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Older Patients Are Immunocompromised by Cytokine Depletion and Loss of Innate Immune Function After HIP Fracture Surgery

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Abstract

Purpose/Introduction: We have examined the immune status of elderly patients who underwent surgery for a hip fracture, an injury associated with poor postoperative outcomes, to identify specific immune defects. **Methods:** In a cohort observational study, 16 patients undergoing surgery for hip fractures had immune function evaluation prior to surgery, and then at 3 and 7 days postoperatively, using flow cytometry for phenotype and for monocyte and granulocyte phagocytic function and respiratory burst. Serum samples were stored and batch analyzed using a human cytokine 25-plex panel. **Results:** We report significant loss of innate immune function, related specifically to reduced granulocyte numbers by day 7 ($P < .0001$, flow cytometry; $P < .05$ white blood cells), and although granulocyte ability to take up opsonized *Escherichia coli* was increased ($P < .05$), the ability of those cells to generate a respiratory burst was reduced at days 3 and 7 ($P < .05$). Monocyte respiratory burst was also significantly reduced ($P < .05$). Serum cytokine levels indicated very poor T-cell function. **Conclusion:** We have demonstrated that the antimicrobial immune response is profoundly reduced after surgery in elderly patients with hip fractures. The effect was sustained up to 7 days postoperatively, identifying these patients as particularly vulnerable to bacterial infections.

Keywords

cellular immunology, monocytes, innate immunity, cytokines, T cells

Introduction

Around 76 000 patients are admitted to hospital every year in the United Kingdom with a hip fracture. The National Health Service (NHS) costs are approximately £1.4 billion (total NHS budget for 2015/2016 is £115.4 billion, http://www.nhs.uk/NHSEngland/thenhs/about/Pages/overview.aspx, last viewed May 6, 2015), and this figure may be doubled when the social care costs related to the hip fracture are included.[1] Additionally, an aging population will continue to present a significant burden in the future.[2,3] Hip fractures occur most commonly in women older than the age of 60 years and are usually treated surgically. A large proportion of patients who have undergone surgical treatment of their fracture fail to attain their previous level of physical function with associated loss of quality of life.[1] There is also a 30-day mortality of 8% and a 1-year mortality of 30% recorded in the NHS-based Scottish Hip Fracture Audit.[4] To date, the effects of hip fracture on the immune system have been poorly documented, but impairments in natural killer (NK) cell and neutrophil activity have been suggested.[5] Of clinical importance is that following surgery, 30% of patients develop an infective complication during their hospital stay, including wound infections, urinary tract infections, and respiratory infections.[6,7] The reasons for this are unclear, but in addition to age-related immune dysfunction, the effects of the causative trauma, the surgery, blood transfusion, the anesthetic

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agents, and impaired nutrition may all be important. These factors are all recognized causes of suppression of the immune system, which predispose to the development of infection.[5,8]

The innate and adaptive immune responses are linked, and efficient functioning of both components is required to clear infections and maintain adaptive memory function as well as maintaining immune homeostasis through immune regulation and tumor surveillance. Any disturbance of the immune signaling processes involved is likely to affect the immune system efficiency and outcome of infection or injury. Changes in the adaptive immune response of healthy elderly humans are well documented, but how the innate immune system is affected by these changes is less well understood.[9,10] Despite involution of the thymus, which reaches 90% by age 40, there is no reduction in the number of T cells in the circulation, but T-cell receptor diversity is lost and proliferative and functional activity, including interleukin (IL) 2 production, is reduced. This is thought to be due to telomere erosion[11] and leads to loss of T-cell memory. Increased morbidity and mortality due to influenza virus, primary bacterial infections such as pneumonia, and reactivation of viral infections such as *Herpes zoster* is well documented[12,13] and has been attributed to loss of T-cell memory.[11] The response to vaccination, requiring cell-mediated adaptive immune function, is also often impaired.[14] In the absence of other health complications, adequate innate immune function appears able to compensate for loss of thymic activity,[15,16] and opportunistic infections in the older, healthy patient are not often seen.

The effect of aging on the innate immune system is less well understood. There are conflicting reports in the literature that may reflect differing health status and consequential effects of cytokine and chemokine production from the T-cell compartment. For example, interferon (IFN) γ is important for macrophage activation and nitric oxide (NO•) generation but reports on its continued production by aged immune cells vary.[17] Some studies have shown a nonspecific increase in the production of proinflammatory cytokines IL-6, IL-8, and tumor necrosis factor (TNF) α but a decrease in IL-1β.[18,19] Some NK cell subsets are reported to increase in number as $CD8^+$ cytotoxic T cells decline, while $CD56^{bright}$ NK cells are reported to decline,[20] and granulocyte function in vitro appears intact but impaired activation in vivo can lead to susceptibility to infection.[21] Dendritic cell function is preserved, and in the absence of disease, innate immune function protects against many pathogens.[22]

With increased longevity, efforts are now being made to understand the complexity of the ageing immune system and to intervene to improve outcomes for patients with infections or undergoing surgery. Recently, studies have confirmed the increased prevalence of malnutrition in elderly patients,[23,24] and we have also confirmed this in our patients with hip fractures.[25] The nutritional status of a patient undergoing surgery predicts in part subsequent postoperative morbidity and mortality,[26] but targeted nutrition, to include certain key nutrients, for example, amino acids (arginine and glutamine), ribonucleic acid/polyribonucleotides, and essential fatty acids, can boost immune function and improve outcomes.[26]

Table 1. Patient Demographics and Comorbidities.

Characteristic	Patient
Age, mean (range)	74 (65-83) yrs
Sex, no (%)	
Female	16 (100%)
Male	0 (0%)
BMI, mean (range)	26.2 (18.8-34.9)
MSQ (/10): no (%)	
9	8 (50%)
0	8 (50%)
Comorbidities: no (%)	
Hypertension	12 (75%)
Raised lipids	8 (50%)
Hypothyroid	4 (25%)
Asthma	3 (19%)
Gastritis	2 (13%)
CVD	2 (13%)
IHD	1 (6%)
AF	1 (6%)
DVT	1 (6%)
Renal impairment	1 (6%)

Abbreviations: BMI, body mass index; MSQ, Mental Status Questionnaire; CVD, cerebrovascular disease; IHD, ischemic heart disease; AF, atrial fibrillation; DVT, deep vein thrombosis.

The purpose of this study was to examine the immunological status of patients undergoing surgery for hip fractures and identify postoperative changes in immune function, which may compromise recovery in this vulnerable patient cohort and act as targets for rational therapeutic intervention.

Methods

Patients

This was a cohort observational study, in which a series of patients who underwent surgery for hip fractures were followed. All had their care at the Trauma Unit, Department of Orthopaedic Surgery at Aberdeen Royal Infirmary between the period October 19, 2009, and August 11, 2010. The patients were postmenopausal female patients with an age of 60 to 85 years and a mental status questionnaire score of at least 8 of 10. Patients taking corticosteroids and those with diabetes mellitus, malignancy, and/or a pathological fracture, other than due to osteoporosis, were excluded. Patient details are shown in Table 1, and demographics including comorbidities were recorded. All patients gave signed, informed consent prior to participating in the study.

Immune function was evaluated prior to surgery, on the day following surgery and then at between days 3 and 7 postoperatively. A total of 2 mL of blood was required for serum/plasma and 7 mL of blood for tests of immune function at each time point. Anthropometric data (height and weight) were recorded. Biochemical assays included serum urea and electrolytes, total proteins, serum albumin, transferrin and retinol binding protein, liver enzymes, and plasma amino acid profiles.

Phenotypic Analysis of Peripheral Blood Leukocytes

Samples of peripheral blood collected in heparin tubes were stained and analyzed in 2 panels by 6 color flow cytometry using standard protocols. Panel 1 for analysis of lymphoid populations comprised directly conjugated murine antihuman monoclonals CD45 PerCP (#345809), CD3 Alexa 700 (#557943), CD69 PE (#555531), CD4 Alexa 488 (#557695), CD8 AmCyan (#339188), and CD19 APC-H7 (#641395). Panel 2 for analysis of myeloid cell populations comprised CD45 PerCP, CD3 Alexa700, CD69 PE, CD14 pacific blue (#558121), CD56 Alexa 647 (#557711), and CD11b APC-Cy7 (#557754). All antibodies were from BD Biosciences (Oxford, United Kingdom), and data from 50 000 events were acquired using a Becton Dickinson LSR II flow cytometer (BD Biosciences, San Jose, California) equipped with blue (488 nm), red (633), and violet (405) lasers and DIVA 6.11 software. FlowJo software was used for analysis of data. Instrument set-up used unstained samples to set voltages and BD CompBeads (BD Biosciences) and fluorescence minus 1 controls to adjust compensation and to set gates to exclude dead or clumped cells. Lymphocyte, monocyte, and granulocyte populations were gated using forward and side scatter, and T cells and B cells were identified by CD3 and CD19 fluorescence, and CD4 and CD8 cells analyzed from the CD3-gated population. In panel 2, myeloid cells within monocyte and granulocyte gates were identified as $CD14^+CD11b^+$ monocytes, $CD14^-CD11b^+$ granulocytes, or $CD3^-CD59^+$ NK cells.

Functional Analysis of Leukocytes

To measure the efficiency of leukocyte function, levels of phagocytic activity (numbers of cells taking up bacteria and the quantity of bacteria ingested per cell) were measured using the Phagotest kit (Orpegen Pharma, D-69115 Heidelberg, FRG, Germany) according to manufacturer's instruction. This measures the percentage of leukocytes ingesting fluorescent fluorescein isothiocyanate (FITC)-labeled *Escherichia coli* and the number of bacteria ingested per cell. Briefly, after ingestion of bacteria at 37°C and cooling on ice to halt phagocytosis, a fluorescence quenching solution was added to eliminate FITC signal from bacteria not fully internalized by the cell. A lysing solution was then added to remove erythrocytes while fixing leukocytes, and a DNA stain was added to discriminate between bacteria and blood leukocytes. Samples were then analyzed by flow cytometry using BD FACSCalibur cytometer with a 488 nm argon-ion laser. Leukocytes were gated using diploid cell DNA signal and monocyte and granulocyte gates set using forward and side scatter. The percentage of each cell type that had phagocytosed FITC-labeled bacteria was counted, and mean fluorescent index (MFI) of these cells used to correlate with number of bacteria per individual cell.

Quantitative analysis of the leukocyte oxidative burst was made using the Phagoburst kit (Orpegen) according to manufacturer's instructions. Briefly, 3 stimulants (*E coli*, phorbol 12-myristate 13-acetate [PMA], and low stimulus N-Formylmethionine-leucyl-phenylalanine [fMLP]) were added to whole blood at 37°C to induce different levels of oxidative burst activity compared to unstimulated control samples. Formation of reactive oxidants was then detected by addition of dihydrorhodamine 123, which produces fluorescent rhodamine 123 upon oxidation. Adding lysing solution, to remove erythrocytes and fix leucocytes, stopped the reaction. A DNA stain was then added to discriminate between bacteria and blood cells. The proportion of monocytes and granulocytes producing oxidative metabolites, plus the level of enzymatic activity in individual cells (MFI rhodamine 123), was then measured using flow cytometry as described earlier.

Serum Cytokine Measurements

Serum samples were stored frozen for batch analysis using the Invitrogen human cytokine 25-plex panel (Lot No. 711252 Invitrogen Life Technologies, Paisley, PA4 9RF United Kingdom) and the Bio-Plex-200 system (BioRad, Hemel Hempstead, United Kingdom). The kit contains optimized reagents to measure human IL-1β, IL-1RA, IL-2, IL-2R, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p40/70, IL-13, IL-15, IL-17, TNF-α, IFN-α, IFN-γ, granulocyte-macrophage colony-stimulating factor (GM-CSF), macrophage inflammatory protein (MIP) 1α, MIP-1β, Interferon-gamma-inducible protein 10 (IP10), Monokine Induced by Gamma-Interferon (MIG), CCL11 (Eotaxin), CCL5 (Regulated on Activation, Normal T Expressed and Secreted [RANTES]), and monocyte chemoattractant protein-1 (MCP-1). Each sample was measured against a standard curve and results expressed as pg/mL.

Statistics

Results were expressed as means ± standard deviation (SD), where means ± standard error of the mean are used, which is noted in the text. The precise number of samples/replicates in each analysis is detailed in the figure legends. Significant differences between the groups were assessed by Student *t* test (unpaired, Welch correction) or by Mann-Whitney *U* test where group sizes differed. $P < .05$ was considered significant.

Results

Phenotypic Analysis of Blood Leukocyte Subsets

The 16 female patients recruited for the study had a mean age of 74 years (range 65-83years) and had a mean body mass index of 26.2 (range 18.8-34.9). We examined the proportions of $CD4^+$ and $CD8^+$ T cells, $CD19^+$ B cells, $CD3^+$ $CD59^{bright}$ NK cells, $CD11^+$ $CD14^+$ monocytes, and $CD11^+$ $CD14^-$ granulocytes to identify any gross abnormalities or changes in leukocyte subpopulations on admission or after surgery. Overall, the proportions of T cells, B cells, NK cells, and granulocytes were within the expected range and showed no major deviations from the expected range over the sampling period.[27] Figure 1A indicates a relative increase in the percentage of lymphocytes in samples taken 7 days postoperatively compared with day 3 postoperative samples

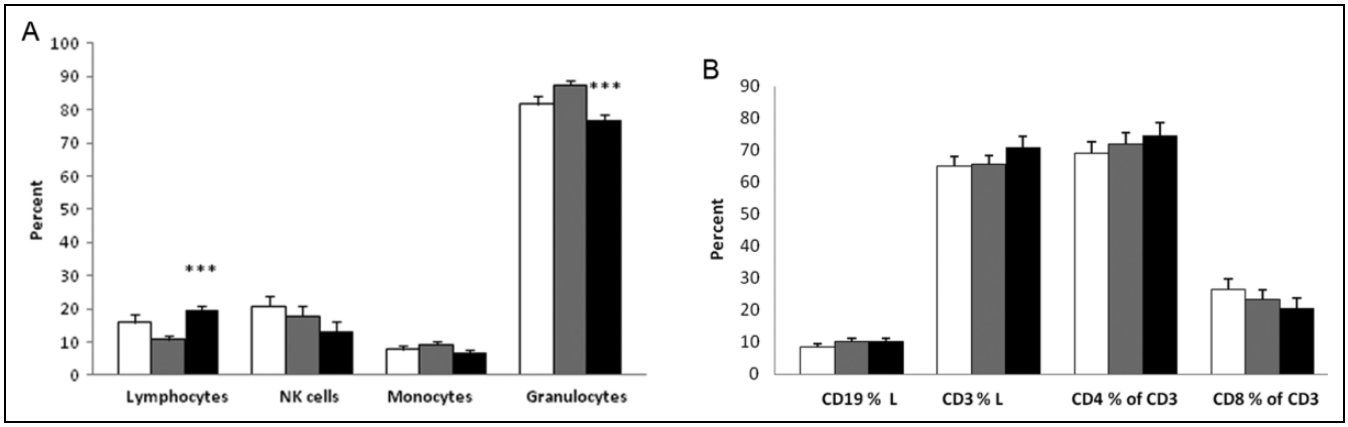

Figure 1. Phenotypic analysis of leucocyte subsets. A, Flow cytometric analysis of major peripheral blood leucocyte subsets; lymphocytes ($CD3^+CD19^+$), NK cells ($CD3^-CD59^+$), monocytes ($CD11b^+$ $CD14^+$), and granulocytes ($CD11b^+CD14^-$). B, Further analysis of gated lymphocytes, $CD19^+$ B cells as a percentage of all lymphocytes, $CD3^+$ T cells as a percentage of all lymphocytes, $CD4^+$ T helper cells as a percentage of $CD3^+$ T cells, and $CD8^+$ cytotoxic T cells as a percentage of $CD3^+$ T cells. For each analysis, 10 000 events were acquired. Data shown as means ± SEM of at least 9 samples. White bars, preoperation; grey bars, day 3 postoperation; black bars, day 7 postoperation. ***$P < .0001$ compared to day 3 postoperation value, Student t test. NK indicates natural killer; SEM, standard error of the mean.

Table 2. Whole Blood Cell Counts ($\times 10^9$/L).

	WBC	Lymphocytes	Monocytes	Neutrophils	Eosinophils	Basophils
Preop	9.8 ± 2.1	1.16 ± 0.5	0.44 ± 0.1	7.9 ± 2	0.14 ± 0.18	0.02 ± 0.01
Postop day 3	9.7 ± 2.5	0.9 ± 0.4[a]	0.49 ± 0.17	8.1 ± 2.1	0.11 ± 0.12	0.022 ± 0.02
Post op day 7	8.2 ± 2.7	1.2 ± 0.6	0.45 ± 0.16	6.2 ± 2.4[b]	0.19 ± 0.09[c]	0.018 ± 0.014

[a] $p < 0.05$ compared with preoperation value.
[b] $p < 0.05$ compared with postoperation day 3 value.
[c] $p < 0.02$ compared with postoperation day 7 value.

(19.45 ± 6.5 vs 10.8 ± 4.4 SD; $P = .0001$, Student t test) and a relative decrease in granulocytes at day 7 compared with day 3 samples (87.4 ± 5.3 vs 77.0 ±7.5; $P = 0.0001$). This decrease in granulocyte number was confirmed by the white blood cell (WBC) counts as shown in Table 2 ($P < .04$). With the exception of lymphocytes (1.16 ± 0.5 × 10^9/L), which were low, WBC counts were within the normal range. These counts also showed a drop in lymphocyte numbers at day 3 postoperation ($P < .05$), which recovered to preoperative levels by day 7. Eosinophil numbers also reduced at day 3 with recovery by day 7 ($P < .02$). No significant changes in NK cell, B cell, or T-cell populations or CD4–CD8 ratios (4.3 ± 4.0 prior to surgery vs 6.6 ± 7.1 at day 7 postoperation) were noted (Figure 1B).

Phagocytic and Respiratory Burst Function of Granulocytes and Monocytes Postsurgery

Quantitative assessment of phagocytic function was made using fluorescently labeled opsonized bacteria (*E coli*-FITC) and the percentage of monocytes or granulocytes able to take up the bacteria as well as the efficiency of uptake (numbers of bacteria engulfed) was measured by flow cytometry. Figure 2A shows an increase in the percentage of granulocytes capable of ingesting bacteria over time and this is significant by day 7 (86.2 ± 3.3 vs 96.3 ± 1.0; $P < .02$), and although there is a trend for greater efficiency of uptake, this did not reach significance (Figure 2C). However, when the ability of these cells to generate an oxidative burst was tested, this was found to be significantly reduced by day 3 ($P < .04$) compared to preoperative values, and this reduction in function was still evident at day 7 ($P < .05$; Figure 3B). No difference in the ability of monocytes to take up bacteria was found (Figure 2B and D), but oxidative burst function was also impaired in these cells at day 3 postoperatively ($P < .04$) and at day 7 ($P < .03$; Figure 3A). No difference in respiratory burst activity over the sampling period was observed in either monocytes or granulocytes stimulated with fMLP (low level receptor mediated stimulus) or PMA (high-level receptor independent priming stimulus; data not shown).

Serum Cytokine Measurements

Serum cytokine measurements were made using luminex technology and a 25-plex panel designed to cover all main immune pathways and identify dominant or dysfunctional cytokine signatures. Samples were taken prior to surgery and on days 3 and 5 postsurgery. The results for cytokines giving values above background are shown in Table 3. Cytokines below the level of detection were IFN-γ, TNF-α, GM-CSF, MIG (CXCL9),

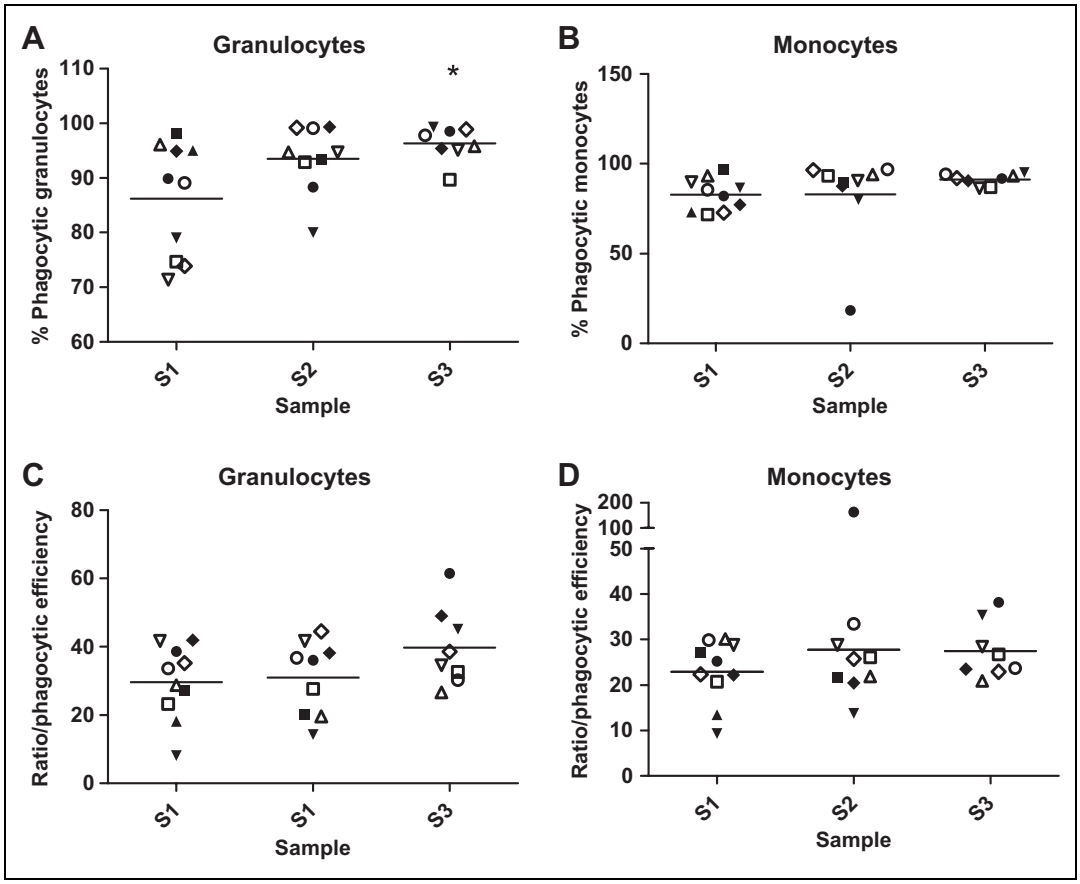

Figure 2. Phagocytic function of patient granulocytes but not monocytes increases postoperatively. The percentage of granulocytes (A) and monocytes (B) able to phagocytose fluorescently labelled *Escherichia coli* was measured using flow cytometry, and the efficiency of uptake quantified as the numbers of fluorescent particles phagocytosed by granulocytes (C) and monocytes (D). A data point for each patient (N = 9) is shown at preoperation (S1), day 3 postoperation (S"), and day 7 post-operation (S3). The bar shows the mean value for the group. *$P < .02$ compared with preoperation value S1, Student *t* test, Welch-corrected.

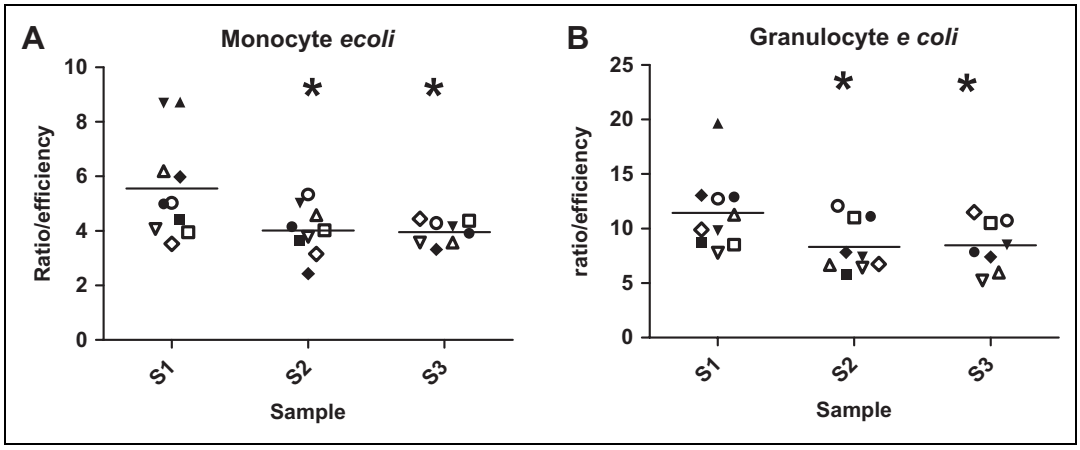

Figure 3. Efficiency of respiratory burst by monocytes and granulocytes is reduced postoperatively. Quantitative determination of the respiratory burst of monocytes (A) and granulocytes (B) in response to uptake of *Escherichia coli* measured by flow cytometric analysis of fluorescent reactive oxidants. Data expressed as a ratio of mean fluorescent intensity (MFI) of test versus control. A data point for each patient (N = 9) is shown at preoperation (S1), day 3 postoperation (S2), and day 7 postoperation (S3). The bar shows the mean value for the group. *$P < .05$ compared with preoperation values S1, Student *t* test, Welch-corrected.

Table 3. Patient Serum Sample Cytokine Levels (pg/mL).[a]

	IL-6	IL-8	MIP-1α	MIP-1β	MCP-I	IL-1RA	RANTES	EOTAXIN	IL-12	IFN-α	IP10	IL-2R
Preop	812 ± 165[a]	1835 ± 857	101 ± 13	219 ± 192	996 ± 209	770 ± 84	15579 ± 2335	103 ± 21	272 ± 14	129 ± 11	65 ± 10	420 ± 73
Postop day 3	1333 ± 224	1647 ± 825	98 ± 19	192 ± 36	729 ± 96	734 ± 58	17913 ± 1843	70 ± 9	251 ± 9	120 ± 10	60 ± 7	553 ± 78
Postop day 7	1042 ± 409	6188 ± 2419	677 ± 349	854 ± 443	670 ± 97	857 ± 61	24472 ± 4406	98 ± 12	269 ± 10	134 ± 12	55 ± 5	755 ± 131[b]

Abbreviations: IFN, interferon; TNF, tumor necrosis factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin.
[a]Cytokines below level of detection: IFN-γ, TNF-α, GM-CSF, MIG, IL-1β, IL-2, IL-4, IL-5, IL-7, IL-10, IL-13, IL-15, and IL-17.
[b]$P < .05$ compared with preoperation value (unpaired student *t* test, Welch correction).

IL-1β, IL-2, IL-4, IL-5, IL-7, IL-10, IL-13, IL-15, and IL-17. Where cytokines or chemokines could be detected, MIP-1α (CCL3), MIP-1β (CCL4), and IL-2R showed a trend for increased levels in samples taken 7 days postoperation. Due to wide variations between individuals at each sampling point, this only reached significance for IL-2R ($P < .04$). Such variation has been previously observed and attributed to underlying medical conditions or genetic influences.[28] Interleukin 8 and CCL5 were markedly upregulated by day 7, but IL-6 and MCP-1, which would be expected to be elevated following surgery,[28] as well as IL-1RA, and CCL11 were present within the normal range and showed no change over the sampling period. Interleukin-12, IFN-α, and IP-10 also showed no change but were present at relatively low levels within the serum.

Discussion

In this study, we have undertaken the first detailed examination of the immune status of elderly female patients who had a hip fracture and underwent surgery, a group known to be vulnerable to infection and poor postoperative outcomes. As the majority of patients with hip fractures presenting to our unit who were able to give consent to participate were women, and our study numbers inevitably small due to laboratory costs, we elected to include only women in order to achieve a more homogeneous study population. Loss of immune function in the elderly patients is well documented, and much research has focused on the decline of adaptive immune responses following loss of thymic function. However, the data are conflicting, possibly due to confounding comorbidity factors in the elderly patients leading to increased chronic inflammatory disease, increased susceptibility to infection, and resistance to effective immunization protocols.[27] The Senior European (SENIEUR) protocol was adopted to provide reference values of immune function in healthy elderly individuals, but our understanding of immune vulnerability in "at risk" groups is still limited, particularly with respect to changes in innate immune function.[15,29,30] Research in mouse models, designed to understand mechanisms and devise therapies to reverse immunosenescence, has provided useful insights but must be interpreted with caution due to fundamental differences in the aging process between mice and humans.[31]

In this study, using a similar multiplex analysis approach, serum levels of IL-6 (>1000 pg/mL) and IL-8 (>4000pg/mL) were considerably higher than levels reported in healthy older adults (<100 pg/mL), and the levels recorded here are consistent with an innate response to the trauma of injury and surgery. Naive T cells produce less IL-2 in the aged,[32,33] and lack of IL-2 or indeed other T cell associated cytokines (IL-4, IL-5, IL-13, IL-17, IFN-γ, and TNF-α) observed in this study would suggest that T-cell function was severely compromised in these patients.[28,34,35] Cytokines are intimately linked to immune responsiveness, and certain gene polymorphisms have been linked to longevity, underlining their importance in maintaining health.[28,36] Phenotypic analysis of circulating leukocytes showed normal CD4/CD8 ratios, with populations of B cells,

NK cells, and monocytes all present. Dysfunctional T-helper cell differentiation would also compromise B cell function and adaptive immunity, although this was not measured in this study. Data also indicated a small but significant decrease in the proportion of circulating granulocytes 7 days postoperatively (Figure 1A), which was confirmed by WBC counts (Table 2). A possible explanation is the high susceptibility of neutrophils to apoptosis. They require GM-CSF and IL-2 or Toll-like receptor (TLR) stimulation to sustain viability in the circulation; both of these cytokines were absent at all time points, despite the trauma of fracture and surgery (Table 1). In particular IFN-γ, secreted by activated T cells and NK cells, and another vital cytokine required for granulocyte survival and function, was also notably absent in the serum. Natural killer cell numbers have been reported to increase in the elderly individuals,[37] and in this study, there was a trend for decreased numbers of $CD3^{+}$ $CD59^{bright}$ NK cells during the postoperative period but this did not reach significance but may have affected IFN-γ levels. In contrast, IL 8 and CCL5 (RANTES), which can be secreted by many tissue types and are associated with chemotaxis and activation of neutrophils and other granulocytes such as basophils and eosinophils and NK cells,[38,39] were both strongly upregulated in the postoperative period in some patients. This may represent an attempt to mobilize the innate immune system in response to injury and surgery.[40,41]

Additional evidence for some activation of the innate immune response postsurgery would be the increase in the percentage of granulocytes able to take up opsonized bacteria by day 7 (Figure 2A) but this was not matched by increased effector function, as the ability to produce the respiratory burst necessary to kill ingested microbes was significantly reduced by day 3 in both granulocytes and monocytes, and this lack of function was sustained at day 7 (Figure 3). The percentage of cells able to produce an oxidative burst was within the normal range (data not shown), so defective TLR may not be the explanation,[29] but the very low levels of oxidizing enzymes generated may be due to abnormally low IFN-γ and TNF-α production evidenced by serum cytokine values.

This is a small pilot study of immune function in a patient group that has not been analyzed in this way before. Although we used standardized, commercially available kits, it is not possible to remove completely the potential for day-to-day variability in the analysis. We have also not been able to correlate the laboratory analysis to clinical outcomes, due to small numbers studied.

In conclusion, we have demonstrated that the ability to mount effective antimicrobial immune responses is reduced in elderly patients undergoing surgery for hip fractures. The effect is sustained up to 7 days postoperatively, identifying these patients as particularly vulnerable to infection, including urinary sepsis, respiratory tract infection, as well as wound infection. Such infective complications contribute to poor outcomes in frail, elderly patients. These patients may constitute a potential population for targeted immune stimulation, but more work is required to clarify the specific impacts of the attenuated immune responses noted.

Authors' Note

This work was conducted at University of Aberdeen.

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Declaration of Conflicting Interests

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