Preoperative systemic inflammation and perioperative myocardial injury: prospective observational multicentre cohort study of patients undergoing non-cardiac surgery

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Running title: Inflammation and perioperative myocardial injury.

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Abstract

Background: Systemic inflammation is pivotal in the pathogenesis of cardiovascular disease. Since inflammation can directly cause cardiomyocyte injury, we hypothesized that established systemic inflammation, as reflected by elevated preoperative neutrophil-lymphocyte ratio (NLR) >4 (a threshold predictive of cardiovascular morbidity/mortality) predisposes patients to perioperative myocardial injury.

Methods: We prospectively recruited 1652 patients aged ≥45 years who underwent non-cardiac surgery in two UK centres. Serum high sensitivity troponin T concentrations (hsTnT) were measured on the first three post-operative days. Clinicians and investigators were blinded to the troponin results. The primary outcome was perioperative myocardial injury, defined as hsTnT ≥14ng.L^{-1} within three days after surgery. We assessed whether myocardial injury was associated with preoperative NLR>4 and/or activated ROS generation in circulating monocytes. Multivariable logistic regression analysis explored associations between age, gender, NLR, Revised Cardiac Risk Index (RCRI), individual leukocyte subsets (monocytes, eosinophils) and myocardial injury. Flow cytometric quantification of ROS in activated circulating monocytes was correlated with NLR (n=21 patients). Data are presented as n (%) or odds ratios (OR:95% confidence intervals).

Results: Preoperative NLR>4 was present in 239/1652 (14.5%) patients. Myocardial injury occurred in 405/1652 (24.5%) patients and was more common in patients with preoperative NLR>4 (OR:2.56 [1.92-3.41]; p<0.0001). Myocardial injury was independently associated with lower absolute preoperative lymphocyte count (OR:1.80 [1.50-2.17];p<0.0001) and higher absolute preoperative monocyte count (OR:1.93 [1.12-3.30];p=0.017). Monocyte ROS generation correlated with NLR (r=0.47; p=0.03).

Conclusions: Preoperative NLR>4 is associated with perioperative myocardial injury, independent of conventional risk factors. Systemic inflammation may contribute to the

development of perioperative myocardial injury.

Introduction

Asymptomatic myocardial injury occurs in more than 30% of patients undergoing non-cardiac surgery, [1, 2] Even apparently minor elevations in troponin are associated with prolonged hospitalisation and higher mortality. The extent and severity of coronary artery disease, however, does not correlate closely with the occurrence of perioperative myocardial injury.[3] Furthermore, pharmacological interventions that are effective in acute coronary syndromes do not prevent myocardial infarction after surgery,[4-6] although longer-term adverse cardiac outcomes may be improved by intensification of medical management for acute coronary syndrome.[7, 8] These data suggest that pathophysiological mechanisms other than ischaemia and/or thrombosis may contribute to perioperative myocardial injury.

Systemic inflammation plays a major role in the development of cardiovascular disease.[9-11] Inhibition of interleukin (IL)-1β, a cytokine that is pivotal to pro-inflammatory pathways, reduces cardiovascular events in patients with established cardiac disease.[12] Mediators of systemic inflammation directly injure cardiomyocytes[13] and/or modulate their response to damage.[14, 15] The systemic inflammatory response is an important contributor to myocardial injury, and to damage of other organs, after myocardial infarction and cardiac surgery. The extent to which systemic inflammation is involved in the pathogenesis of myocardial injury after non-cardiac surgery is not known.

The neutrophil-lymphocyte ratio (NLR) is a readily available and inexpensive marker of systemic inflammation driven by elevated levels of circulating cytokines which have been shown to modulate myocardial injury.[16, 17] NLR enhances the Framingham-based risk prediction of cardiovascular mortality,[18] with higher NLR associated with adverse outcomes after acute coronary syndromes[19] and decompensated heart failure.[20] NLR is also associated with worse perioperative outcomes,[21] albeit for ill-defined, mechanistically unclear reasons.

Other leukocyte subsets, including higher monocyte counts,[22] and lower eosinophil counts,[23] may also contribute to the development of cardiovascular morbidity.

Here, we hypothesized that the development of perioperative myocardial injury may be modified by mechanisms invoked by systemic inflammation. We assessed prospectively whether systemic inflammation, as reflected by elevated preoperative NLR and/or leukocyte subsets implicated in the pathogenesis of cardiomyocyte dysfunction, may promote perioperative myocardial injury. As a further test of this hypothesis, we assessed whether circulating monocytes, which are pivotal for the repair of myocardial injury, generated more injurious ROS when activated ex vivo in patients with higher NLR.

Methods

We report the results of a prospectively designed sub-study from two UK centres which captured leukocyte data where investigators, patients and healthcare providers were blinded to troponin results throughout the study period. The study was approved by UK NRES Committee London (MREC:10/WNo03/25). It was conducted in accordance with the principles of the declaration of Helsinki and institutional guidelines. Participants undergoing elective noncardiac surgery gave written informed consent before surgery.

Participants

Participants were aged 45 years or older and underwent non-cardiac surgery under general or regional anaesthesia at The Royal London Hospital (Barts Health NHS Trust) or at University College Hospital (University College London Hospitals NHS Foundation Trust) who required at least one night in hospital after surgery. Participants were excluded if they refused consent or if they had previously enrolled in the VISION study. [1]

Data Collection

Researchers collected a detailed and standardised dataset from patients and their medical records, before and during the 30 days after surgery, including postoperative morbidity (defined by PostOperative Morbidity Survey and Clavien-Dindo grading). Full definitions of the variables included in this analysis are documented in the supplementary file. Blood samples were taken before, between 6-12 hours after the end of surgery, and on postoperative days one, two and three.

Exposures

Leukocyte subsets were measured preoperatively by observers masked to troponin results (Sysmex XE2100 analyzer, Sysmex, Milton Keynes, UK). NLR >4 was defined prospectively as an established median threshold value associated with subclinical inflammation and adverse clinical outcomes derived from studies totalling 40,559 patients.[24] Moreover, a population-based study reported mean NLR 1.76 (95% confidence intervals: 0.83–3.92) in >8500 participants.[25] We assessed the contributions of specific leukocyte subsets to perioperative myocardial injury using absolute counts and thresholds associated with cardiovascular risk in the general cardiovascular literature. Lymphopaenia was defined as $<1x10^9$ lymphocytes.L^{-1}. [26] The following thresholds for absolute subset counts were used: neutrophil count $\geq 6x10^9$cells.L^{-1},[22] eosinophil count <0.05 cells.10^9L^{-1},[23] and monocyte count $\geq 0.7x10^9$cells.L^{-1}. [22]

Primary outcome

The primary outcome measure was perioperative myocardial injury defined as serum high sensitivity troponin T concentration ([hs TnT]) ≥14ng.L^{-1}, measured by high sensitivity troponin T assay (Elecsys, Roche, Basel, Switzerland) within three days after surgery. This hsTnT assay enables the detection of cTnT at the 99th percentile of an apparently healthy reference population with <10% variability, with a 5ng/L limit of detection.[27] We did not seek to define ischaemic versus non-ischaemic causes of hsTnT elevation, since elevated troponin is linked to poorer clinical outcomes regardless of its aetiology.[28] The secondary clinical outcome was length of hospital stay, stratified by NLR</>4 and/or hs-TnT ≥14ng.L^{-1}.

Preoperative monocyte reactive oxygen species generation

Preoperative monocyte reactive oxygen species (ROS) generation was measured by flow cytometry in a subset of 21 patients. Investigators undertaking flow cytometry were masked to NLR values. Monocytes were identified by forward/side scatter characteristics and CD14+ CD16- surface staining (Supplementary Figure 1). Using fresh whole blood samples obtained preoperatively, loaded with dihydrorhodamine (DHR)-123 (10μm), monocyte ROS were quantified after dimethyl sulfoxide (control) or phorbol 12-myristate 13-acetate (PMA) incubation for 10 minutes at 37°C in a CO_2 incubator (Phagoburst, Orpegen, Heidelberg, Germany).[29] The ROS-reactive dye DHR is converted to cationic green fluorescent rhodamine-123 upon oxidation by PMA, trapping it intracellularly. Median fluorescence intensity was quantified by flow cytometry (CyAn ADP flow cytometer, Beckman Coulter, Wycombe, UK). PMA-induced ROS was expressed as fold-change over each individual's unstimulated (time-matched control) sample.

Statistical analysis

The statistical analysis was prospectively planned and registered on a public database (Research Registry:3927). We used NCSS 11 (Utah, USA) and STATA version 14 (StataCorp LP, Texas, USA) to analyse the data. We ordered the sample according to integer values of NLR</>4 and stratified the baseline characteristics of the cohort according to this threshold. Binary data were expressed as percentages, normally distributed continuous data as mean with standard deviation (SD), and non-normally distributed continuous data as median with interquartile range (IQR). We used multivariable logistic regression analysis to test for associations between leukocyte subsets (absolute counts) and perioperative myocardial injury, taking into account established conventional risk factors (age, gender, and the Revised Cardiac Risk Index (RCRI),).[30-33] The selection of covariates was based on prior evidence of association with the dependent variable or similar clinical outcomes, rather than using univariable analysis or p-value based approaches.[34, 35] Covariates were treated as categorical variables. Missing data were handled by list-wise deletion. Fold-change in monocyte ROS (stimulation/basal ROS levels) was correlated with NLR using simple linear regression.

Sample size estimation

Our previous work in four UK centres showed that preoperative NLR>4 is present in approximately 20% of patients undergoing noncardiac surgery.[36] Observations from the main VISION study, suggest that ~25% of patients develop hsTnT ≥14ng.L^{-1} within 72h after surgery.[1] We therefore estimated that at least 1554 patients would be required to detect a 10% absolute difference in incidence of hsTnT ≥14ng.L^{-1} within 72h after surgery between patients with and without preoperative NLR>4 (α=0.05; 1-β=0.9; estimated dropout rate of ~7%). For the monocyte ROS experiment, we estimated a total sample size ≥19 subjects would be required, assuming a correlation coefficient r=0.6 (α=0.05; 1-β=0.8).

Results

The study included 1682 patients, who were recruited between March 2011 and May 2014. (Figure 1). Preoperative NLR >4 was present in 239 (14.2%) patients (Supplementary Figure 2). A RCRI score >2 was more common in 52/239 (21.8%) patients with NLR>4 (odds ratio: 2.36 (95% CI: 1.66-3.36); p<0.001), but age, gender and other perioperative and preoperative cardiovascular parameters were similar (table 1). Measures of frailty, cardiovascular medication or surgical subspecialty were similar as stratified by NLR>4 (Supplementary figures 3-5).

Primary outcome: myocardial injury and neutrophil-lymphocyte ratio

Perioperative myocardial injury was sustained in 405/1652 (24.5%) patients. Signs and/or symptoms of myocardial ischaemia were reported in 2/405 (0.49%) patients. Median troponin rise was 18 (14-26) $ng.L^{-1}$ in patients with troponin $\geq 14 ng.L^{-1}$ within 72h after surgery. No patients required coronary angiography during the same hospital stay. Perioperative myocardial injury was more frequent in 99/239 (41.4%) patients with preoperative NLR>4, compared to 306/1413 (21.7%) patients with NLR ratio <=4 (odds ratio:2.56 [95%CI:1.92-3.41]; p<0.0001). Adjusting for RCRI, age and gender, NLR was independently associated with perioperative myocardial injury either when dichotomised by NLR</>4 (odds ratio:2.27 (95%CI:1.67-3.09); p<0.001) or considered as a continuous variable (odds ratio:1.06 (95%CI:1.02-1.10); p<0.001); Supplementary Tables 1-3). The combination of high preoperative NLR and myocardial injury was associated with prolonged length of stay (hazard ratio:1.45 (95%CI:1.17-1.80); p<0.001; Figure 2). This relationship was similar across surgical subspecialties (Supplementary Figure 6).

Leukocyte subsets and myocardial injury

Using thresholds described in the wider cardiovascular literature, we found that myocardial injury was more frequent among 45/105 (42.6%) patients with preoperative lymphopaenia, compared to 345/1549 (22.3%) patients with a lymphocyte count >$1x10^9$ cells.L^{-1} (odds ratio:2.62 (95%CI:1.75-3.92); p<0.0001). Similarly, a higher proportion of patients with an absolute neutrophil count >$6x10^9$cells.L^{-1} (88/294; 29.9%) sustained myocardial injury (odds ratio:1.5 (95%CI:1.14-1.99); p<0.0001). For monocytes, myocardial injury was more frequent in 129/440 (29.3%) patients with a monocyte count ≥$0.7x10^9$cells.L^{-1}, [22] compared to 261/1213 (21.5%) patients with a monocyte count <$0.7x10^9$cells.L^{-1} (odds ratio:1.51 (95%CI:1.18-1.94); p<0.0001). No relationship was found between previously described thresholds for eosinophil count (<0.05 cells.10^9L^{-1}) and myocardial injury (odds ratio:0.99 (95%CI: 0.69-1.43); p=0.55).

Multivariable logistic regression analysis of leukocyte subsets and myocardial injury.

Adjusting for RCRI, age and gender (Table 2), elevated postoperative troponin was independently associated with lower preoperative absolute lymphocyte count (odds ratio:1.80 [95%CI:1.50-2.17]; p<0.0001) and higher preoperative absolute monocyte count (odds ratio:1.93 [1.12-3.30]; p=0.017).

Monocyte ROS generation in relation to preoperative NLR

Clinical characteristics for these patients are shown in Supplementary Table 4. Higher preoperative NLR was significantly associated with more ROS release from activated monocytes (n=21 patients; r=0.47;.p=0.03; Figure 3).

Discussion

The principal finding of this prospective observational cohort study involving more than 1600 elective patients undergoing noncardiac surgery showed that elevated preoperative NLR, which reflects established systemic inflammation, was associated with increased risk of myocardial injury as measured by a high-sensitivity, cardiac-specific troponin assay. Our data implicate specific leukocyte subsets, suggesting that relative lymphopaenia and higher levels of circulating monocytes may play a mechanistic role in the development of perioperative myocardial injury. Monocytes, when obtained from patients with higher NLR, generate more injurious ROS when activated ex vivo. Taken together, these data suggest that a common, readily attainable leukocyte-based biomarker indicates that dysregulated inflammation may contribute to perioperative troponin rises.

These data reflect the findings of several studies exploring NLR in the cardiovascular literature, although these have primarily focussed on longer term outcomes rather than short-term risk of cardiac injury.[37] Our data provide further mechanistic insight, since preoperative NLR appears to be associated with a potentially more injurious monocyte phenotype. Murine models of myocardial infarction show that monocytes produced in the bone marrow and spleen are recruited to the injured myocardium in two distinct phases.[38] Within 24 hours after myocardial infarction, angiotensin II mediates the release of monocytes from the spleen. The first phase involves B lymphocyte coordinated recruitment of monocytes; depletion of B cells reduces monocyte infiltration and infarct size.[39] At least in mice, this first phase is characterised by Ly-6c^{high} monocytes which give rise to inflammatory macrophages that clear damaged tissue by phagocytosis and secretion of proteolytic enzymes. This is followed by the infiltration of Ly-6c^{low} macrophages that promote wound healing, angiogenesis and myofibroblast differentiation. Mobilisation of circulating monocytes in patients at higher risk

of PMI that are capable of releasing more injurious ROS may contribute to more extensive myocardial tissue damage.

By contrast, relative higher lymphocyte counts were associated with less myocardial injury. These findings are consistent with previous prospective work we have undertaken showing that lymphopaenia is associated with excess cardiovascular morbidity after elective orthopaedic surgery.[26] Genetic deficiency, or depletion of $CD4^+$ T-cells impairs wound healing in murine models of myocardial infarction.[40] $CD4^+$ T-cells modulate monocyte infiltration; their absence increases left ventricular dilatation and mortality after murine myocardial infarction.[41] We have previously shown that lymphopaenia in perioperative patients is characterised by lower T and B cell populations,[26] so the relationship between protective T cell and deleterious B cell phenotypes requires further elucidation. However, reduced NLR is also associated with poor cardiorespiratory reserve,[36] which may also contribute to perioperative myocardial injury.[42]

A strength of our large, prospective, generalizable study is the blinded measurement of high sensitivity troponin, an objective biomarker linked to adverse outcome.[1] The exploration of widely available leukocyte measurements adds further generalisability. Since causality cannot be inferred from observational data, we are unable to discount that intraoperative management and drug therapy may play an important role in mitigating or exacerbating the influence of chronically elevated systemic inflammation. While the aim of this study was to identify a broadly defined inflammatory phenotype linked to myocardial injury, a further limitation is the lack of additional, integrative functional immune data. Although numbers of circulating leukocytes may correlate with function, they do not necessarily reflect their phenotype at sites of tissue injury. We cannot discount that frailty contributes to poorer outcome, given that in elderly cancer patients frailty is positively

correlated with higher NLR.[43] Similarly, other unmeasured confounders may influence these data. Further investigations are required to establish how a number of inflammatory mechanisms reflected by higher NLR may be involved in perioperative myocardial injury. We have previously shown that non-classical monocyte subsets, which produce the highest levels of inflammatory cytokines in response to toll-like receptor ligands,[44] are more prevalent in patients with higher perioperative risk.[45] Upregulation of endothelial adhesion molecules, chemokines and/or cytokines may promote arrhythmias.[46] Excess inflammation may destabilize atherosclerotic plaques[47] or exacerbate microvascular pathophysiology.[48]

In summary, these data provide support for an alternative hypothesis that may, in part, explain the high incidence of perioperative myocardial injury being related to chronically elevated systemic inflammation.

Author contributions

GLA designed the analysis plan. GLA, TEFA performed the data analysis independently. The manuscript was drafted by GLA TEFA, RMP and revised following critical review by all authors.

Declaration of competing interests

RP holds research grants, and has given lectures and/or performed consultancy work for Nestle Health Sciences, BBraun, Medtronic, GlaxoSmithKline, Intersurgical and Edwards Lifesciences, and is a member of the Associate editorial board of the British Journal of Anaesthesia; PD has received other funding from Roche Diagnostics and Abbott Diagnostics for investigator initiated studies; GLA is a member of the editorial advisory board for Intensive Care Medicine Experimental, Editor for British Journal of Anaesthesia and has undertaken consultancy work for GlaxoSmithKline; there are no other relationships or activities that could appear to have influenced the submitted work.

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Figure Legends

Figure 1. Patient flow diagram showing cases included in the primary analysis.

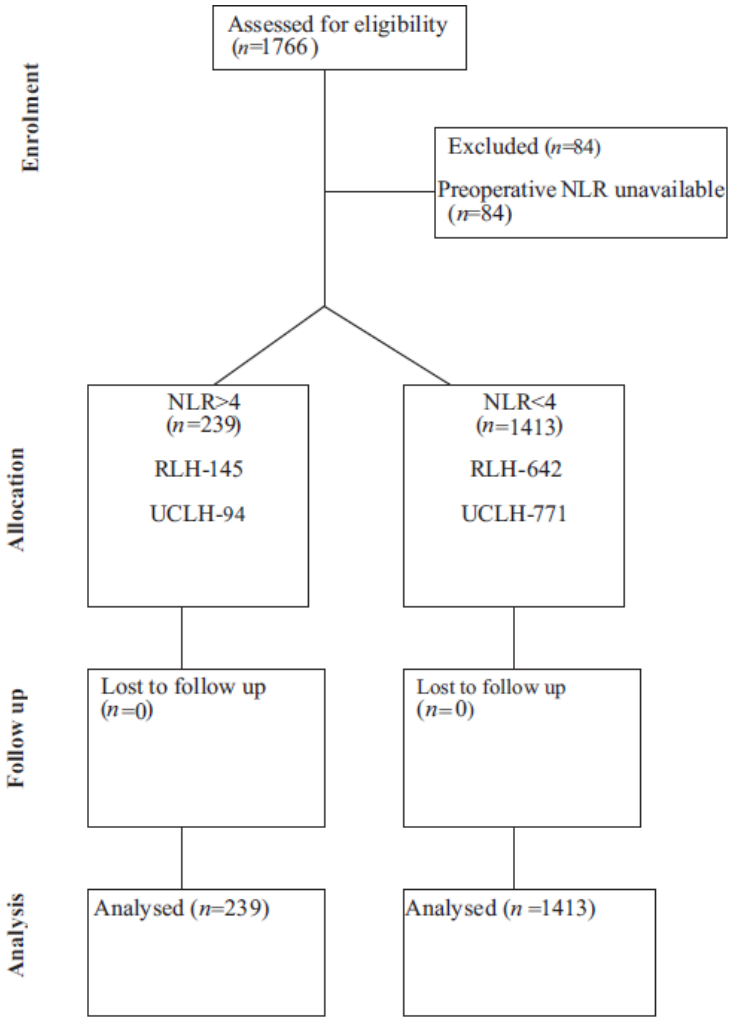

Figure 2. Length of hospital stay in relation to troponin events and neutrophil:lymphocyte ratio.

Kaplan-Meier plot showing time to hospital discharge, stratified by patients preoperative NLR and/or development of raised troponin. Numbers at risk for each category are matched to coloured lines shown in graph panel.

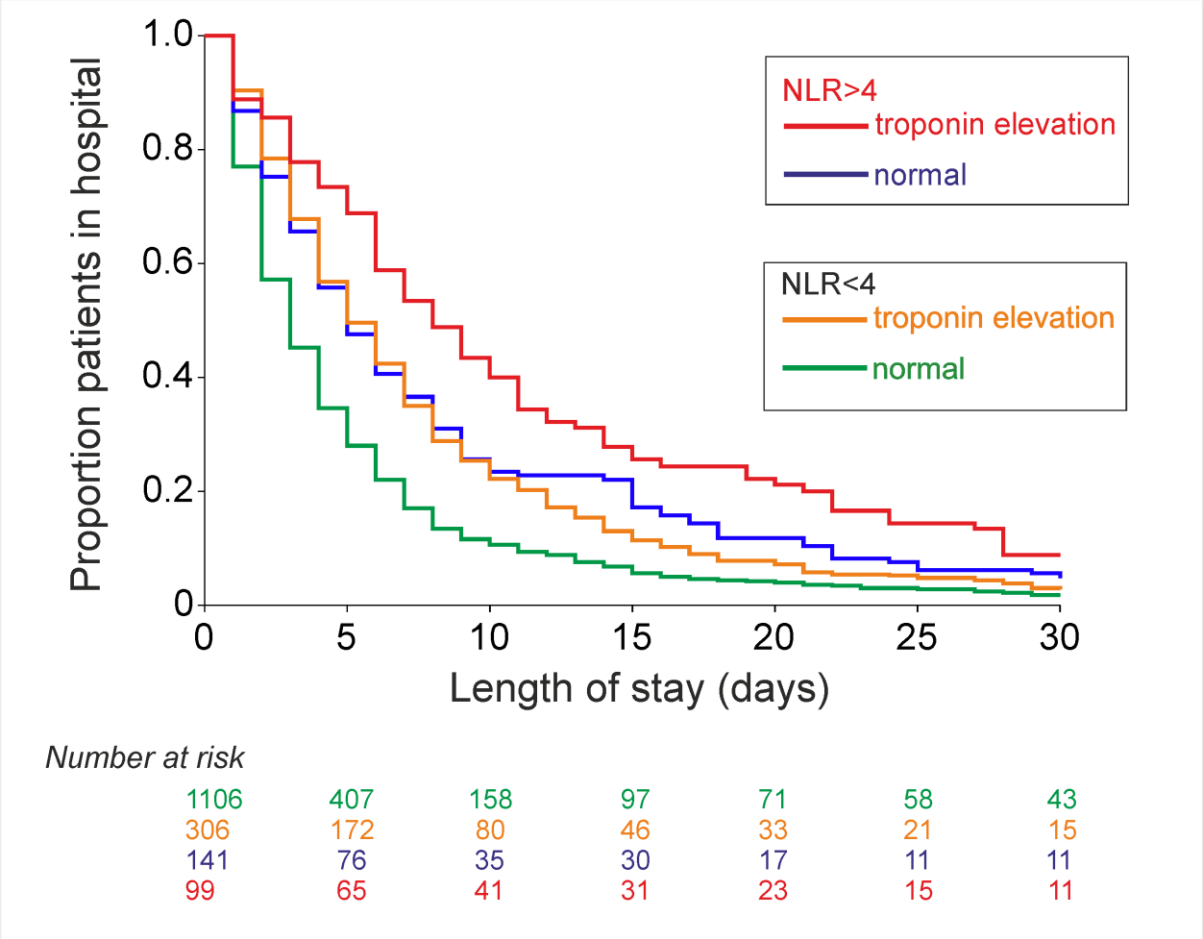

Figure 3. Neutrophil:lymphocyte ratio and monocyte reactive oxygen species generation.

A. Histograms showing monocyte intracellular reactive oxygen species before (grey) and after (red) whole blood samples obtained preoperatively from 21 patients were stimulated with PMA. Monocyte responses are shown from patients with high and low preoperative NLR. For clarity, unstained samples are not shown.

B. Correlation between preoperative NLR and fold-change (over basal values) in monocyte ROS after PMA stimulation.

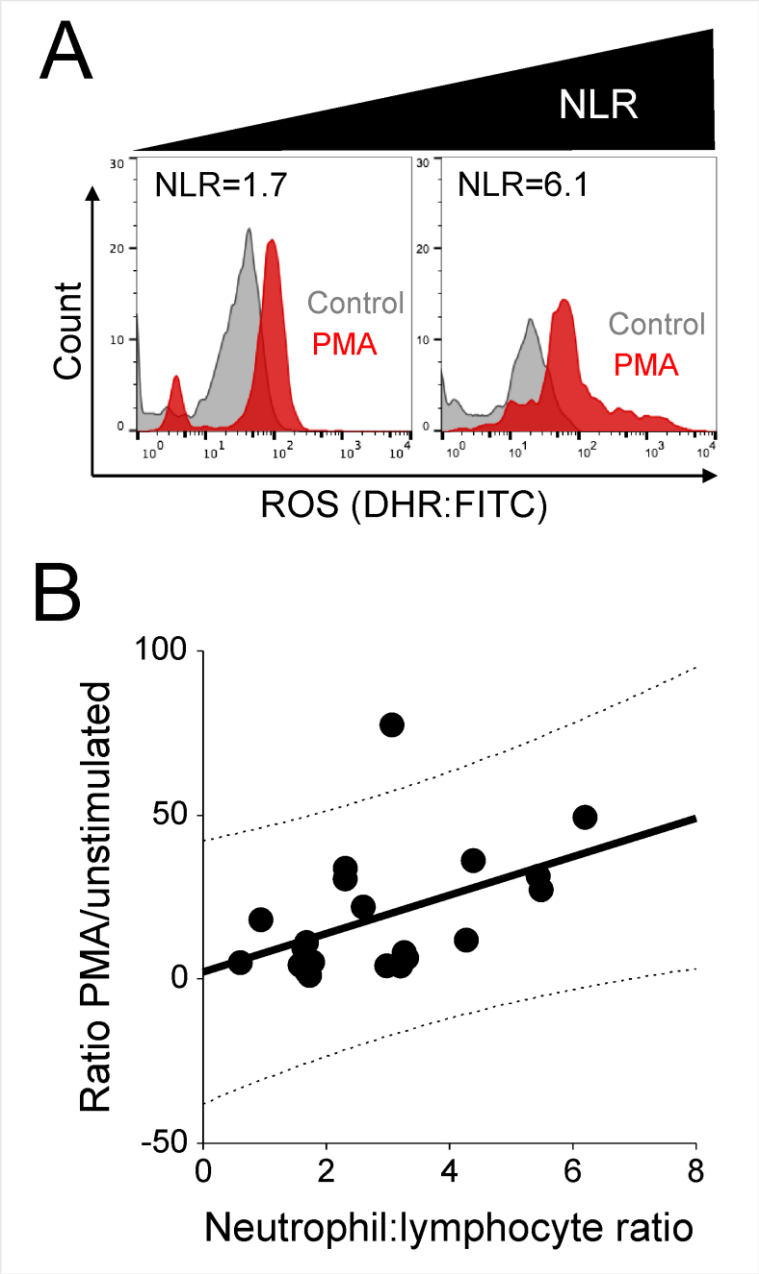

Table 1. Patient characteristics.

Descriptive data stratified by neutrophil-lymphocyte ratio </>4, presented as numbers with percentages (%) or means with standard deviations (SD). Age is rounded to nearest whole number. Neutrophil-lymphocyte ratio (NLR). Estimated glomerular filtration rate (eGFR). * Preoperative CRP available in 241 patients.

Characteristic	**NLR>4**	**NLR<4**	**P**
Number of patients	239	1413	
Age (years)	65 (12)	67 (12)	0.03
Female gender (n;%)	98 (41.0%)	715 (50.6%)	0.005
Body Mass Index (SD)	30 (7)	28 (7)	0.02
Systolic blood pressure (mmHg)	138 (21)	135 (21)	0.09
Diastolic blood pressure (mmHg)	75 (12)	73 (13)	0.004
Heart rate (beats.min^{-1})	76 (13)	77 (13)	0.36
RCRI ≥2 (n; %)	52 (21.8%)	185 (13.1%)	<0.001
Estimated GFR (ml.min^{-1})	81 (21)	79 (33)	0.23
Haemoglobin (g L^{-1})	123 (21)	134 (16)	<0.001
White cell count (cells.10^{9} L^{-1})	9.6 (4.3)	7.1 (2.1)	<0.001
Neutrophil count (cells.10^{9} L^{-1})	7.5 (3.9)	4.2 (1.5)	<0.001
Lymphocyte count (cells.10^{9} L^{-1})	1.2 (0.5)	2.1 (0.8)	<0.001
Monocyte count (cells.10^{9} L^{-1})	0.68 (0.34)	0.56 (0.23)	<0.001
Basophil count (cells.10^{9} L^{-1})	0.02 (0.03)	0.04 (0.1)	<0.001
Eosinophil count (cells.10^{9} L^{-1})	0.14 (0.15)	0.2 (0.19)	<0.001
Platelets (cells.10^{9} L^{-1})	275 (118)	255 (75)	0.004
C reactive protein* (mg.L^{-1})	16 (4-72)	3 (1-14)	<0.001
Albumin (g.dL^{-1})	41 (6)	45 (4)	<0.001
Moderate or high risk surgery (n;	214 (89.5%)	1289 (91.2%)	
Hepatobiliary	7 (2.9%)	75 (5.3%)	
Gastrointestinal	31 (13.0%)	241 (17.1%)	
Vascular	22 (9.2%)	59 (4.2%)	
Urology	38 (15.9%)	246 (17.4%)	
Neurosurgery	16 (6.7%)	85 (6.0%)	
Gynaecological	20 (8.4%)	129 (9.1%)	
Head/neck/ear, nose,	12 (5.0%)	54 (3.8%)	
Orthopaedic	71 (29.7%)	440 (31.1%)	
Duration of surgery (minutes)	135 (96-204)	130 (90-195)	0.47
Allogenic blood products (n;%)	14 (5.8%)	53 (3.8%)	0.15
Postoperative critical care admission			
Level 2 (n;%)	39 (16.3%)	239 (16.9%)	0.52
Level 3 (n;%)	15 (6.2%)	57 (4.0%)	

Table 2. Multivariable logistic regression model of leukocyte subsets associated with myocardial injury after non-cardiac surgery.

Dependent variable is myocardial injury within first three days of surgery. Lower absolute lymphocyte, and higher absolute monocyte counts, were independently associated with myocardial injury. Absolute leukocyte subset counts were treated as continuous variables. Results presented as odds ratios with 95% confidence intervals.

	Odds ratio (95% CI)	**P value**
Age	1.03 (1.02-1.04)	<0.0001
Male gender	1.08 (0.84-1.38)	0.553
RCRI *(compared to zero score)*		
1	1.64 (1.27-2.12)	<0.0001
2	3.55 (2.28-5.53)	<0.0001
≥3	6.29 (2.47-16.05)	<0.0001
Neutrophils	1.04 (0.98-1.10)	0.202
Lymphocytes	0.56 (0.46-0.67)	<0.0001
Monocytes	1.93 (1.12-3.30)	0.017
Eosinophils	1.58 (0.70-3.58)	0.274
Basophils	3.81 (0.13-109.80)	0.436