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Investigating immunological, transcriptomic and metabolomic signatures and their association with pneumonia after upper gastrointestinal cancer surgery

Short Title: Biomarkers of post-operative pneumonia

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Abstract

Upper gastrointestinal (UGI) cancer is an increasing global health problem. Surgical resection is the primary curative intervention yet is associated with high-risk of post-operative pneumonia. This study aims to characterise how UGI cancer impacts biological ageing, and evaluate their association with post-operative pneumonia. A single-centre observational pilot study recruiting UGI cancer patients prior to elective oesophagectomy surgery and twenty age- and sex-matched healthy controls. Reduced frequencies of naïve CD8 T cells and expansion of CD28⁻ CD57⁺ CD8 senescent T cells were observed pre-operatively and persisted post-operatively in UGI patients, compared to healthy controls. Increased inflammatory cytokines and transcriptomic and metabolomic signatures associated with reduced autophagy, metabolic programming and microbiome dysbiosis were also observed. Post-operative pneumonia developed in 65% (n=13) of the UGI cohort. Despite being chronologically younger, patients with pneumonia had significantly raised pre-operative inflammatory profile, identified by inflammatory cytokines, transcriptomic and metabolomic signatures. This exploratory pilot study identifies evidence of enhanced immune ageing in UGI surgical patients at pre-operative baseline, which persists post-operatively. Pre-operative inflammatory profiles potentially contribute to the susceptibility of post-operative pneumonia. While validation in larger cohorts is necessary, these findings are hypothesis-generating and provide early insight into potential immunological targets to mitigate post-operative risk.

Key words: Surgical complications, biomarkers, metabolomics, immunity, oesophagectomy, ageing

Background

Cancer is a major global public health problem, responsible for almost one in six deaths worldwide and associated with substantial societal and economic burden across all ages, genders and geographic regions¹. The incidence and burden of upper gastrointestinal (UGI) cancers are rising rapidly, with oesophageal cancer exhibiting a global prevalence rate of 9.1 per 100,000 and the third highest mortality-to-incidence ratio among all cancer types². It is projected that by 2040 there will be a 63.5% increase in new cases and an 86% increase in deaths attributable to oesophageal cancer globally³.

Oesophageal cancer is an aggressive malignancy that is frequently undetected until advanced stages⁴. Surgical resection via oesophagectomy remains the primary curative treatment; however, post-operative complications pose significant clinical challenges, adversely affecting both short-term recovery and long-term outcomes^{4,5}. Among post-operative pulmonary complications, pneumonia is particularly consequential, with rates as high as 70% in high-risk procedures such as those required for UGI cancer⁶. Post-operative pneumonia is associated with poor prognosis, including a three- to five-fold increase in in-hospital mortality rates and lower five-year survival^{7,8}. Furthermore, post-operative pneumonia increases the duration of mechanical ventilation, in addition to length of admission to the intensive care unit (ICU) and hospital, subsequently increasing healthcare costs⁷. This highlights a pressing need to better characterise early predictors and the biological mechanisms underlying susceptibility to post-operative pneumonia after UGI surgery.

T cells play a central role in shaping the tumour immune microenvironment in gastrointestinal malignancies; with cytotoxic CD8⁺ T cells acting as the primary anti-tumour effectors and CD4⁺ helper subsets orchestrating and sustaining these responses through regulatory,

1 pro-inflammatory, and immunosuppressive functions⁹⁻¹². Beyond the tumour site, systemic T
2 cell dysregulation is well documented in cancer patients, with an emerging concept of
3 accelerated immune senescence. Although immune senescence is typically associated with
4 chronological ageing, increasing evidence shows that inflammation and chronic illness can
5 precipitate premature immune ageing¹³⁻¹⁷. The key molecular processes of immune ageing,
6 termed as the 'Hallmarks of Ageing', are closely intertwined with the fundamental biology of
7 cancers, driving inflammatory dysregulation¹⁸⁻²⁰.

8
9 Immune ageing is characterised by the loss of naïve T cells, accumulation of terminally
10 differentiated memory T cells with reduced proliferative capacity, and increased senescent-like
11 T cells. Senescent T cells display a loss of co-stimulatory molecule CD28 and gain of CD57,
12 exhibiting a senescence-associated secretory phenotype (SASP), marked by upregulation of
13 pro-inflammatory cytokines such as interleukin (IL)-6, IL-1 β , tumour necrosis factor (TNF)- α ,
14 and interferon (IFN)- γ . This phenotype is driven by activation of NF- κ B, p38 mitogen-
15 activated protein kinase (MAPK), and mechanistic target of rapamycin (mTOR) signalling
16 pathways, and is further associated with increased DNA damage response (DDR) signalling
17 and mitochondrial dysfunction^{21,22}. However, whether UGI cancer patients already exhibit
18 features of premature immune ageing, and whether this is associated with outcomes after
19 surgical intervention, remains poorly understood²³. Clarifying these dynamics is important, as
20 post-operative immune vulnerability has been linked to increased risk of post-operative
21 pneumonia and adverse outcomes in other surgical cohorts, and immunesenescence may
22 represent an under-recognised biological contributor²⁴⁻²⁷.

23
24 By assessing features of immune ageing through immune phenotyping and contextualising
25 these findings alongside established transcriptomic and metabolomic signatures of the

Hallmarks of Ageing, this study aims to; 1) characterise how UGI cancer influences biological ageing; 2) determine whether UGI surgery induces measurable acceleration of ageing-associated immune signatures, and; 3) evaluate their association with post-operative pneumonia. Through a multidimensional interrogation of the ageing–cancer axis, this study seeks to inform risk stratification and support the development of future personalised interventions to reduce post-operative infectious complications in patients with UGI cancer.

Results

Participant Demographics & Clinical Characteristics

Thirty patients undergoing UGI surgery consented to participate, with 20 patients completing follow-up at hospital discharge and included in the final analysis [Supplementary Figure 2]. The final cohort (n=20) had a mean age of 63.60 ± 11.70 years (range 42 – 78 years; 15 males) with a mean APACHE score of 11.0 ± 5.83 and mean SOFA score of 4.50 ± 4.14 [Supplementary Table 1]. The mean hospital length of stay was 23.50 ± 17.89 days, with an observed positive association between APACHE scores and length of stay ($R^2=0.51$, $p=0.02$) [Supplementary Figure 3]. Twenty healthy age- and sex-matched controls (mean age 63.60 ± 11.70 years [range 44 – 81 years], 15 males) were also recruited to the study.

The impact of surgical stress on features of T cell ageing in UGI surgical patients

On assessing subset distribution within the T cell pool, we observed a downregulation in absolute numbers of circulating CD8 ($p=0.02$) and CD4 ($p=0.008$) T cells in UGI surgical patients at day 2-5 post-operatively compared to healthy controls [Supplementary Table 2 and 3]. Within the CD8 T cell pool, we observed a significantly lower frequency ($p=0.005$) and absolute number ($p=0.02$) of circulating naïve T cells in UGI surgical patients at pre-operative baseline in comparison to healthy controls [Figure 1A; Supplementary Table 2], which was not observed in the CD4 T cell pool [Supplementary Table 3]. This loss of naïve T cells was accompanied by an expansion of terminally differentiated memory EMRA CD8 T

cell frequency ($p=0.003$) in UGI surgical patients pre-operatively compared to healthy controls [Supplementary Table 2]. However, unlike the CD8 subset, we observed a significantly higher frequency and absolute number of central memory CD4 T cells ($p=0.01$ and $p=0.0002$, respectively) and effector memory CD4 T cells ($p<0.0001$ and $p<0.0001$, respectively) in UGI surgical patients pre-operatively compared to healthy controls [Supplementary Table 3].

We also observed an enrichment of CD69 in surgical patients at all post-operative time points, an early activation marker, in CD8⁺ T cells ($p=0.002$) [Figure 1B; Supplementary Table 2] and CD4 T cells ($p=0.003$) [Supplementary Table 3] compared to healthy controls. Our transcriptomic analysis revealed increased Inducible Co-Stimulator (ICOS) at pre-operative baseline and day 2-5 post-operatively compared to healthy controls ($p=0.04$ and $p=0.05$ respectively) [Figure 1C; Supplementary Table 4a and 5a]. ICOS is a co-stimulatory molecule known to promote the T cell activation and proliferation; particularly of cytotoxic CD8⁺ T cells, which is gaining attention as a promising immunotherapeutic target²⁸.

In addition, a higher frequency and absolute numbers of senescent-like CD28⁻ CD57⁺ CD8⁺ T cells was observed in UGI surgical patients compared to healthy controls at all time points ($p<0.0001$ and $p=0.004$ respectively) [Figure 1D and Supplementary Table 2]. Furthermore, we detected a higher gene expression of ANAPC1 ($p=0.03$) and ANAPC10 ($p=0.01$) at pre-operative baseline compared to healthy controls, which have been implicated in pathways related to cell cycle and cellular senescence markers (CDKN1A, CCND3) that can be induced by DNA damage through the p53 pathway in PBMCs [Figure 1C; Supplementary Table 4a]. A key trait of these senescent-like T cells is the secretion of pro-inflammatory and tissue remodelling factors, termed senescence-associated secretory phenotype (SASP). We observed an upregulation of sixteen genes of the SASP signature [including IL6 ($p=0.03$), IL1 β ($p=0.02$), SERPING1

($p=0.02$) and CCL2 ($p=0.05$)] in UGI surgical patients pre-operatively compared to healthy controls; these genes are a part of the recently published gene set that identifies senescent cells and predicts senescence-associated pathways (SenMayo) [Figure 1C; Supplementary Table 4b]^{29,30}. Interestingly, we have observed a positive association between systemic frequency of these senescent CD4 T cells at baseline and hospital length of stay $R^2 = 0.48$, $p = 0.007$, ICU length of stay $R^2 = 0.39$, $p = 0.028$, and the Clinical Frailty Score $R^2 = 0.39$, $p = 0.02$ [Supplementary Figure 3].

Furthermore, we observed a higher frequency of Th17 cells (ROR γ^+) and regulatory CD4 T cells (FoxP3 $^+$) in UGI surgical patients compared to healthy controls at all time points ($p<0.0001$ and $p<0.0001$ respectively); indicating a persistently skewed helper CD4 T cell landscape as early as the peri-operative period [Figure 1E and 1F; Supplementary Table 3].

The IMM-AGE score is a composite clinically relevant metric that quantifies an individual's immune age based on T cell phenotype³¹. IMM-AGE scores indicated a significantly higher immunological age in UGI surgical patients pre-operatively ($p=0.03$), day 2-5 ($p=0.01$) and at hospital discharge ($p=0.02$) compared to healthy age-matched controls [Figure 1G].

The impact of surgical stress on systemic inflammation in UGI surgical patients

We observed systemic inflammation and elevated levels of inflammatory cytokines IL1RA ($p<0.0001$) [Figure 1H], IL-6 ($p<0.0001$) [Figure 1I] and IL-8 ($p<0.0001$) at day 2-5 after UGI surgery, compared to healthy controls [Supplementary Table 7]. We also observed lower levels of IL-4 in UGI surgical patients compared to healthy controls at all time points ($p=0.01$) [Supplementary Table 7]. Clinical data also identified elevated CRP levels post-operatively, with clinical blood-tests reporting significant higher CRP at day 2-5 post-

operatively in UGI surgical patients compared to healthy controls (273.9 ± 120.6 vs 9.47 ± 8.82 , $p < 0.0001$). In this study we have observed a positive association between APACHE score and systemic levels of IL1 β , IL6 and TNF levels in surgical patients ($R^2 = 0.52$, $R^2 = 0.008$, $p = 0.03$ and $R^2 = 0.35$, $p = 0.04$ respectively) [Supplementary Figure 3]. Similarly, we have observed a positive association between SOFA score and systemic levels of IL1 β , IL6, IL17 and TNF levels in these patients ($R^2 = 0.52$, $p = 0.007$; $R^2 = 0.34$, $p = 0.02$; $R^2 = 0.35$, $p = 0.04$, and; $R^2 = 0.37$, $p = 0.03$ respectively) [Supplementary Figure 3].

These findings are in line with our transcriptomics data [Figure 2], which revealed an upregulation of genes such as INPP5D ($p = 0.03$), AIM2 (Absent in Melanoma 2) ($p = 0.01$) and NCF4 ($p = 0.01$) in surgical patients pre-operatively compared to healthy controls. These genes are known as inflammasome activators that can trigger inflammation via release of pro-inflammatory cytokines, such as IL-1 β , IL-18, further influencing tumour behaviour³². We also observed pre-operative upregulation of inflammation promoting mediators such as NF-kB1 ($p = 0.03$), and other signalling pathways such as JAK-STAT ($p = 0.01$) and MAPK ($p = 0.04$), that are critical players in inflammation and tumour development [Supplementary Table 4b]. Interestingly, we observed a positive association between systemic IL-6 and NFkB expression levels ($R^2 = 0.34$, $p = 0.04$) [Figure 2].

The impact of surgical stress on transcriptomic signature in UGI surgical patients

Analysis of 770 genes demonstrated the impact of surgical stress in transcriptomic signatures [Supplementary Tables 4a – 6b and Figure 1C and Figure 2]. Firstly, we observed an upregulation of fifteen genes associated with tumour progression in the UGI cohort pre-surgery which declined post-surgery, such as CREB1 a known driver of cancer cell proliferation ($p = 0.03$ and $p = 0.01$ respectively); CYFIP1 (Cytoplasmic FMRP Interacting

Protein 1) that plays a role in metastasis ($p=0.01$ and $p=0.04$ respectively); and FGL2 (Fibrinogen-like protein 2) that acts as an immunosuppressive factor that can dampen anti-tumour immune responses, particularly of cytotoxic $CD8^+$ T cells, promoting the recruitment and activation of immunosuppressive cells within the tumour microenvironment ($p=0.003$ and $p=0.04$ respectively)³³. Cytotoxic $CD8^+$ T cells carry out their killing of tumour cells by releasing cytotoxic protein such as granzymes GZM A and GZM B, whose expression levels were higher in the UGI patients compared to healthy controls across all three timepoints ($p=0.03$, $p=0.03$, $p=0.03$ at pre-operative, day 2-5 post-operative and hospital discharge respectively). We have also observed a pre-operative upregulation of Natural cytotoxicity triggering receptor (NCR1) ($p=0.01$), that plays a vital role in immune surveillance by NK cells with known implications in cancer^{34,35}. Importantly, we also observed a pre-operative upregulation of markers of exhaustion, such as LAG3 ($p=0.03$) and PTGER4 ($p=0.03$), hinting towards a state of T cell dysfunction characterized by the progressive loss of effector functions and the inability to effectively combat cancer cells [Figure 2]³⁶.

Autophagy is a physiological intracellular degradative process that eliminates damaged organelles in adaptation to several stressful conditions, including starvation, nutrient deprivation to maintain cellular homeostasis. The mechanism of autophagy initiates the formation of autophagosomes that capture degraded components and then fuse with lysosomes to recycle these components. We have observed a reduction of autophagy related genes in UGI surgical patients pre-operatively and day 2-5 post-surgery, including ATG2B ($p=0.05$ and $p=0.006$) which is necessary for LC3 processing and conjugation, and others such as CFLAR ($p=0.03$ and $p=0.02$) and VAMP8 ($p=0.04$ and $p=0.02$), crucial for autophagosome formation^{37,38}. Mammalian target of rapamycin (mTOR) is associated with cell proliferation, stress, and cancer progression. In this study we have observed an upregulation of LAMTOR2 ($p=0.02$),

LAMTOR4 ($p=0.001$) and mTOR ($p=0.04$) in UGI patients pre-operatively, which is known to suppresses autophagy by inhibition of autophagosome formation³⁹. This pattern is consistent with dysregulated autophagy, which can contribute to apoptosis resistance (e.g., reduced caspase-3 expression), defective clearance of damaged cellular components and antigens, and accumulation of inflammatory cells within the tissue microenvironment. Together, these findings point to a role for aberrant mTOR–autophagy signalling in UGI disease development and progression.

Lastly, we have observed altered expression of genes such as ABCA1 ($p=0.04$) and PLTP (Phospholipid Transfer Protein) ($p=0.01$) pre-operatively, that are involvement in metabolic reprogramming, particularly in lipid metabolism, which has a key aspect of its influence upon cancer cell behaviour⁴⁰⁻⁴². Furthermore, an upregulation of Insulin Receptor (INSR) ($p=0.05$) and Aldolase B (ALDOB) ($p=0.03$) was observed pre-operatively in UGI surgical patients, which are known to play a role in glucose uptake, the process of glycolysis and cancer development⁴³.

The impact of surgical stress on metabolomics signature in UGI surgical patients

243 metabolites were significantly different between healthy controls and UGI surgical patients at pre-operative baseline. Seventy-one of the metabolites were selected as biologically meaningful with established relevance to immune, inflammatory and metabolic pathway, based upon the Kyoto Encyclopaedia of Genes and Genomes database, with the remaining identified as drug, drug-related compounds and exometabolites (coming from diet or life course exposure) [Supplementary Table 8-10 and Figure 3). From the 71 significantly altered metabolites, we focused our discussion on those forming the two dominant clusters of metabolomic signatures, the first reflecting microbiome dysbiosis.

We observed higher pre-operative levels of indole-3-acetic acid-O-glucuronide (IAA-G) ($p=0.04$) [Figure 3A], a microbiota-derived tryptophan metabolite whose elevation reflects increased microbial indole flux and a shift towards host glucuronidation⁴⁴⁻⁴⁶. The concurrent rise in LysoPC ($p=0.04$) [Figure 3B] and LysoPE [Supplementary Table 8-10] provides biochemical evidence of epithelial membrane degradation and mucosal stress; with LysoPC declining post-surgery ($p=0.87$). Interestingly, we have observed a positive association between systemic Lyso PC levels, and the length of hospital ($R^2 = 0.65$, $p=0.002$) and ICU stay ($R^2 = 0.60$, $p=0.005$) and also with systemic frequency of senescent T cells ($R^2 = 0.43$, $p=0.03$) [Supplementary Figure 3]. Furthermore, we observed elevated levels of pyrogallol-2-O-glucuronide, a detoxification product of oxidised polyphenol, possibly driven by the inflammatory surge [Figure 3C]. We also observed elevated Tyramine-O-sulfate, a detoxification product of microbially derived tyramine, in UGI surgical patients at all three time points ($p=0.01$; $p=0.03$; $p=0.01$ respectively), which also reflects a state of dysbiosis [Supplementary Table 8-10]. In contrast, indole-3-lactic acid, a protective Lactobacillus-derived AhR ligand ($p=0.04$) [Figure 3D], hippuric acid ($p=0.04$), a host-microbiome co-metabolite reflecting polyphenol metabolism [Figure 3E], and Docosahexaenoyloxy-4-butanoate ($p=0.0008$) [Supplementary Table 8-10] were reduced in UGI patients and remained low post-surgery, although 5-methyl-4-Isoxalepropionic acid levels increased post-surgery ($p=0.003$) [Supplementary Table 8-10]; suggesting an incomplete mucosal recovery. Furthermore, we observed a negative association between systemic hippuric acid levels and age ($R^2 = -0.51$, $p=0.03$) and BMI ($R^2 = -0.42$, $p=0.05$) [Supplementary Figure 3]. S-allylcysteine, a dietary organosulfur compound with antioxidant properties, was markedly decreased ($p=0.01$) [Figure 3F] alongside 11-nitro-1-undecene ($p=0.002$), which has anti-inflammatory and redox-modulating properties [Supplementary Table 8-10]^{47,48}.

Together with the loss of microbiome-derived metabolites such as ILA, our data also points to a broader collapse of immune-metabolic homeostasis. Reduced levels of 6-hydroxy-3-oxo-8-tetradecenoic acid indicate accelerated consumption of fatty-acid oxidation intermediates, ($p=0.008$) [Figure 3G], in addition to the parallel reduction in 3,4-dimethyl-5-pentyl-2-furanpropanoic acid ($p=0.03$) [Figure 3H]. Post-operative increases in 3-hydroxy-hexanoyl-carnitine ($p=0.0001$), hexadecanedioic acid L-carnitine ester ($p=0.002$) is indicative of a restoration of mitochondrial metabolic efficiency following tumour removal, while reduced methionyl-phenylalanine and increased γ -glutamyl-valine ($p=0.0001$) point to altered amino-acid turnover [Supplementary Table 8-10]⁴⁹. Elevated pyridoxamine ($p=0.0002$), a vitamin B6 vitamer involved in transamination, further supports this interpretation, likely reflecting a heightened demand for B6-dependent amino-acid and nucleotide metabolism⁵⁰.

Post-operative pneumonia status amongst UGI surgical patients

In this study, 65% ($n=13$) of the surgical patients developed pneumonia during their post-operative hospital admission, based on clinician's diagnosis reported in the patient's EHR. Retrospective calculation of the Clinical Pulmonary Infection Score (CPIS), using the same clinical datasets from patient EHRs, demonstrated complete concordance with the clinical classification, with all patients diagnosed with pneumonia having a CPIS score ≥ 6 , and patients without pneumonia scoring <6 (8.54 ± 1.71 vs 3.29 ± 1.89 , $p<0.0001$). Due to overlapping clinical information for both pneumonia assessment, the CPIS provides a standardised supportive measure, rather than an independent validation tool. We observed a positive association between APACHE scores and CPIS scores ($R^2 = 0.34$, $p=0.04$) [Supplementary Figure 3]. The median timepoint of pneumonia onset was day four post-operatively (range 2-12 days).

Those with pneumonia were significantly younger than patients without pneumonia (59.92 ± 12.39 years vs 70.43 ± 6.55 years, $p=0.047$) and had lower predicted Clinical Frailty Scores pre-admission (1.71 ± 0.75 vs 2.54 ± 0.77 , $p=0.049$) [Supplementary Table 1]. Patients with pneumonia had significantly longer ICU (15.08 ± 15.18 days vs 3.71 ± 2.29 days, $p=0.038$) and hospital length of stays (29.23 ± 19.59 days vs 12.86 ± 6.52 days, $p=0.024$) and required longer periods mechanically ventilated (9.23 ± 13.30 days vs 0.00 ± 0.00 days, $p=0.038$) than patients without pneumonia. Patients with pneumonia had higher illness severity, measured by APACHE-2 scores (13.08 ± 6.22 vs 7.14 ± 1.86 , $p=0.011$) and SOFA scores (6.23 ± 4.16 vs 1.42 ± 1.51 , $p=0.0018$) than non-pneumonia counterparts [Supplementary Table 1].

Pre-operative profile of UGI surgical patients with and without subsequent post-operative pneumonia

On comparison of T cell subset distribution between UGI surgical patients who developed post-operative pneumonia compared to those who did not, we observed no significant differences at pre-operative baseline [Supplementary Table 11 and 12]. There were no statistically significant differences in pre-operative IMM-AGE scores between UGI surgical patients who developed pneumonia and those who did not develop pneumonia ($p=0.25$) [Figure 4A]. However, on assessing circulating levels of cytokines we observed elevated levels of pre-operative pro-inflammatory cytokines IFN α ($p=0.01$), IL1RA ($p=0.04$) [Figure 4B], IL1 β ($p=0.04$) in patients who developed post-operative pneumonia, alongside elevated levels of regulatory cytokines IL-10 ($p=0.02$) and IL-4 ($p=0.007$), compared to those without pneumonia [Supplementary Table 13].

Metabolomics revealed elevated inflammatory markers such as 3-hydroxytetradecanoic acid [Figure 4C], alongside reduced dimethyluric acid ($p=0.03$) and hydroxypropionylcarnitine

(p=0.06) [Supplementary Table 14]; indicating strong endotoxin-driven inflammation and impaired energy metabolism at pre-operative baseline in those who developed post-operative pneumonia. In parallel, increased corticosterone (p=0.04) [Figure 4D], L-tyrosine (p=0.07), methionylphenylalanine (p=0.08), methylimidazoleacetic acid (p=0.08), and N-acetylputrescine (p=0.008) reflect a state of elevated stress, protein catabolism, inflammation, and epithelial injury in those who later developed post-operative pneumonia [Supplementary Table 14].

Finally, we also observed altered expression of 46 genes between UGI patients that did and did not develop post-operative pneumonia at pre-operative baseline [Supplementary Table 15a-b and Figure 4E]. There was an upregulation of Adenosine Deaminase 2 (ADA2), AKT1 (p=0.04), BIRC3 (p=0.04), DAPK1 (p=0.01), which are known to play a role in the inflammatory response during pneumonia by activating p38/NFkB and other signalling pathways. There were also lower levels of AIM2 (p=0.05), a known regulator of macrophages homeostasis that can block TLR-induced secretion of TNF and IL-1 β while increasing IL-10 levels, thereby downregulating macrophages inflammation. We also observed lower IRAK3 levels (p=0.03) in patients that developed pneumonia, which is known to dampen down excessive inflammation by inhibiting the activation of NF- κ B. The inflammatory response in pneumonia involves signalling pathways such as an upregulation of senescence related genes CDKN1A (p=0.04) and CREBBP (p=0.04)⁵¹⁻⁵³.

Discussion

This exploratory pilot study investigated immunological, inflammatory, transcriptomic and metabolomic signatures in elective UGI surgical patients before and after surgery. Compared with healthy controls, UGI surgical patients demonstrated features of immune ageing,

1 including increased senescent CD8⁺ T cells, elevated inflammatory cytokines, and
2 transcriptomic and metabolomic signatures associated with reduced autophagy, metabolic
3 programming and microbiome dysbiosis. Pneumonia was a common complication following
4 UGI surgery, occurring in 65% of patients post-operatively. Patients who developed
5 pneumonia exhibited a significantly heightened inflammatory profile pre-operatively. Despite
6 being younger and having lower predicted clinical frailty scores, patients who developed
7 pneumonia experienced worse post-operative outcomes than those without pneumonia,
8 including longer durations of mechanical ventilation and prolonged ICU and hospital stays.
9 These findings reinforce the importance of establishing early, standardised and quantifiable
10 methods for identifying patients at risk of pneumonia. However, given the pilot study design
11 and small sample size, these findings should be considered hypothesis-generating and require
12 large-scale validation.

13
14 CD8⁺ cytotoxic T cells are essential components of the anti-tumour immune response,
15 functioning via release of cytolytic molecules such as perforin and granzymes, inducing
16 caspase-mediated apoptosis in tumour cells. In this study, we observed clear evidence of
17 accelerated pre-operative immunesenescence in UGI surgical patients compared with healthy
18 controls. This was characterised by a marked loss of naïve CD8⁺ T cells alongside an expansion
19 of terminally differentiated EMRA and senescent CD8⁺ T-cell subsets. Our transcriptomic data
20 further revealed up-regulation of senescence and cytotoxicity-associated genes together with
21 increased expression of exhaustion markers. Overall, these findings indicate an aged and
22 functionally exhausted immune phenotype with reduced effector competence pre-surgery.
23 Notably, this pattern mirrors findings reported in colorectal cancer, where reduced naïve CD8⁺
24 T cells, accumulation of EMRA and senescent T cells, and up-regulation of
25 senescence-associated inflammatory pathways are well described^{54,55}.

1
2 This immunosenescent profile persisted post-operatively, with transcriptomic data showing
3 sustained expression of senescence-related and inflammatory genes, and a further increase in
4 immune activation and inflammatory signalling after surgery. Based on our data, we
5 hypothesise persistent antigen stimulation, hypoxia, and metabolic stressors within the tumour
6 microenvironment contribute to a T cell senescent-like pro-inflammatory phenotype and a
7 dysfunctional state of exhaustion. Surgical stress triggers systemic inflammation, followed by
8 compensatory immune suppression. This immunosuppressive response is characterised by T
9 cell redistribution, T cell activation, and a shift towards a dysfunctional phenotype. This leads
10 to disrupted homeostasis and weakened pathogen clearance, creating a post-operative window
11 of vulnerability with increased susceptibility to infections such as pneumonia. While functional
12 assays were not performed due to limited blood volumes, the combined phenotypic and
13 transcriptomic signatures observed are consistent with an immune system exhibiting hallmarks
14 of accelerated ageing and exhaustion.

15
16 Although at a cellular level we did not identify significant differences in patients developing
17 pneumonia, at a molecular level we observed down-regulation of autophagy alongside
18 up-regulation of mTOR signalling in UGI surgical patients. This pattern aligns with the key
19 Hallmarks of Cancer and Ageing, which is closely linked to immunesenescence and inflamm-
20 ageing, potentially contributing to the accumulation of dysfunctional T-cell subsets and
21 exaggerated peri-operative inflammatory responses⁵⁶. We observed that patients who
22 developed pneumonia showed even higher pre-operative mTOR signalling and lower
23 autophagy activity alongside elevated NLRP3 signalling pathway. These features have been
24 reported in models of lung injury and pneumonia pathogenesis, supporting the plausibility of
25 this pathway contributing to susceptibility^{57,58}. Interestingly, a genetic variant (rs4845987) that

1 reduces mTOR expression in activated T cells, has been recognised as a critical regulator in
2 improving survival in pneumonia-driven sepsis⁵⁹. However, the results of this exploratory pilot
3 identify hypothesis-generating biomarker signals, and thus they do not establish causality.

4
5 Together, these findings raise the possibility that geroprotective agents targeting the
6 immunesenescence and mTOR–autophagy axis could help restore immune resilience and
7 improve post-operative outcomes in this high-risk population. Preclinical studies suggest that
8 agents such as rapamycin and metformin modulate mTOR signalling, enhance autophagy and
9 reduce inflammatory and senescence-associated pathways^{60,61}. In addition, senolytic therapies
10 and autophagy-promoting compounds (such as spermidine, dasatinib and quercetin) have
11 demonstrated potential to improve immune function and attenuate lung injury in experimental
12 and early clinical settings⁶²⁻⁶⁵. However, this exploratory study did not directly assess autophagic
13 activity or establish causality, and therefore the link between the mTOR-autophagy axis and
14 pneumonia are speculative. Further rigorous prospective studies are required, prior to clinical
15 application.

16
17 Our metabolomic data indicate a shift toward accelerated fatty-acid oxidation, which aligns
18 with the increased expression of ACSL4, FABP5 and related fatty-acid activation genes,
19 suggesting rapid channelling of long-chain fatty acids into oxidative pathways at the expense
20 of intermediate hydroxy-oxo species that normally support redox balance. Such
21 reprogramming mirrors ageing-associated metabolic phenotypes characterised by impaired
22 epithelial resilience, increased susceptibility to oxidative injury, and promotion of T cell
23 senescence^{41,66,67}. Complementary changes in ABCA1, PLTP and ATF4 further support a
24 broader rewiring of lipid handling and stress-adaptive metabolism, while ADCY7 upregulation
25 highlights concurrent modulation of cAMP-dependent immune signalling during bacterial

challenge^{68,69}. Together, these alterations point to a regulated shift toward inflammatory and lipid oxidation, reinforcing an oxidative, ageing-like metabolic state that may exacerbate tissue vulnerability and immune dysfunction. These findings highlight several potential intervention points, including inhibition of ACSL4, which has demonstrated protective effects in models of lung injury, and enhancement of antioxidant pathways through the microbiome-derived metabolite butyrate. Butyrate has been reported to suppress mitochondrial ROS production in vitro in T cells, offering an additional strategy to modulate redox balance and mitigate metabolic dysfunction within an inflamed microenvironment^{70,71}. Beyond immune phenotypes, metabolomic profiling revealed evidence of systemic metabolomic reprogramming, consistent with accelerated ageing.

This pilot study has several limitations. Firstly, due to the small sample size, results have limited statistical power and increased risk of type two error, particularly when comparing pneumonia and pneumonia subgroups which were unmatched and unequal in size. The integrative approach involved multiple comparisons across immunological, transcriptomic and metabolomic datasets, increasing the risk of type one error. Bias may also be introduced by the convenience sampling methods from a single-centre, therefore further impairing the generalisability and external validity of the results. Pneumonia status was determined by the presence or absence of a clinician-documented diagnosis in the patient's medical records, rather than a standardised adjudication protocol. As such, diagnosis may be subject to inter-clinician variability and may introduce heterogeneity, particularly as diagnosis is dependent on the accuracy and availability of the EHR and open to potential misinterpretation during retrospective data retrieval. However, clinician diagnosis was confirmed by retrospective calculation of the CPIS, completed by a researcher who was blinded to the patient's clinical status. Groups were compared by their pneumonia status, however there are numerous other

1 potential confounding variables, such as drug history, past medical history or life-style factors,
2 that may influence results. In addition, 50% of patients were identified to have post-operative
3 delirium and anastomotic leak, which may also contribute to the altered post-operative immune
4 profile. The management and medical treatment of pneumonia between clinicians and patients
5 may also vary and influence patient outcomes. The observational design precludes causal
6 inference, and the associations identified cannot be directly attributed to post-operative
7 pneumonia and do not establish mechanistic pathways. Overall, the small sample size in
8 addition to the complexity and heterogeneity of the clinical cohort is likely to impede the
9 validity of the results. Accordingly, these findings should be interpreted as exploratory and
10 hypothesis-generating, pending validation in a larger multicentre mechanistic study that is
11 adequately powered.

13 **Conclusions**

14 Cancer represents a substantial and growing public health challenge, due to its adverse effects
15 on patient outcomes and the burden it places on global healthcare resources. There is therefore
16 an urgent need to transition from reactive to proactive care strategies within cancer populations,
17 with improved stratification of surgical risk and biological vulnerability. This exploratory pilot
18 study suggests that UGI surgery induces profound peri-operative immune and metabolic
19 disruption consistent with accelerated biological ageing. Patients demonstrated persistent
20 immunesenescence marked by loss of naïve CD8⁺ T cells, expansion of senescent and
21 exhausted subsets, heightened inflammation, altered mTOR–autophagy signalling, and broad
22 metabolic reprogramming. Post-operative pneumonia was frequent and associated with worse
23 outcomes, with affected patients exhibiting distinct pre-operative features of inflamm-ageing,
24 including inflammatory, transcriptomic, and metabolic vulnerability, despite lower frailty.
25 Together, these findings should be interpreted as exploratory and hypothesis-generating,

1 suggesting that surgical stress may amplify pre-existing immune ageing and create a window
2 of susceptibility to post-operative complications. However, given the small sample size, limited
3 statistical power and multiple comparisons, these observations require cautious interpretation.
4 This integrative multi-omics approach identifies potentially modifiable ageing-related
5 pathways that merit validation in larger, mechanistic and interventional studies.

6 7 **Methods**

8 *Study Design & Setting*

9 A single-centre observational pilot study was conducted. Adult patients (aged ≥ 18 years)
10 were recruited consecutively from an UGI surgical assessment clinic at the Queen Elizabeth
11 Hospital in Birmingham (QEHB) over a period of 15-months (May 2022 and December
12 2023). A pragmatic convenience sampling method was used to facilitate preliminary
13 investigation within the available study timeframe and resources.

14 15 *Participants & Ethical Considerations*

16 This study was designed as an exploratory pilot study intended to identify preliminary
17 biological signals, rather than provide definitive efficacy estimates. A target enrolment of 20
18 participants was therefore considered appropriate for exploratory within-subject
19 immunological analyses, informed by published variability estimates for immune activation
20 markers and the feasibility constraints typical of early-phase mechanistic studies.

21
22 Patients were deemed eligible to participate if they were attending the hospital outpatient
23 clinic prior to elective oesophagectomy surgery for UGI cancer (termed ‘UGI surgical’ from
24 here forwards). Written informed consent was obtained in pre-operative clinic from patients
25 who agreed to participate. Patients were followed-up at two further time-points, including

day 2-5 post-operatively and hospital discharge. Ethical approval for this study was granted by the Yorkshire and The Humber Research Ethics Committee (IRAS reference number: 251882), via the centralised UK Integrated Research Application System (IRAS). In accordance with UK national research governance, this independent committee provides centralised ethical oversight, independent of the authors' specific institutional affiliations.

Age- and sex-matched healthy controls were also recruited, approved by the North Staffordshire Research Ethics Committee, via the centralised UK IRAS (IRAS reference number: 301974). The exclusion criteria for healthy controls included: self-reported current infection, history of cancer, type 1 diabetes, chronic inflammatory or auto-immune disorders or the use of immune suppressive medication. Written informed consent was obtained from healthy controls who participated in a single study visit. All experiments were performed in accordance with relevant named guidelines and regulations.

Data Collection

Participant demographics including age, sex, body mass index (BMI), pre-operative chemotherapy history, smoking history, predicted clinical frailty scores and Charlson comorbidities index were retrieved from patient electronic health records (EHR). Post-operative clinical data including ICU length of stay, number of days ventilated, post-operative complications, hospital length of stay, discharge destination, in-hospital mortality and 3-month mortality were also recorded. Post-operative complications included pneumonia, anastomotic leak and delirium status during the patient's hospital admission after surgery, based upon reporting in the EHR. Pneumonia was defined based on a documented diagnosis by the treating surgical or intensive care clinician, during routine clinical care, as recorded in the HER. In clinical practice, this diagnosis typically reflects a combination of clinical

features (such as new or worsening respiratory symptoms, fever, or rising oxygen requirements), radiological findings (such as new or worsening infiltrates on chest imaging) and where available, microbiological data. No additional study-specific criteria were applied, and diagnostic status was retrieved retrospectively. Clinical data from EHR was also used to retrospectively calculate the acute physiological and chronic health evaluation (APACHE 2), sequential organ failure assessment (SOFA) and the clinical pulmonary infection score (CPIS) at day four post-operatively (coinciding with mean time of pneumonia clinical diagnosis). These scores were calculated retrospectively with blinding of pneumonia status.

Blood Sample Processing

Blood samples (18 ml) were collected from each patient at three time points: pre-operative, day 2-5 post-surgery and at hospital discharge. Patients were excluded from post-operative follow-up if they had known SARS-CoV-2 infection, if the surgical procedure was abandoned (such as due to advanced disease), anticipated end of life care within the 48-hours or if the patient declined ongoing participation. Blood samples were collected from healthy controls at one time-point. For each blood sample collected, the complete blood differential counts were measured from 1ml of whole blood using an automated haematology analyser (Sysmex, UK). Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll density centrifugation as previously described and frozen in freezing medium (HiFCS with 10% DMSO) [14]. Whole blood collected in vacutainers without a coagulant was centrifuged (3000g, 10 minutes) and serum was removed. The PBMC and serum samples were stored at -80°C until further analysis.

T Cell Subsets & Immunological (IMM-AGE) Scoring using Flow Cytometry

Frozen PBMCs (1×10^6 cells/ml) were thawed and immunostained with a combination of fluorochrome-conjugated antibodies, including cell-surface antibodies anti-human CD3 PE cy7 (clone: SK7; Invitrogen, Paisley, UK), anti-human CD4 Violet (clone: OKT4; Invitrogen), anti-human CD8 PE (clone:QA18A37; Biolegend, London, UK), anti-human CCR7 FITC (clone:GO43H7; Biolegend), anti-human CD45RA APC (clone: HI100; Biolegend), anti-human CD28 APC (clone:CD28.2; Biolegend), anti-human CD57 FITC (clone:HNK-1; Biolegend) and anti-human CD69 (clone:FN50; Biolegend) for 30 min at 4 °C. Post immunostaining cells were washed in PBS twice and analysed using the MACS Quant® Analyzer 10 flow cytometer (Miltenyi Biotec, Bergisch Gladbach, Germany). Data were then analysed using FlowJo software and unstained PBMCs were run to set gating parameters. CD3⁺ cells were defined as T cells, 10,000 T cells were gated and were divided into CD4⁺ and CD8⁺, which were further divided into four subsets based on CD45RA and CCR7 expression and denoted as naive (CD45RA⁺ CCR7⁺), central memory (CD45RA⁻ CCR7⁺), effector memory (CD45RA⁻ CCR7⁻) and terminally differentiated effector memory (CD45RA⁺ CCR7⁻). Senescent CD4⁺ and CD8⁺ T cells were denoted as CD28⁻ CD57⁺ cells. Activated CD4⁺ and CD8⁺ T Cells were denoted as CD69⁺. [Gating strategy; Supplementary Figure 1A for CD8 subsets and 1B for CD4 subsets].

Eight immune parameters based on frequencies of T cells (total T cells, naive CD4 T cells, effector memory CD4 and CD8 T cells, EMRA CD8 T cells, CD28^{-ve} CD8 T cells, CD57^{+ve} CD8 T cells and regulatory T cells) were used to calculate a modified profile of Immunological Age (IMM-AGE) score, an indication of the overall degree of ageing of the immune system [15, 31].

Intracellular Staining for Regulatory T cells

Thawed PBMCs (1×10^6 cells/ml) were resuspended in 50 μ l of PBS and stained with anti-human CD3 PE cy7, anti-human CD4 Violet and anti-human CD45RA Pcy5.5 (clone HI100; Biolegend) for 30 min at 4 °C. Post incubation, the cells were washed in PBS twice and fixed with Foxp3 Fix Perm solution (Thermo Fisher) for 30 min at room temperature, followed by a wash and staining with anti-human Foxp3 PE (clone:206D; Biolegend) and RORy APC (clone: 2A2; Biolegend) in diluted permeabilization buffer (Thermo Fisher Scientific, Loughborough, UK) for 30 min at 4°C. Post incubation, the cells were washed and analysed using a MACS Quant® Analyzer 10 flow cytometer (Miltenyi Biotec). Regulatory CD3⁺ CD4⁺ T cells were defined as Foxp3⁺ cells (Tregs), naïve Tregs as CD45RA⁺ Foxp3⁺ subset, and RORy⁺ cells were defined as Th17 cells [gating strategy Supplementary Figure 1B].

Serum Cytokine Measurement

Concentrations of circulating cytokines (IFN α , IL-1RA, IL-6, TNF- α , IL-8, IFN- γ , IL-10, GM-CSF, IFN- β , IL-1 β , IL-4, IL-17) were measured in thawed serum samples using commercially available human premixed multi-analyte magnetic Luminex Assays (R&D systems, Abingdon, UK). The absorbance of the wells was measured on a Bio-Plex™ 200 suspension array system (Bio-Rad Laboratories, Watford, UK) using Bio-Plex Manager software v6.2 (Bio-Rad Laboratories). Protein concentrations were extrapolated from a standard curve from known concentrations using GraphPad PRISM software v10 (GraphPad Software Inc., San Diego, CA, USA).

RNA Isolation & Nanostring nCounter® Gene Expression Analysis

To elucidate molecular signalling pathways contributing toward this state of enhanced immune ageing, the Nanostring nCounter gene expression assay was used to detect 770 genes in PBMCs from twelve healthy controls and twelve UGI patients at three timepoints. RNA extractions from two million frozen PBMC's were completed using the RNAeasy Mini Isolation Kit (Qiagen, Hilden, Germany) according to manufacturer instructions. The quantity of RNA was assessed immediately using a NanoDrop One Spectrophotometer (Thermo Fisher Scientific). The quality of the RNA was determined using the Agilent 2100 BioAnalyzer. Gene expression analysis was performed using the fibrosis panel from Nanostring nCounter®. In the absence of an ageing-specific panel, the fibrosis panel was selected. Unlike available cancer-focused panels, which prioritise oncogenic signalling, the fibrosis panel provides coverage of several pathways relevant to immune ageing, including inflammation, cellular activation, senescence, oxidative stress, autophagy, MAPK signalling and cellular stress-response pathways. These pathways overlap with established Hallmarks of Ageing and are highly relevant to UGI cancer biology.

For each sample, 80ng of total RNA, with a maximum of 7µL (>28.6 ng/µL), was used. Hybridisation was performed at 65°C for 17 hours using a SimpliAmp Thermal Cycler (Applied Biosystems, Abingdon, UK). The nCounter Flex system (NanoString, Seattle, WA, USA) was used for sample preparation. Raw gene counts were normalised using the most stable housekeeping genes from the panel. Differential expression of genes between PBMC from the two cohorts was tested with Mann-Whitney U tests and Benjamini-Hochberg (BH) procedures were used to correct for multiple testing. The BH false discovery method was used and a p-value cut-off of <0.05 (FDR) was set as significant. A heatmap was generated by clustering analysis of the differentially expressed genes.

Metabolomics

Untargeted metabolomic profiling on serum samples was conducted using Ultra-High-Performance Liquid Chromatography Mass Spectrometry (UHPLC-MS), which was coupled to an Orbitrap Exploris 240 (Thermo Fisher Scientific). The LC-MS method selected in the experiment was originally optimised using the Orbitrap/Exploris platform⁷². Chromatographic separation employed a Vanquish Accucore C18⁺ UHPLC column (100 mm × 2.1 mm, 2.6 µM; Thermo Fisher Scientific), operated at a flow rate of 400 µL min⁻¹. Mobile phase A consisted of 99.9% water with 0.1% formic acid, and mobile phase B of 99.9% methanol with 0.1% formic acid. The gradient started at 99 percent A and 1 percent B, held briefly, then increased to 50 percent B by 2.0 minutes, before ramping to 99 percent B at 10.5 minutes and holding for column washing. The system was subsequently re equilibrated back to initial conditions, with a total run time of 15 minutes. Electrospray ionization (ESI) was used for ion generation, with voltages set at 3900 V (positive mode) and 2500 V (negative mode). Source-dependent parameters were configured as follows: sheath gas at 40 Arb, auxiliary gas at 10 Arb, sweep gas at 1 Arb, ion transfer tube temperature at 300 °C, and vaporizer temperature at 280 °C. Pierce™ FlexMix™ calibration solution (Thermo Fisher Scientific) was used to perform instrument calibration following vendor-recommended procedure. Mass spectrometry data were collected via data-dependent acquisition (DDA) mode, enabling MS/MS-based metabolite identification to MSI level 2.

Mass Spectrometry Data Processing

For initial pre-processing of untargeted metabolomics data, Thermo Scientific Xcalibur format raw data files (.RAW) were imported into Compound Discoverer software version 3.2 (Thermo Fisher Scientific). The raw files were filtered to retain positive-ion spectra and were

1 subsequently subjected to adaptive retention-time alignment using a mass tolerance of 2 ppm
2 and a maximum allowable shift of 0.3 minutes. Compound detection was performed using
3 stringent quality filters, including a signal-to-noise threshold of 5, a minimum peak intensity
4 of 500,000, and an extensive ion list encompassing common adducts, dimers, and neutral
5 losses. Detected features were consolidated across samples using combined mass and
6 retention-time tolerances, and preferred fragment ions were selected for downstream
7 identification.

8
9 Compound annotation was performed using multiple complementary evidence layers.
10 Spectral matching was carried out against mzCloud reference and auto-processed libraries
11 using DDA spectra and cosine similarity scoring with strict precursor and fragment mass
12 tolerances. Molecular formula prediction was constrained using defined elemental
13 boundaries, RDBE limits, isotopic pattern matching, and fragment consistency. Resulting
14 annotations were prioritised using integrated evidence from mzCloud, predicted
15 compositions, ChemSpider, mass list searches, and pathway-based annotation. The workflow
16 was completed with gap filling, QC-based signal correction, background subtraction using
17 blank samples, and log-transformed differential analysis, resulting in a high-confidence,
18 QC-controlled dataset suitable for downstream statistical and biological interpretation.

20 ***Statistical Analysis***

21 Statistical analysis was performed using GraphPad Prism® software v10. A Kolmogorov-
22 Smirnov test was performed to determine the normality of the data. Unpaired students T-test
23 (parametric test) was used for normally distributed data and Mann Whitney U tests were used
24 (non-parametric test) when data was skewed, when comparing two groups. A repeated-
25 measures one-way analysis of variance (ANOVA) with Bonferroni correction was used to

compare biomarker levels over sequential timepoints. To determine the strength and direction of associations across the full multi-omics dataset, integrating all pairwise combinations of clinical variables, immune phenotypes, cytokine profiles, transcriptomic signatures, and metabolomic features, associations between continuous variables were assessed using Pearson's correlation coefficient, as the data met assumptions of approximate normality and linearity. For each pairwise comparison, we report R^2 values and the corresponding p-value. All data tables are presented as mean $\pm$ standard deviation (SD). Statistical significance was accepted at $p < 0.05$ for all data sets.

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Additional Information

Data availability statement:

The data that support the findings of this study are available from University of Birmingham, but restrictions apply to the availability of these data, which were used under licence for the current study and so are not publicly available. The data are, however, available upon request via the corresponding author.

Ethics approval and consent to participate:

This study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from surgical patients in pre-operative clinic and reviewed at each post-operative visit (day 2-5 post-operatively and hospital discharge). This study was approved by Yorkshire and The Humber Research Ethics Committee (REC) (IRAS reference number: 251882). Written informed consent was also obtained from age- and sex-matched healthy controls, as approved by the North Staffordshire REC (IRAS reference number: 301974). Ethical approval for both study groups was granted via the centralised UK Integrated Research Application System (IRAS). In accordance with UK national research governance, this independent committee provides centralised ethical oversight, independent of the authors' specific institutional affiliations.

Consent for publication, clinical trial registration and permission to reproduce material from other sources

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List of Abbreviations

ANOVA: a one-way analysis of variance

APACHE: acute physiological and chronic health evaluation

BH: Benjamini-Hochberg

BMI: body mass index

CPIS: clinical pulmonary infection score

DDR: DNA damage response

EHR: electronic health records

FGL2: Fibrinogen-like protein 2

GI: gastrointestinal

GZM: granzymes

ICU: intensive care unit

IFN: interferon

IL: interleukin

IMM-AGE: immunological age

MAPK: mitogen-activated protein kinase

mTOR: mechanistic target of rapamycin

NCR1: natural cytotoxicity triggering receptor

PBMC: peripheral blood mononuclear cells

QEHB: Queen Elizabeth Hospital Birmingham

SASP: senescence-associated secretory phenotype

SOFA: sequential organ failure assessment

TNF: tumour necrosis factor

UGI: upper gastrointestinal

UHPLC-MS: Ultra-High-Performance Liquid Chromatography Mass Spectrometry

Figure Legends

Figure 1: T-cell subset distribution, transcriptomic signatures, and serum cytokine levels in surgery patients and healthy controls.

(A) Frequency of CD8 T cell subsets; naïve ($CCR7^{+ve} CD45RA^{+ve}$), central memory ($CCR7^{+ve} CD45RA^{-ve}$), effector memory ($CCR7^{-ve} CD45RA^{+ve}$) and terminally differentiated effector memory expressing RA, TEMRA ($CCR7^{+ve} CD45RA^{+ve}$) T cells (B) Frequency of activated $CD69^{+ve}$ CD8 T cells % (D) senescent $CD28^{-ve} CD57^{+ve}$ CD8 T cells (E) $ROR\gamma^{+ve}$ CD4 T cells (F) $FoxP3^{+ve}$ CD4 T cells in PBMCs (G) Calculated IMM-AGE scores (G) Serum IL1RA (I) Serum IL6 levels in UGI surgery patients pre-operatively, day2-5 post surgery and at hospital discharge (n = 20) compared with age and sex matched healthy controls (n =20) (C) Heat map showing mean expression levels of significantly differentially expressed senescence and inflammation associated genes in PBMCs isolated from 12 age and gender matched healthy controls and 12 UGI patients across the three timepoints. Overall group differences were assessed using a one-way ANOVA with multiple comparisons, comparing the mean of each UGI surgical patient timepoint with the mean of the healthy control group. Statistically significant: $p \leq 0.05$ indicated by *; $p \leq 0.01$ indicated by **; $p \leq 0.001$ indicated by ***; $p \leq 0.0001$ indicated by ****.

Figure 2: Transcriptomic signature in upper gastrointestinal (UGI) surgery patients across timepoints and healthy controls.

Heat map showing mean expression levels of significantly differentially expressed genes associated with hallmarks of immune ageing, key signalling pathways and tumour progression related genes in PBMCs isolated from 12 age and gender matched healthy controls and 12 UGI patients across the three timepoints. Overall group differences were assessed using a one-way ANOVA with multiple comparisons, comparing the mean of each UGI surgical patient timepoint with the mean of the healthy control group.

Figure 3: Metabolomics signature in upper gastrointestinal (UGI) surgery patients across timepoints and healthy controls.

Serum levels of metabolites (A) Indole 3 acetic acid P glucuronide; (B) LysoPC; (C) Pyrogallol 2 O glucuronide; (D) Indole 3 lactic acid; (E) Hippuric acid; (F) S-Allylcysteine; (G) 6 hydroxy 3 oxo 8 tetradecenoic acid; (H) 3,4-Dimethyl-5-pentyl-2-furanpropanoic acid in UGI surgery patients pre-operatively , day2-5 post surgery and at hospital discharge (n = 20) compared with age and sex matched healthy controls (n =20). Overall group differences were assessed using a one-way ANOVA with multiple comparisons, comparing the mean of each UGI surgical patient timepoint with the mean of the healthy control group. Statistically significant: $p \leq 0.05$ indicated by *; $p \leq 0.01$ indicated by **.

Figure 4: Comparing pre-operative immunological age, inflammatory transcriptomic, and metabolic signatures between patients with and without pneumonia.

(A) Calculated IMM-AGE scores (B) Serum IL1RA cytokine levels (C) Serum metabolite levels of 3-Hydroxytetradecanoic acid (D) Corticosterone (E) Heat map showing mean expression levels of significantly differentially expressed anti-viral immunity , signalling

1 pathway, senescence and inflammation associated genes in PBMCs isolated from 12 age and
2 gender matched healthy controls and 6 UGI patients who developed pneumonia and 6 UGI
3 patients who did not develop pneumonia. Overall group differences between pneumonia and
4 non-pneumonia were assessed using an unpaired students T-test (parametric test) was used for
5 normally distributed data and Mann Whitney U tests were used (non-parametric test) when
6 data was skewed, when comparing two groups. Not statistically significant $p>0.05$ indicated
7 by ns. Statistically significant: $p\leq 0.05$ indicated by *

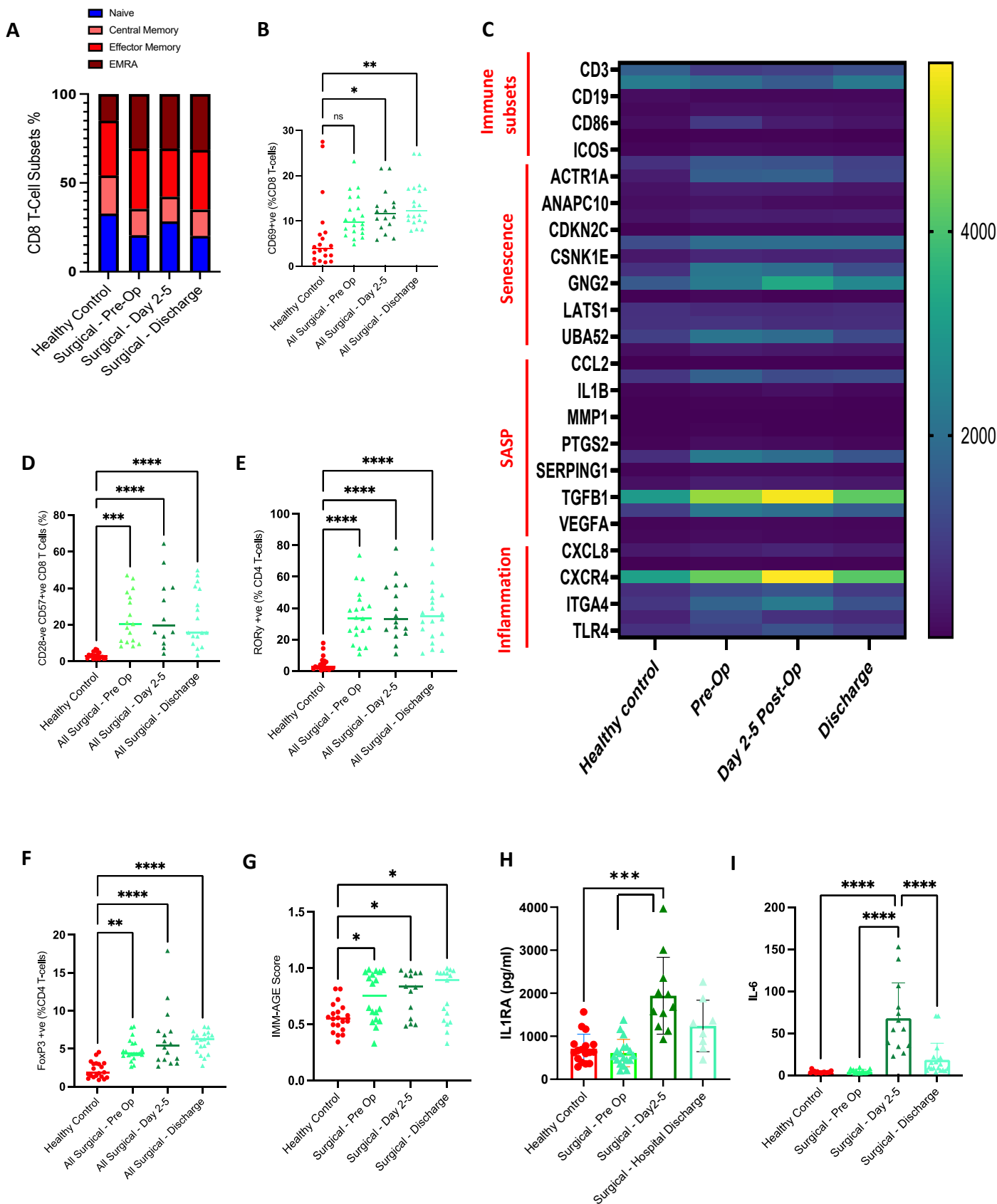


Figure 1: T cells subset distribution, transcriptomic signatures and serum cytokine levels in upper gastrointestinal (UGI) surgery patients (pre-operatively, day 2-5 post-operatively, hospital discharge) and healthy controls.

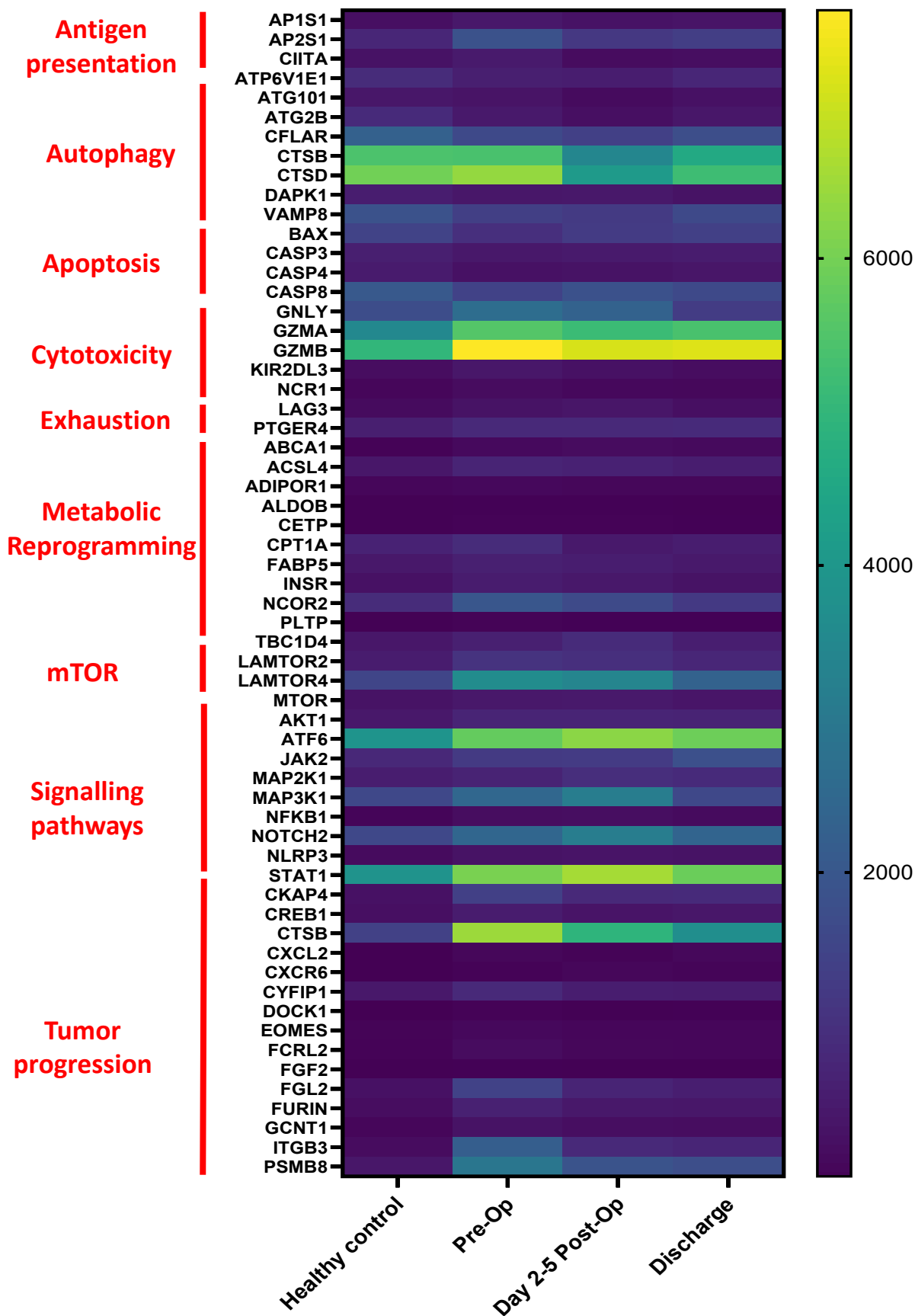


Figure 2: Transcriptomic signature in upper gastrointestinal (UGI) surgery patients (pre-operatively, day 2-5 post-operatively, hospital discharge) and healthy controls.

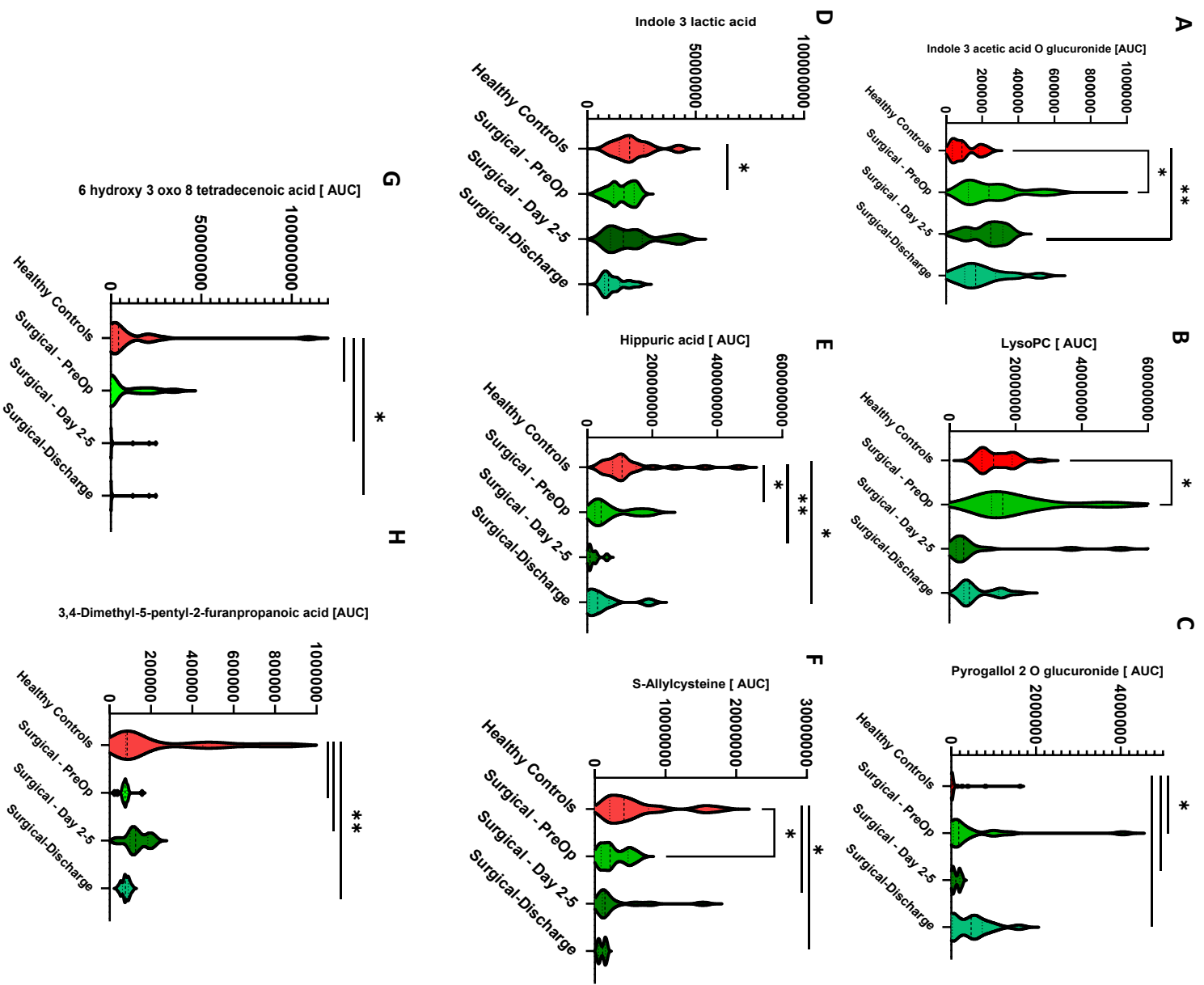


Figure 3: Metabolomics signature in upper gastrointestinal (UGI) surgery patients (pre-operatively, day 2-5 post-operatively, hospital discharge) and healthy controls.

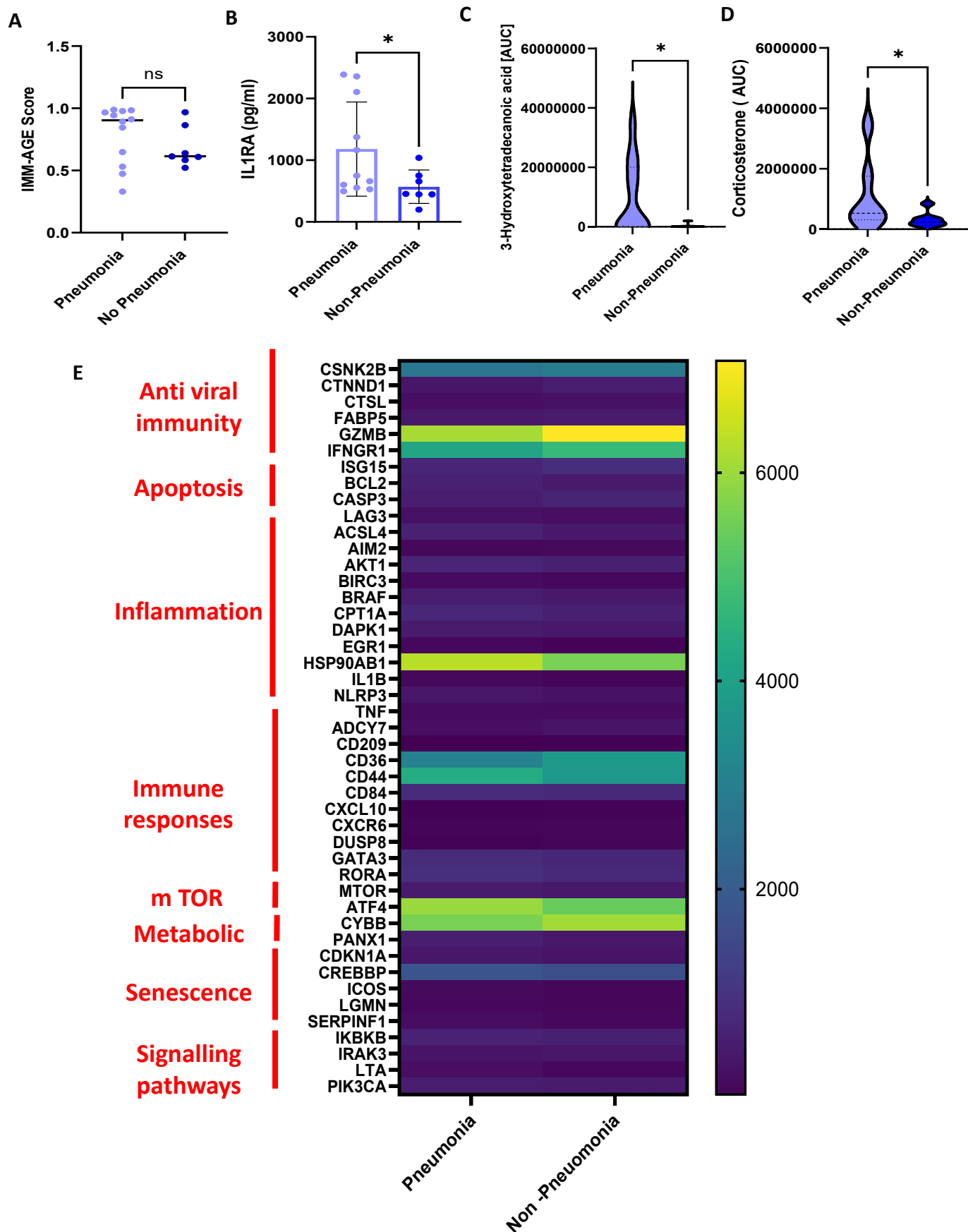


Figure 4: IMM-AGE, serum cytokines, metabolomic and transcriptomic signatures in upper gastrointestinal (UGI) surgery patients pre-operatively; post-operative pneumonia vs no pneumonia.

Supplementary Files

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