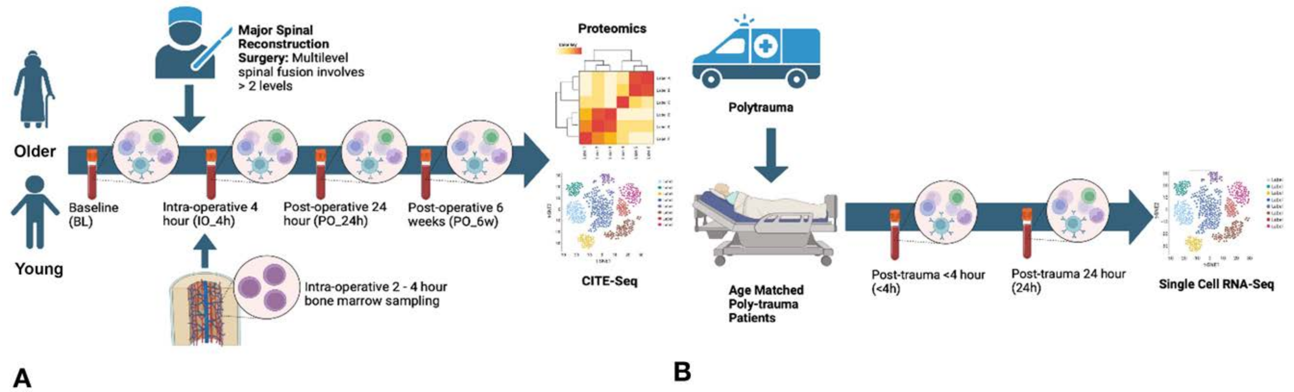


**Figure 1.** Experimental design for the analysis of cells and/or plasma from major elective surgery or polytrauma patients. (A) Older and young patients undergoing multilevel spinal fusion operations involving more than 2 levels were enrolled after informed consent. Blood samples were collected on the day of operation before incision (baseline [BL]), 4 hours into the intraoperative period (IO_4h), 24 hours after the operation (PO_24h), and 6 weeks after the operation (PO_6w). Additionally, at 2 to 4 hours into the intraoperative period, a bone marrow sample was collected from the instrumented vertebral bodies. Plasma samples were subjected to proteomics, peripheral blood mononuclear cells (PBMC) underwent cellular indexing of transcriptomes and epitopes sequencing (CITEseq) analysis, and bone marrow mononuclear cells were subjected to single-cell RNA sequencing (scRNAseq) analysis. (B) PBMC scRNAseq data from subset of polytrauma patients matched to the major elective surgery patients for age, sex, and injury severity was used for comparing the cellular response between trauma and major elective surgery. Data from PBMC isolated at less than 4 hours after the trauma (<4h), and 24 hours after the trauma (24h) were used for the comparative analysis.

hours of injury and at 24 hours after injury (Fig. 1B). A control dataset was established with blood samples from 7 healthy age- and sex-matched uninjured volunteers.

### Single-cell cDNA library preparation and sequencing

For the samples obtained from patients undergoing elective surgery, PBMC and BMMC were isolated by standard Ficoll centrifugation. Single-cell 3′ cDNA libraries were prepared following 10× Genomics protocol (human: v3). Cell surface protein expression data were captured in conjunction with transcriptomic data for PBMCs from the surgery group (CITEseq) following the BioLegend TotalSeq and human universal antibody cocktail protocol. Cases with corresponding controls were processed in parallel within the same batch. Libraries were paired-end sequenced on an Illumina HiSeq platform, with a read length of 150 bp at each end. On average 180 million reads were sequenced for each sample. scRNAseq was previously performed on PBMC isolated from polytrauma patients as described.[18]

### Single-cell sequencing data processing

The raw sequencing data were processed using the 10× Genomics CellRanger pipeline, cellranger count/aggr (v 7.0.1, mapped to GRCh38), and then by Seurat (v 4.3.0) to generate a unique molecular identifier count matrix and perform clustering analysis. Genes expressed in at least 3 cells were retained. Cells with less than 200 genes, more than 5,000 gene counts, or >25% mitochondria genes were filtered out. The number of detected molecules per cell, an unwanted source of variation, was regressed out by the ScaleData() function. Principal component (PC) analysis was performed on variable genes. The threshold for the number of significant PCs was determined via elbow plot. Based on the significant PCs, Uniform Manifold Approximation and Projection (UMAP) and clustering analyses were performed. The resolution for graph-based clustering was tuned back-and-forth until the identified clusters were biologically meaningful. Differentially expressed genes (DEGs) between clusters were identified using DESeq2 R package standard workflow[25] that performs an internal normalization where the geometric mean is calculated for each gene across all samples. This workflow fits negative binomial generalized linear models for each gene and uses the Wald test for significance testing. The p values are corrected for multiple testing using the Benjamini–Hochberg method by default. For specific cell populations of interest, we extracted the unique molecular identifier count submatrix and repeated the steps of secondary analysis to analyze the differences at a higher resolution.

### Gene set score calculation

Gene Ontology Biological Process (GOBP) pathways were used to calculate activation scores—that is the degree of upregulation in inflammatory behavior—for specific cell populations. GOBP-positive regulation of myeloid leukocyte–mediated immunity gene set (GO:0002888) was used to calculate the pro-inflammatory activation score of

**Table 2.** Clinical Characteristics of Elective Spinal Reconstruction Surgery Patients

| Patient identification | Major comorbidity | Diagnosis | Operation | AIS-external | AIS-chest (thoracic spine) | AIS-abdomen (lumbar spine) | AIS-extremities (including pelvis) | ISS | Major complication |
|---|---|---|---|---|---|---|---|---|---|
| Older-1 | HTN, GERD, gastrointestinal bleeding, scoliosis | Proximal junctional kyphosis with prior instrumentation for scoliosis lumbar radiculopathy | T3-pelvis PSF | 2 | 3 | 3 | 3 | 27 | None |
| Older-2 | HTN, HLD, asthma, GERD, scoliosis, osteoporosis | Radiculopathy with prior instrumentation for scoliosis | T2-pelvis PSF | 2 | 3 | 3 | 3 | 27 | Subsegmental pulmonary embolism, arrest |
| Older-3 | HTN, AF, coronary artery disease, transient ischemic attack, hypothyroidism, osteoporosis, scoliosis | Pain syndrome s/p prior instrumentation 3 times for scoliosis | T3-pelvis PSF | 2 | 3 | 3 | 3 | 27 | L3 nerve palsy treated with IV steroids |
| Older-4 | HTN, HLD, GERD, osteopenia, scoliosis | Degenerative scoliosis | T4-pelvis PSF | 2 | 3 | 3 | 3 | 27 | None |
| Older-5 | HTN, HLD, AF | Spinal stenosis, pseudoarthrosis of earlier instrumentation | L1-S1 PSF | 2 | 0 | 3 | 3 | 22 | Coagulopathy |
| Young-1 | asthma, seizure, GERD, Crohn's disease, pulmonary embolism, scoliosis | Pain and radiculopathy s/p earlier instrumentation for scoliosis | L5-S1 PSF | 2 | 0 | 3 | 3 | 22 | Durotomy s/p primary repair, left foot drop |
| Young-2 | None | Scoliosis | T4-L4 PSF | 2 | 3 | 3 | 0 | 22 | None |
| Young-3 | Asthma, migraine | Scoliosis | T3-L3 PSF | 2 | 3 | 3 | 0 | 22 | None |
| Young-4 | Hepatitis C, opioid-use disorder, depression | Scoliosis | T2-L2 PSF | 2 | 3 | 3 | 0 | 22 | None |
| Young-5 | GERD, depression | Scoliosis, pain after earlier instrumentation | T4-S1 PSF | 2 | 3 | 3 | 3 | 27 | None |
| Young-6 | GERD, depression | L5-S1 spondylolisthesis | L3-pelvis PSF | 2 | 0 | 3 | 3 | 22 | None |

Extent of the operation, AIS score for each anatomical location, and total ISS are demonstrated in the table.

AF, atrial fibrillation; AIS, Abbreviated Injury Scale; HLD, hyperlipidemia; HTN, hypertension; ISS, Injury Severity Score; PSF, posterior spinal fusion; s/p, status post.

bone marrow myeloid cells. GOBP natural killer (NK) cell activation gene set (GO:0030101) was used to calculate the pro-inflammatory activation score of NK cells. For each patient, the activation scores were calculated as follows: (1) the $\log_2$-transformed expression values of selected gene sets from the specific cells of interest were extracted; and (2) the values were then further transformed to *z*-scores calculated across all selected genes; the corresponding *z*-score–transformed values were averaged to get the activation score. The results are shown in boxplots.

## Enrichment analysis

For enrichment between 2 gene sets or protein sets, a preranked list was prepared from the output of DEGs or differentially expressed proteins. The Fgsea (v 1.26.0) R package standard workflow[26] was used to obtain p values from permutation testing with correction for multiple hypothesis testing. For validation, the ClusterProfiler (v 4.8.1) R package workflow[27] was used to support the functional characterization of both coding and noncoding transcriptomic data. The hypergeometric p values for enrichment were computed and adjusted with the Benjamini–Hochberg method for multiple testing. An adjusted p value of <0.05 was considered statistically significant. The ggplot2 (v 3.4.2) R package[28] was used for customized data visualization.

### Plasma proteomics selection

The proteomic profiles of the plasma samples were characterized using the SOMAScan-modified oligonucleotide aptamer–based assay, which detected 6,967 proteins and the O-link Bioscience antibody-based platform using 2 antibodies to detect individual targeted proteins, which detected 2,926 proteins. To carry out cross-platform validation, a subset of 1,170 proteins with between-platform Spearman (rho) correlation values greater than 0.3 were classified as "strongly correlated" across platforms and selected for further analysis. Raw intensity values were $\log_2$-transformed and scaled for all downstream analyses.

### Senescence-associated secretory phenotype proteomic selection

Of the 1,170 correlated proteins, we identified those that showed significantly (adjusted $p < 0.05$) higher levels in older patients compared with the young patients at the BL. These proteins were then cross-referenced in the senescence-associated secretory phenotype (SASP) Atlas[29] to validate their association with increasing age. There were 10 proteins correlated across platforms, associated with increasing age, and identified as SASP proteins used for further analysis.

### Proteomic visualization

Standardized values (*z*-scores) of proteins were used to generate heatmaps visualizing relative changes in protein levels over time, based on either timepoints or age groups. For each protein family, the proteins whose values were significantly different between older and young patients (Benjamini–Hochberg adjusted p value of <0.05) at each of the timepoints were identified.

## RESULTS

### Major elective surgery induces a dramatic, but delayed transcriptomic shift in CD14+ monocytes

To interrogate the transcriptomic response within specific circulating cell populations during and after major elective surgery, we performed CITEseq on PBMC isolated from patients undergoing spinal reconstruction surgery (63,090 total cells). Patient demographics for the surgery cohort are displayed in Table 1. No significant differences were observed in operative time, estimated blood loss, or transfusion requirement between the older (n = 5) and young (n = 6) groups. As shown in Table 2, all the older patients experienced major postoperative complications, whereas only 1 young patient experienced a complication (foot drop).

We confirmed cell identities across the PBMC populations using surface protein expression and transcriptomic patterns in single cells from the CITEseq analysis (see feature plots in **Supplemental Digital Content 2A, 2B**, http://links.lww.com/JACS/A326). Transcriptomic data from PBMC across all analyzed patients and timepoints are displayed by UMAP plot and colored by cell type (Fig. 2A) or timepoint (Fig. 2B). Transcriptomic shifts after the initiation of surgery were observed in multiple cell types but were most dramatic in cluster of differentiation 14 (CD14+) monocytes. A minor transcriptomic shift was observed in monocytes at the IO_4h timepoint; however, this shift became much more dramatic by the PO_24h timepoint. The PO_24h pattern was associated with 3,201 significant DEGs compared with BL. By PO_6w, CD14+ monocyte clusters from patients undergoing surgery overlapped with clusters of cells obtained at BL, and there were no DEGs detected between BL and PO_6w (Fig. 2C). The remaining cell types showed differential gene expression only at the PO_24h timepoint, and all had much lower DEG counts than CD14+ monocytes.

### Transcriptomic responses in CD14+ monocytes demonstrate insult and age-dependent differences

To further characterize the changes in the transcriptomic responses over time, CD14+ monocytes were extracted from the CITEseq data and analyzed separately. Consistent with shifts seen in the pseudobulk analysis of PBMC, CD14+ monocytes extracted from patients undergoing major elective surgery began to demonstrate separate clustering at the IO_4h timepoint and formed a completely separate cluster at the PO_24h timepoint (Fig. 3A). To characterize these transcriptomic shifts, we performed gene set enrichment analysis (GSEA) using DEGs at each timepoint relative to BL. The major pathways showing significant (p-adjusted <0.05) differential expression at IO_4h, PO_24h, and PO_6w compared with at BL are demonstrated using dot plots. By the IO_4h timepoint, a subset of genes associated with inflammation and myeloid leukocyte activation were increased in monocytes (Fig. 3B). This pattern markedly expanded to include an additional 449 DEGs known to be involved

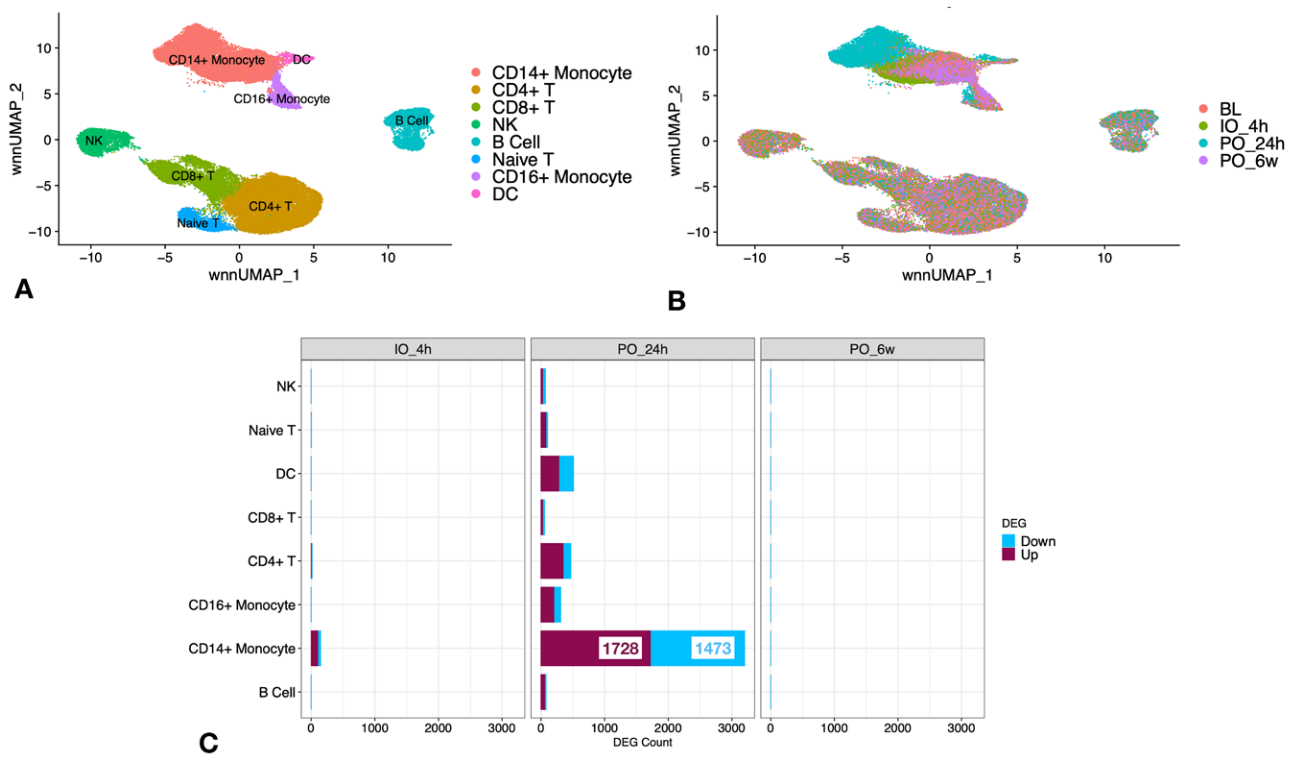


**Figure 2.** Transcriptional changes in peripheral blood mononuclear cells (PBMCs) after major elective surgery. Uniform Manifold Approximation and Projection (UMAP) plots of transcriptional patterns in PBMC visualized by cell type (A) and timepoint (B) for elective spinal reconstructive surgery (n = 6). The number of significant (adjusted $p < 0.05$) differentially expressed genes (DEGs) in each major cell type at each timepoint compared with the BL (C) are displayed by a bar graph. BL, baseline; IO_4h, 4 hours into the intraoperative period; NK, natural killer; PO_24h, 24 hours after the operation; PO_6w, 6 weeks after the operation.

in inflammation and monocyte activation at PO_24h (see **Supplemental Digital Content 3**, http://links.lww.com/JACS/A327, for the list of significantly increased genes). The number of significant gene sets increased from 2 to 12 all centering around macrophage or myeloid leukocyte activation and acute inflammatory responses at PO_24h. There was a notable suppression in the transcription of genes involved in the type-I interferon response and major histocompatibility complex-class 2 (MHC-II)-dependent antigen presentation. By the PO_6w timepoint, there were no differences in transcriptomic patterns assessed by GSEA compared with BL.

To identify age-dependent alterations in the response to major elective surgery, we compared CD14+ monocytes from older patients with young patients across time. UMAP plot showed that CD14+ monocytes form unique clusters correlating with age groups (Fig. 3C). GSEA revealed that CD14+ monocytes of older patients had a significantly greater pro-inflammatory profile and leukocyte activation along with less suppression of transcripts for MHC-II compared with young patients at PO_24h (Fig. 3D). To determine if the surface protein expression of MHC-II molecules on CD14+ monocytes correlated with the transcriptional changes, we assessed the ADT data from the CITEseq analysis. CD14+ monocyte MHC-II (human leukocyte antigen isotypes DR and DQ) surface expression dropped significantly compared with BL in young patients at PO_24h; however, the drop was notably less in cells from older patients and not significantly different from BL (**Supplemental Digital Content 4A, 4B**, http://links.lww.com/JACS/A328). By PO_6w monocytes from older patients showed higher MHC-II levels compared with BL and cells from young patients. Monocytes from older patients exhibited an imbalance with an exaggerated inflammatory response and less suppression of antigen presentation pathways compared with cells from young patients.

We have previously published the scRNAseq landscape for PBMC isolated from polytrauma patients.[18] Although major transcriptomic shifts in CD14+ monocytes were also the most prominent change after trauma, the timing of the maximum shift was much earlier than that seen after major elective surgery. The maximal transcriptomic change and number of DEG compared with healthy controls (HCs) was observed at admission. To directly compare PBMC responses between major elective surgery

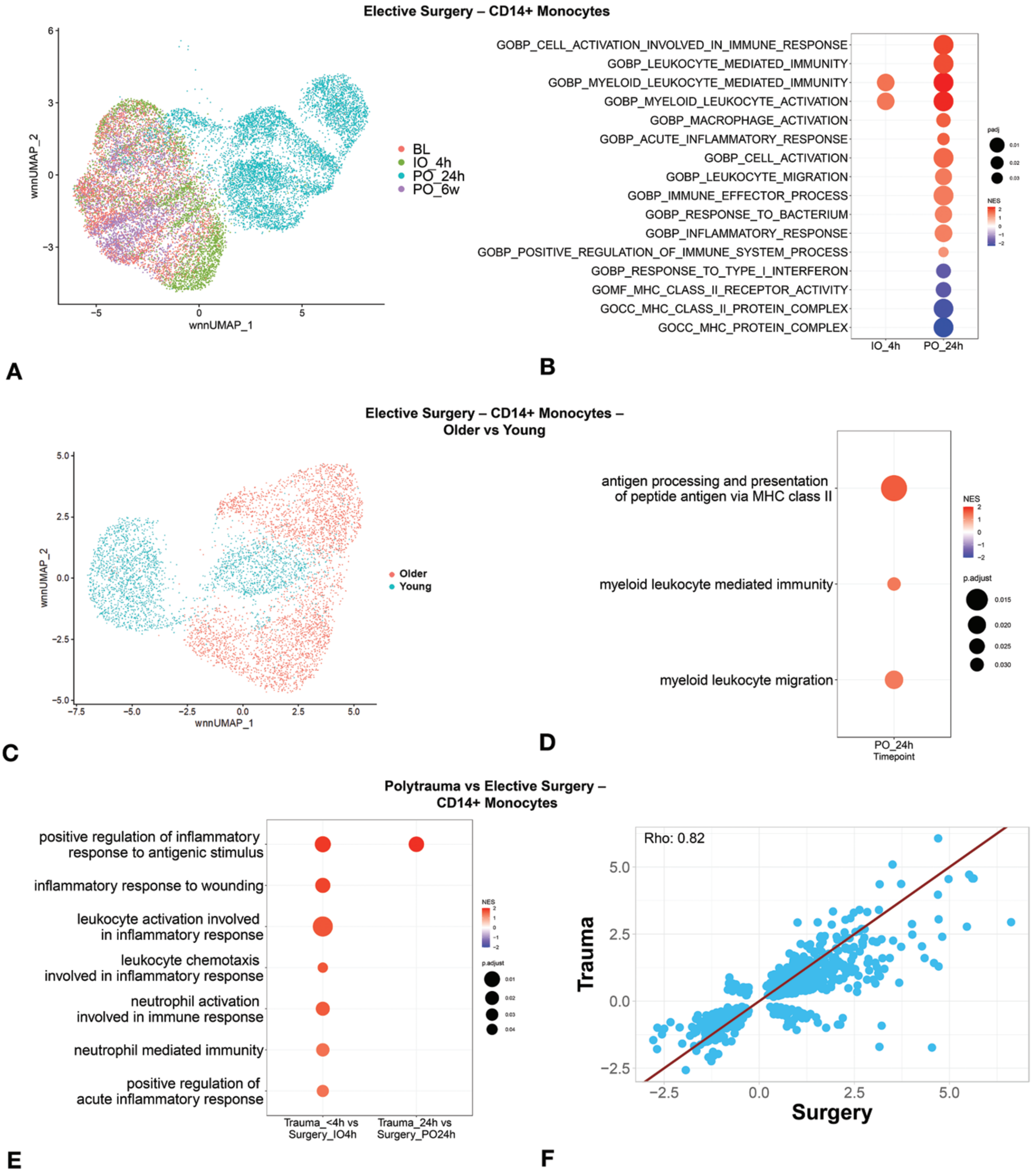


**Figure 3.** Transcriptomic changes in circulating CD14+ monocytes after major elective surgery or polytrauma. (A) Uniform Manifold Approximation and Projection (UMAP) plot of transcriptomic patterns in CD14+ monocytes from elective surgery patients (n = 6) color-coded by timepoint. (B) Dot plot of significant gene ontology (GO) terms derived by gene set enrichment analysis (GSEA) of the transcriptomic changes observed at each timepoint compared with baseline (BL) in CD14+ monocytes from the elective surgery patients. (C) UMAP plot of transcriptomic differences in CD14+ monocytes from young (n = 3) and older (n = 3) elective surgery patients at 24 hours after the operation (PO_24h). (D) Dot plot of significant GO terms derived from the transcriptomic differences observed by GSEA between young and older elective surgery patients at PO_24h. (E) Dot plot of GO terms derived by GSEA of the differentially expressed gene (DEG) in CD1+ monocytes from the polytrauma patients (n = 8) at timepoints <4h and 24h, compared with DEG identified in CD14+ monocytes from the major elective surgery patients at IO_4h and PO_24h timepoints, respectively. (F) Spearman correlation analysis of significant DEG of CD14+ monocytes in polytrauma patients at less than 4 hours vs major elective surgery patients at 24 hours. GOBP, gene ontology biological process; GOCC, gene ontology cellular component; GOMF, gene ontology molecular functions; IO_4h, 4 hours into the intraoperative period; PO_6w, 6 weeks after the operation; MHC, major histocompatibility complex; NES, normalized enrichment score.

and trauma, we reanalyzed a subset of patients from our previously published trauma PBMC dataset.[18] To match the severity of tissue injury between elective surgery patients and polytrauma patients, we estimated ISSs for the spine surgery patients to be between 22 and 27. We then selected 8 polytrauma patients with ISS between 17 and 27 and analyzed data from 10,8461 PBMCs obtained at admission (average time from injury 76 minutes) and at 24 hours. Data from 7 HCs matched for age and sex of the trauma patients were used as controls for the trauma patients' data. The demographics of the trauma patients and HC is shown in **Supplemental Digital Content 1** (http://links.lww.com/JACS/A325).

We compared the DEG in CD14+ monocytes from trauma at less than 4 hours with those identified in monocytes from major elective surgery at IO_4h and showed CD14+ monocyte transcriptomic response of the polytrauma cohort was statistically more pro-inflammatory with increased leukocyte activation, chemotaxis, and acute inflammatory response. By 24 hours monocytes from polytrauma patients were still significantly more pro-inflammatory; however, the number of significant pro-inflammatory gene sets decreased from 7 to 1. We next compared the levels of common significant DEGs in CD14+ monocytes from polytrauma patients at less than 4 hours and major elective surgery patients at PO_24h via Spearman correlation analysis. The significant 1,061 CD14+ monocyte DEGs of these peak timepoints for polytrauma and major elective surgery response showed a correlation coefficient of 0.82 between cohorts. These results show that the CD14+ monocyte activation response, although later in major elective surgery, has considerable overlap with polytrauma.

### NK and T-cell responses are suppressed after major elective surgery

To characterize the NK cell activation state after spinal reconstructive surgery, we separately analyzed NK cells by UMAP (**Supplemental Digital Content 4E**, http://links.lww.com/JACS/A328), GSEA, and activation score calculation by using genes specific to NK cell activation (GO:0030101). NK cell activation scores were decreased at PO_24h across both age groups, with greater suppression relative to BL in the older group (Fig. 4A). The top genes that affect the NK cell activation profile after major spinal surgery are shown in Fig. 4B. GSEA revealed a significant suppression in the inflammatory, proliferative, and cytotoxic pathways at PO_24h compared with BL, consistent with the decrease in activation pathway scores (Fig. 4C). This suppressed profile was resolved by the PO_6w timepoint. We then compared NK cells between age groups of patients undergoing spinal reconstructive surgery and demonstrated a significantly greater suppression of NK activation state in older patients compared with the young at the PO_24h timepoint with decreased cytotoxicity and NK cell immunity gene set scores (Fig. 4D). CD8+ T and B cells also showed significantly lower pro-inflammatory pathway enrichment at PO_24h in response to major elective surgery, indicating transient postoperative suppression of these cell types (**Supplemental Digital Content 5B, 6B**, http://links.lww.com/JACS/A328). CD8+ T cells from older patients demonstrated an even greater suppression of the key proinflammatory pathways in comparison to young patients (**Supplemental Digital Content 5D**, http://links.lww.com/JACS/A328), whereas B cells from older patients demonstrated less suppression than those from young patients (**Supplemental Digital Content 6D**, http://links.lww.com/JACS/A328). Naïve T cells exhibited a significantly activated adaptive immune response and T-cell–mediated immunity transcriptomics at the PO_24h timepoint (**Supplemental Digital Content 7B**, http://links.lww.com/JACS/A328). Additionally, this pro-inflammatory response was notably more robust in older patients at the PO_24h timepoint (**Supplemental Digital Content 7D**, http://links.lww.com/JACS/A328). CD4+ T cells showed no significant time- or age-dependent differential gene expression after elective surgery (**Supplemental Digital Content 8A, 8B**, http://links.lww.com/JACS/A328).

The suppression of NK cell transcriptomic responses after elective surgery was in marked contrast to the early activation of NK cells observed after trauma. NK cells formed a cluster distinct from HC at less than 4 hours after trauma. This separation was largely resolved by 24 hours (**Supplemental Digital Content 4E**, http://links.lww.com/JACS/A328). The GSEA demonstrated a dramatic enrichment of transcriptomic patterns representing immune cell activation, an immune defense response, cytokine production, and enhanced cytotoxicity early after trauma (Fig. 4E). The opposing NK cell responses observed between major elective surgery and polytrauma were confirmed with the Spearman correlation analysis of the DEGs. Using the 1,182 DEGs that were significant in major elective surgery (PO_24h timepoint) or trauma (<4 hour timepoint; **Supplemental Digital Content 6E**, http://links.lww.com/JACS/A328), our analysis did not reveal any substantial correlation, as indicated by a rho correlation coefficient of only 0.14 (Fig. 4F).Unlike the common gene expression patterns observed in CD14+ monocytes between major elective surgery and polytrauma, NK cell responses markedly diverged between the 2 insults.

### Early responses of bone marrow myeloid progenitor cells during elective spinal reconstructive surgery are higher in older patients

We next analyzed the single-cell transcriptomes of BMMC (33,711 total cells) sampled intraoperatively between 2 and 4 hours from the time of incision. Myeloid progenitor cells were identified as those

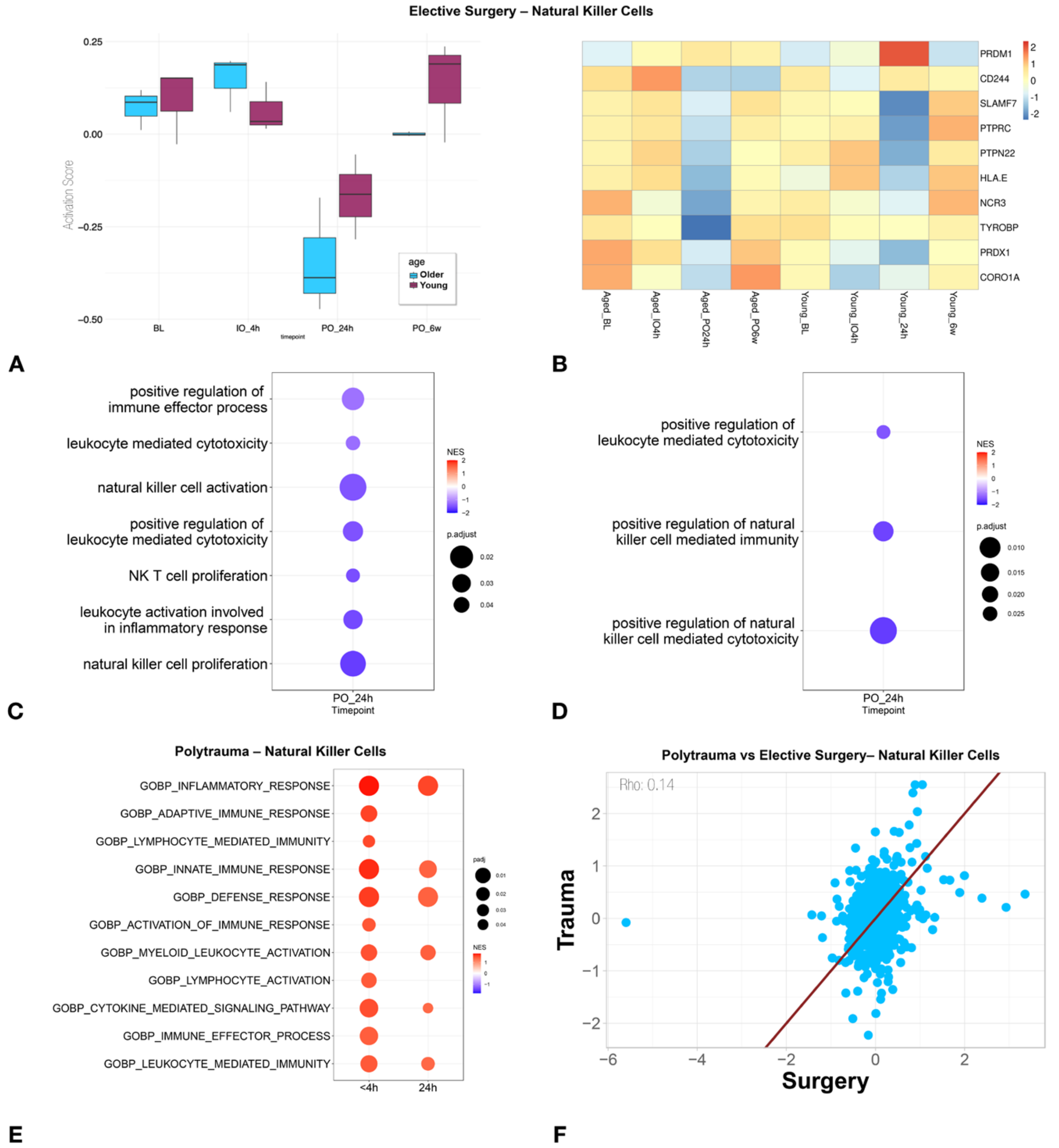


**Figure 4.** Transcriptomic changes in circulating natural killer (NK) cells after major elective surgery and polytrauma. (A) NK activation scores displayed by box plot for young and older patients undergoing elective spine reconstruction surgery using the Gene Ontology Biological Process (GOBP) NK cell activation gene set (GO:0030101). (B) Normalized *z*-scores of top genes in the GOBP NK cell activation gene set (GO:0030101) displayed by heatmap for young and older patients. (C) Dot plot showing gene set enrichment analysis (GSEA) of significant differentially expressed genes (DEGs) from NK cells from elective spine reconstruction patients comparing 24 hours after the operation (PO_24 h) timepoint to baseline (BL; n = 6 patients). (D) Dot plot showing GSEA of significant DEG comparing NK cells from older (n = 3) vs young patients (n = 3). (E) Dot plot of significant gene ontology (GO) terms derived by GSEA of the transcriptomic changes observed in NK cells from the polytrauma patients compared with healthy controls (HC) at less than 4 hours (<4h) and 24 hours (24h). (F) Spearman correlation analysis of DEG for NK cells from major elective surgery patients at 24 hours compared with NK cells from polytrauma patients at 24 hours. GOBP, gene ontology biological process; GOMF, gene ontology molecular functions; NES, normalized enrichment score.

expressing elastase, neutrophil expressed and myeloperoxidase (**Supplemental Digital Content 9D**, http://links.lww.com/JACS/A328). Coexpression of CD34 was used to identify myeloid-committed hematopoietic stem cells (HSC). Although these progenitor and stem cells did not cluster separately based on age (Figs. 5A, 5B), GSEA revealed significantly greater enrichment in inflammation, cytokine responses, and innate immune response pathways in older patients compared with young patients (Figs. 5C, 5D). One of the pathways that was significantly enriched in HSCs in older patients was a receptor for advanced glycation end-product binding. This pathway propagates cellular dysfunction in several inflammatory disorders and has been associated with inflammaging.[30] To measure the inflammatory tone of HSCs, myeloid progenitors, and CD14+ monocytes in bone marrow, we calculated the inflammatory profile score for each cell type using the positive regulation of the myeloid leukocyte mediated immunity gene set (GO:0002888). All three bone marrow myeloid cell types had a higher inflammatory profile with increased transcription of inflammatory genes in the older patients compared with the young (Fig. 5E). These data support the notion that the pathways observed to be activated during major surgery originate at least partially in the bone marrow.

### Plasma-based proteomics demonstrate age- and time-dependent alterations in response to major elective surgery

To further characterize the systemic response to major elective surgery we carried out O-link and SomaLogic plasma proteomic analysis in the patients undergoing major elective surgery ($n = 11$). The SomaLogic platform detected 6,967 human proteins and the O-link platform 2,926 proteins, with an overlap of 2,042 proteins between the 2 platforms (Fig. 6A). To select proteins for further analysis, cross-validation was performed by calculating the Spearman correlation coefficients of the 2,042 common proteins. Proteins with a correlation coefficient greater than 0.3 ($n = 1,170$) between the 2 platforms were used for analyzing the responses to major elective surgery (Fig. 6B). Significant alterations were observed in the plasma as early as the IO_4h timepoint (Fig. 6C). However, the most dramatic differences in the proteomic profile occurred at the PO_24h timepoint. At PO_24h, 276 proteins were significantly lower and 139 proteins were higher ($p < 0.05$) compared with BL levels. Significant differences in the plasma proteomics remained evident at PO_6w with 86 proteins differentially expressed from BL. We analyzed the proteomic profile of each timepoint compared with BL for both platforms and examined enriched protein pathways. The major changes at the PO_24h timepoint included, suppression of biosynthetic, metabolic, and cell cycle functions along with increased expression of inflammatory defense response and leukocyte activation pathways (Fig. 6D). By 6 weeks, there was significant evidence for ongoing inflammation as well as a conversion to an adaptive immune phenotype with activation of adaptive immune pathways, immunoglobulin-mediated immune response, lymphocyte-mediated inflammation, and the complement pathways. Of note, the statistical significance of the differentially expressed protein pathways was consistent across the O-link and SomaLogic platforms, providing a measure of validation for the observed differences between timepoints (Fig. 6D).

To evaluate age-dependent alterations in the proteomic response to major surgery, we analyzed the differences in plasma proteins at each timepoint in older patients compared with young patients (Fig. 6E). Pathway enrichment analysis revealed that at BL, circulating plasma proteins were significantly more pro-inflammatory in older patients, with increased responsiveness to tumor necrosis factor (TNF) even in the absence of tissue injury. Further enhanced pro-inflammatory responses to tissue injury in older compared with young patients were observed as early as the IO_ 4h timepoint. This age-related divergence in the response increased in magnitude by PO_24h, with 126 proteins significantly ($p < 0.05$) higher and 133 lower in the older patients. These proteins corresponded to a higher number of significantly enriched pro-inflammatory pathways and higher normalized enrichment scores in older patients, perhaps reflecting the resolution of inflammation in the young patients and persistent inflammation in the older patients. By PO_6w, older patients demonstrated a higher level of inflammation with significantly less metabolic and biosynthetic activity and less cell death and apoptosis, possibly contributing to ineffective cell clearance and delayed normalization of functional capacity in older patients (Fig. 6F).

### Differences in the responses between older and young patients can be seen in inflammatory mediators and senescence-associated secretory phenotype proteins

Next, we examined changes within specific protein families of interest. Among the 1,170 proteins that demonstrated a strong correlation between the O-link and SomaLogic platforms, we identified 10 SASP proteins that were significantly higher in the circulation of older patients compared with young patients at BL (Figs. 7A, 7B). The pattern of SASP proteins in circulation of older patients is

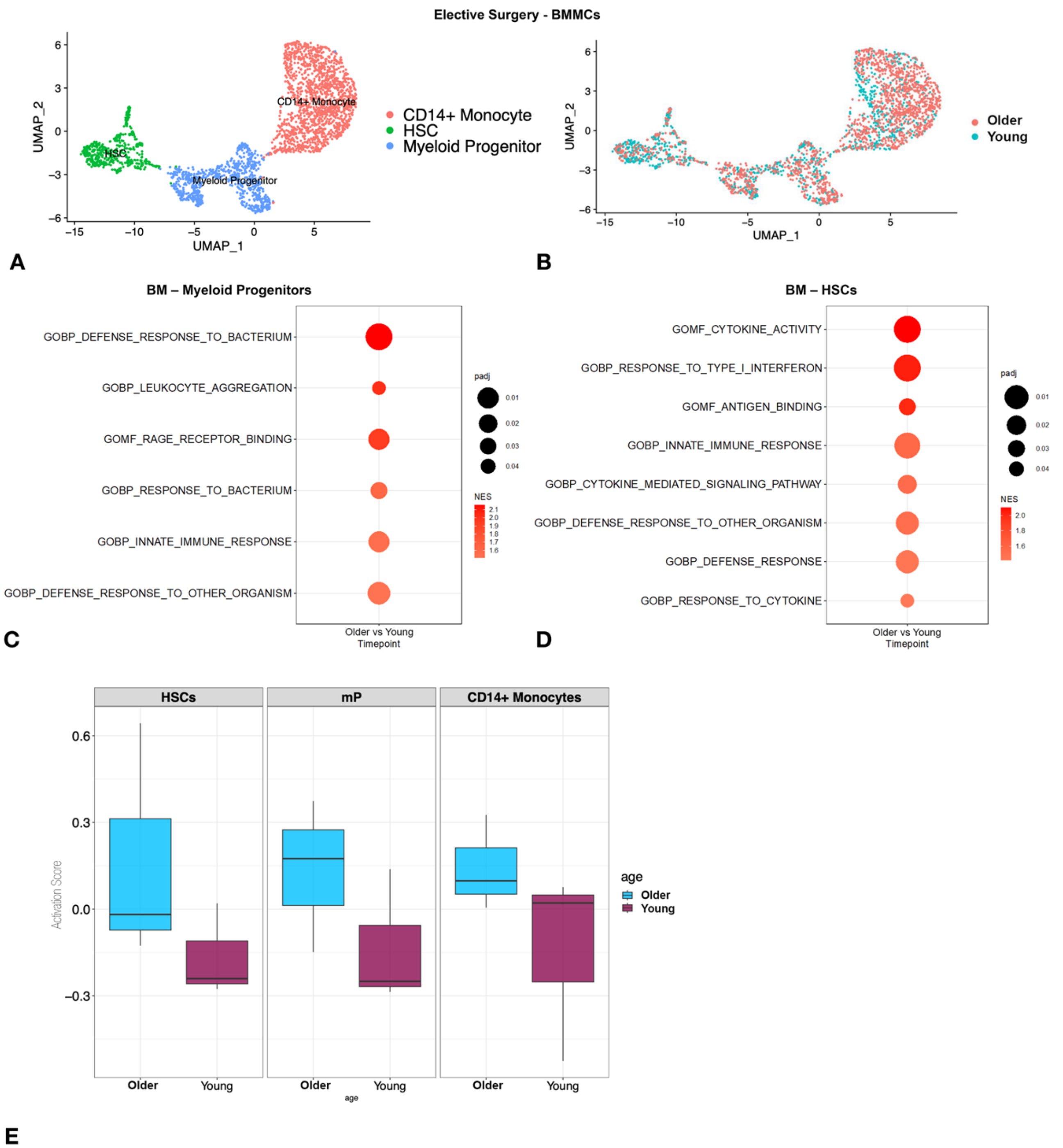


**Figure 5.** Transcriptomic changes in bone marrow (BM) cells after major elective surgery. Uniform Manifold Approximation and Projection (UMAP) plots of the bone marrow myeloid cells (BMMCs) color-coded by cell type (A) and age group (B). Dot plots show the gene ontology (GO) terms for the most significant transcripts that were higher in myeloid progenitor cells (C) and CD34+ hematopoietic stem cells (HSCs) (D) in older patients compared with young patients. Immune activation scores for myeloid committed HSC, myeloid progenitor cells (mP), and CD14+ monocytes of BM cells from older and young patients (E). GOBP, Gene Ontology Biological Process.

summarized in Fig. 7E with red arrows indicating the proteins that were significantly higher in older patients compared with young patients (significance validated by both O-link and SomaLogic platforms). The levels of all 10 SASP proteins were higher in older patients at BL. A subset of these proteins remained higher in older patients during

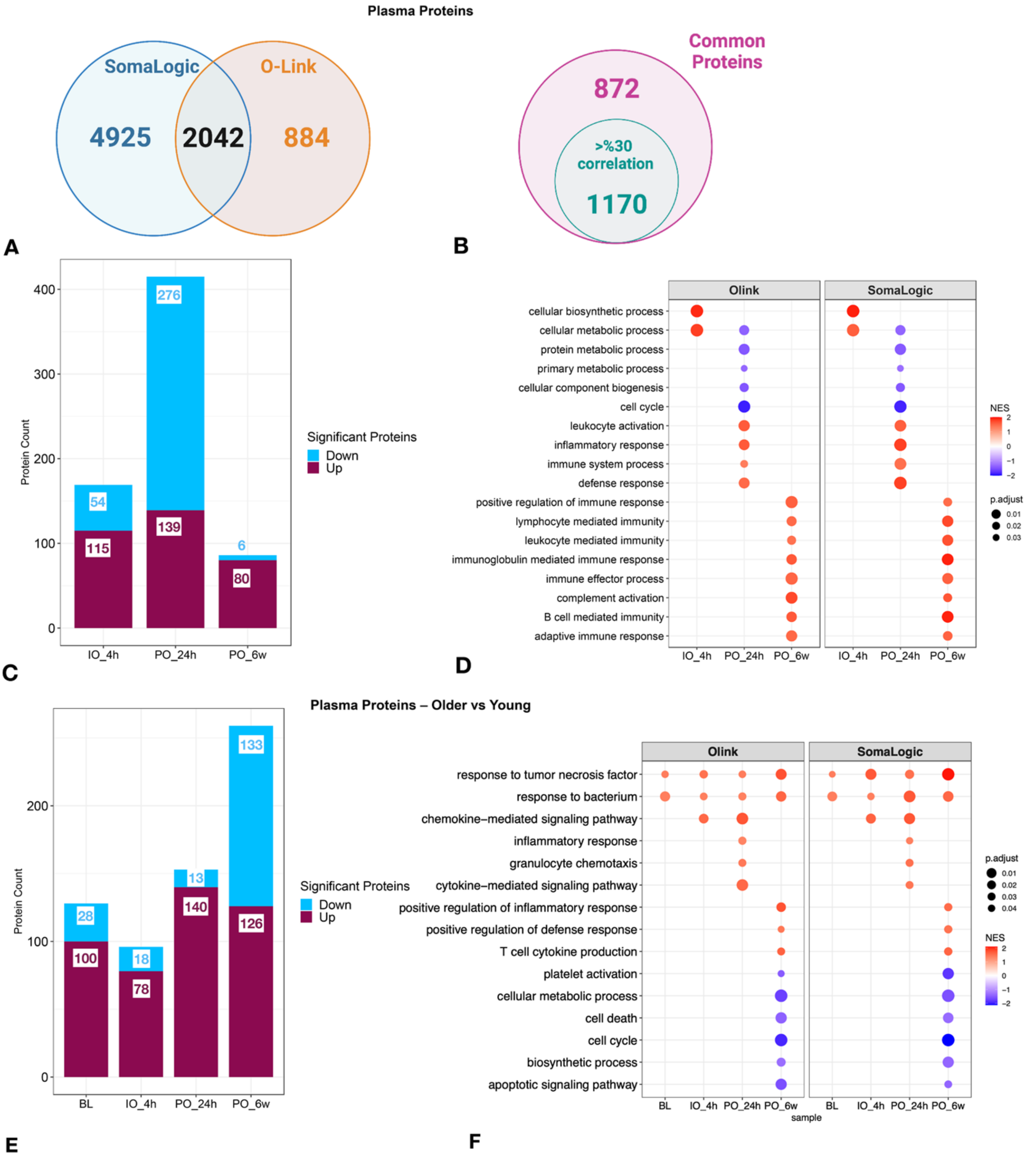
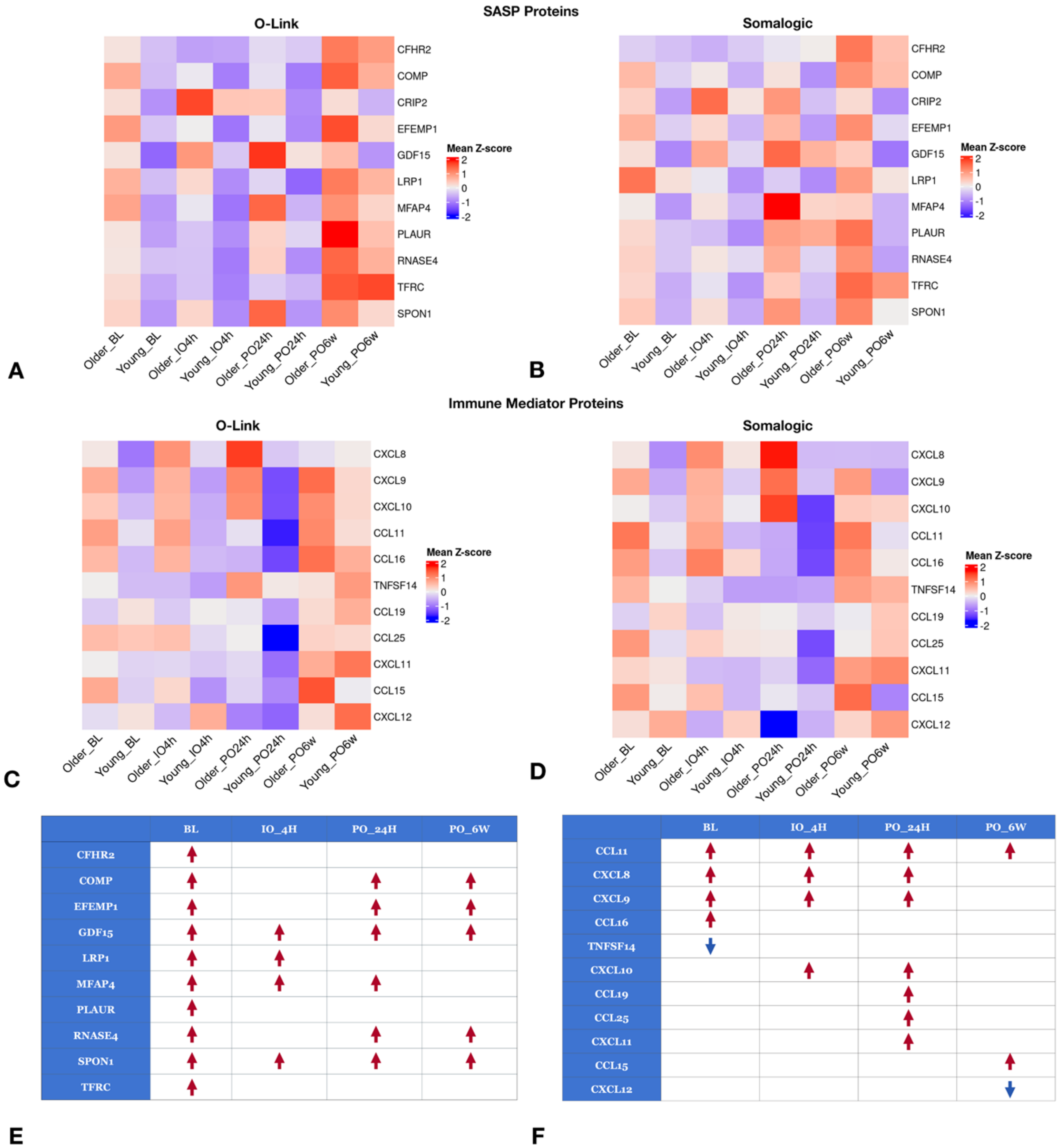


**Figure 6.** Plasma proteomic analysis of the age- and time-dependent responses to major elective surgery. (A) Venn diagram showing the total proteins detected in the plasma by the SomaLogic platform (n = 6,967) and O-link platform (n = 2,926) and the number of proteins detected by both platforms (n = 2,042). (B) Depiction of the 1,170 proteins that had a correlation of >30% between the 2 platforms. These 1,170 proteins were selected for downstream analysis. (C) Number of proteins significantly different during and after operation compared with the baseline (BL) (p-adjusted < 0.05). (D) Enrichment analysis by platform of the proteins significantly different from BL. (E) Number of proteins significantly different between young (n = 6) and older (n = 5) patients over time (p-adjusted < 0.05). (F) Enrichment analysis by platform of the proteins significantly different in the older patients compared with young patients before, during, and after major elective surgery. IO_4h, 4 hours into the intraoperative period; PO_24h, 24 hours after the operation; PO_6w, 6 weeks after the operation.



| | BL | IO_4H | PO_24H | PO_6W |
|---|---|---|---|---|
| CFHR2 | ↑ | | | |
| COMP | ↑ | | ↑ | ↑ |
| EFEMP1 | ↑ | | ↑ | ↑ |
| GDF15 | ↑ | ↑ | ↑ | ↑ |
| LRP1 | ↑ | ↑ | | |
| MFAP4 | ↑ | ↑ | ↑ | |
| PLAUR | ↑ | | | |
| RNASE4 | ↑ | | ↑ | ↑ |
| SPON1 | ↑ | ↑ | ↑ | ↑ |
| TFRC | ↑ | | | |

| | BL | IO_4H | PO_24H | PO_6W |
|---|---|---|---|---|
| CCL11 | ↑ | ↑ | ↑ | ↑ |
| CXCL8 | ↑ | ↑ | ↑ | |
| CXCL9 | ↑ | ↑ | ↑ | |
| CCL16 | ↑ | | | |
| TNFSF14 | ↓ | | | |
| CXCL10 | | ↑ | ↑ | |
| CCL19 | | | ↑ | |
| CCL25 | | | ↑ | |
| CXCL11 | | | ↑ | |
| CCL15 | | | | ↑ |
| CXCL12 | | | | ↓ |

**Figure 7.** Specific protein families showed significantly different expression between age groups. The heatmaps illustrate the normalized *z*-scores of senescence-associated secretory phenotype (SASP) proteins, which were detected with a high correlation in both O-link (A) and SomaLogic (B) platforms and 11 inflammatory mediators identified as significantly different during or after operation in both O-link (C) and SomaLogic (D) platforms. SASP proteins (E) and inflammatory mediators (F) were significantly different in both platforms in older patients (n = 5) compared with young patients (n = 6). Red arrow indicates significantly higher and blue arrow indicates significantly lower proteins in older patients compared with young patients at the indicated timepoint. CFHR2, complement factor H–related 2; COMP, cartilage oligomeric matrix protein; CRIP2, cysteine rich protein 2; EFEMP1, EGF-containing fibulin extracellular matrix protein 1; GDF15, growth differentiation factor 15; LRP1, LDL receptor-related protein 1; MFAP4, microfibril-associated protein 4; PLAUR, plasminogen activator, urokinase receptor; RNASE4, ribonuclease A family member 4; TFRC, transferrin receptor; SPON1, Spondin 1; CXCL, C-X-C motif chemokine ligand; CCL, C-C motif chemokine ligand; TNFSF, TNF superfamily member.

and after surgery. It is noteworthy that young patients had an increase in a subset of SASP compared with BL by the PO_6w timepoint (Figs. 7A, 7B). These findings suggest that older patients have less ability to clear senescent cells at BL and this pattern remains postoperatively.

We also investigated the levels of the soluble immune mediator proteins within the dataset. We detected 27 soluble immune mediators among the 1,170 proteins well correlated across the 2 platforms. Most are chemokines and 11 of these demonstrated significant differences at 1 or more timepoints in older patients compared with young patients (Figs. 7C, 7D) and at each timepoint compared with BL (**Supplemental Digital Content 10B**, http://links.lww.com/JACS/A328). The pattern of immune mediator differences between the older and young patients is summarized in Fig. 7F. Multiple chemokines were elevated at BL in the older patients with C-X-C motif chemokine ligand 8 (CXCL8) the most significantly different. All showed an additional significant upregulation at PO_24h timepoint in older patients compared with young patients. The immune mediators showed generally high correlation with SASP levels with correlation coefficients greater than 0.5 (**Supplemental Digital Content 11A**, http://links.lww.com/JACS/A328), indicating the senescence profile is positively associated with the inflammatory profile. These observations confirm that older patients have a higher inflammatory activation at BL and after surgery.

## DISCUSSION

By characterizing the immune response to spinal reconstructive surgery, this study provides insights into the impact of age on this response and, importantly, how the response to major elective surgery differs from that of polytrauma. The differences between elective surgery and polytrauma were observed not only in the timing of the onset of the immune-inflammatory response but also in the cell-specific activation patterns. Major elective surgery is not a suitable substitute to study the early responses to trauma. We also conclude that spine reconstructive surgery is an excellent platform for the study of age-related differences in the host response to major surgery. With ready access to blood over time (including pre-op and long-term post-op) as well as bone marrow, it was straightforward to identify major differences in how older patients differ at BL and through PO_6w. Our results provide insights into biologic processes, including inflammation and cell senescence, that could be targeted to reduce complications after spinal reconstructive surgery. We suggest that elective spine reconstructive surgery is an excellent model for further study of factors that influence patient outcomes after major elective surgery.

Although early studies suggested that monocyte function may be suppressed after surgery and anesthesia,[31,32] more recent studies indicate an upregulation in the pro-inflammatory functions of monocytes after major surgery.[33-35] Here, we confirm that CD14+ monocytes show the greatest changes after elective major surgery, and although delayed compared with trauma, exhibit patterns (upregulation of inflammation pathways and suppression of interferon signaling and MHC-II expression) in common polytrauma patients. MHC-II molecules play a crucial role in the induction of both adaptive immune responses through antigen presentation and the enhancement of innate immune responses via toll-like receptors.[36] It has been proposed that the suppression of adaptive immune responses, particularly antigen presentation,[37] serves as a protective mechanism to prevent overactivation of deleterious immune responses after tissue injury. Our study findings suggest that this protective mechanism might be compromised in the older population due to a failure to adequately suppress MHC-II expression. Notably, young patients undergoing elective surgery in our study exhibited a significant downregulation of MHC-II expression in addition to less CD14+ monocyte activation compared with cells from the older population. Moreover, we observed a higher level of suppression in B cells from young patients, which rely on MHC-II for their activation, differentiation, and proliferation.[38]

The differences in the immune response between major elective surgery and trauma were especially evident in the diverging NK cell activation patterns: significant enhancement of cytotoxicity and cytokine responses early after trauma and suppression of NK cell activation after elective surgery. In addition to NK cells, CD8+ T cells showed suppression after major elective surgery that was more robust in older patients. Anesthesia has suppressive effects on NK cell and T lymphocyte functions, including cytokine release and chemotaxis,[39] and this could be one of the explanations for the pattern observed after spinal reconstruction surgery.

Our observation that the inflammatory response was higher in myeloid bone marrow progenitors and HSCs in the elderly raises the possibility that the difference in the magnitude and nature of the inflammatory response in the older group is encoded in bone marrow cells. Others have shown in mice models that a pro-inflammatory environment induced by TNF-α exposure impairs HSC function, contributes to myeloid-biased differentiation of HSCs, and induces pro-inflammatory gene expression in HSCs.[40,41] The enhanced pro-inflammatory patterns in HSCs and myeloid progenitors that we observed in older patients were associated with increased TNF-α signaling. Further research will be required to define the mechanistic bases for the heightened myeloid inflammatory responses in older patients undergoing surgery; however, age-associated epigenomics changes are a possibility.[42]

Limitations of our study are the small number of subjects and the absence of an analysis for sex-dependent differences. The small sample size poses limitations in correlating changes in transcriptomics or proteomics with patient outcomes. For proteomics, we addressed this by focusing on a single operation performed by a single surgeon (DOO). We also applied 2 validated proteomic platforms that use distinct protein detection methods (SomaLogic: aptamers, and O-link: antibodies) and included only the 1,170 proteins that were well correlated between the 2 platforms in the analysis. By focusing on patterns and biologic pathways (and not specific proteins), we were able to confirm that older patients have a higher inflammatory tone and evidence for higher ongoing cell senescence at BL. The inflammatory responses detected in the proteomic patterns aligned well with the immune cell transcriptomic patterns in the first 24 hours after major surgery. However, changes in the proteomic patterns at 6 weeks continued to evolve, suggesting the ongoing tissue repair and remodeling may be compartmentalized over time.

Cellular senescence is a terminal arrest of proliferation triggered by various cellular stresses. Senescence cells are unable to undergo apoptosis[43] and are known to secrete SASP proteins, including pro-inflammatory cytokines, chemokines, growth factors, and proteases that recruit macrophages, NK cells, neutrophils, and T lymphocytes. This way, the SASP increases immune surveillance of damaged cells, maintaining tissue homeostasis. The short-term presence of senescent cells is beneficial largely due to their roles during development and tissue repair, regeneration and reprogramming, and interaction with neighbor cells.[44-46] These actions may explain the presence of higher SASP in young patients at 6 weeks. However, prolonged presence of senescent cells can be deleterious, leading to chronic exposure to relatively high SASP levels that can induce chronic inflammation after tissue injury and delay recovery during the subacute phase.[47] Importantly, eliminating senescent cells in transgenic mouse models delays age-related tissue dysfunction and increases health span.[48] Prolonged exposure to SASP and increased senescence cell burden are associated with dysfunctional immune cells that were unable to clear senescence cells.[49] Our findings of higher SASP in older patients at BL and over time suggest that the presence of more senescent cells is one explanation for the sustained inflammatory response of older patients after elective spinal reconstruction surgery. It will be interesting to follow patterns in patients over longer time periods and correlate differences based on functional outcomes.

## CONCLUSIONS

Our study reveals time- and age-dependent shifts in the circulating transcriptomic and proteomic patterns to major elective surgery. The PBMC transcriptomic patterns after elective surgery vary significantly from that of polytrauma response. We posit that the differences are enough to conclude that major elective surgery should not be viewed as a surrogate for the response to trauma. Older patients exhibit an imbalance between the pro- and counter- inflammatory response after elective spinal reconstructive surgery, and this could contribute to the high complication rates in these patients. Elective spine reconstructive surgery is a useful model to understand the impact of patient-specific factors on the human response to major spine surgery.

### Author Contributions

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Funding acquisition: Bonaroti, Reitz, Kar, Billiar
Investigation: Bonaroti, Ozel, Billiar
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Software: Bonaroti, Ozel
Visualization: Bonaroti, Ozel
Writing – original draft: Bonaroti, Ozel, Billiar
Writing – review & editing: Bonaroti, Ozel, Chen, Reitz, Moheimani, Darby, Sun, Das, Kar, Zuckerbraun, Okonkwo, Billiar
Validation: Chen, Das
Conceptualization: Zuckerbraun, Okonkwo, Billiar
Project administration: Zuckerbraun, Okonkwo, Billiar
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Supervision: Okonkwo, Billiar

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