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Reticulated Platelets as Predictor of Myocardial Injury and 30 Day Mortality After Non-cardiac Surgery

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WHAT THIS PAPER ADDS

This study shows the additional value of measuring pre-operative young, reticulated platelets (pRP) in patients undergoing major non-cardiac surgery to predict post-operative myocardial injury (PMI) and 30 day mortality. Measuring pRP could identify patients with an increased risk of PMI and 30 day mortality. Future, prospective studies with consequent adjustment of cardiovascular risk management after pRP measurement, should determine the clinical relevance.

Objective: A pre-operative marker for identification of patients at risk of peri-operative adverse events and 30 day mortality might be the percentage of young, reticulated platelets (pRP). This study aimed to determine the predictive value of pre-operative pRP on post-operative myocardial injury (PMI) and 30 day mortality, in patients aged $\geq$ 60 years undergoing moderate to high risk non-cardiac surgery.

Methods: The incidence of PMI (troponin I $>$ 0.06 μg/L) and 30 day mortality was compared for patients with normal and high pRP ($\geq$2.82%) obtained from The Utrecht Patient Orientated Database. The predictive pRP value was assessed using logistic regression. A prediction model for PMI or 30 day mortality with known risk factors was compared with a model including increased pRP using the area under the receiving operator characteristics curve (AUROC).

Results: In total, 26.5% (607/2289) patients showed pre-operative increased pRP. Increased pRP was associated with more PMI and 30 day mortality compared with normal pRP (36.1% *vs.* 28.3%, $p < .001$ and 8.6% *vs.* 3.6%, $p < .001$). The median pRP was higher in patients suffering PMI and 30 day mortality compared with not (2.21 [IQR: 1.57–3.11] *vs.* 2.07 [IQR: 1.52–1.78], $p = .002$, and 2.63 [IQR: 1.76–4.15] *vs.* 2.09 [IQR: 1.52–3.98], $p < .001$). pRP was independently related to PMI (OR: 1.28 [95% CI: 1.04–1.59], $p = .02$) and 30 day mortality (OR: 2.35 [95% CI: 1.56–3.55], $p < .001$). Adding increased pRP to the predictive model of PMI or 30 day mortality did not increase the AUROC 0.71 *vs.* 0.72, and 0.80 *vs.* 0.81.

Conclusion: In patients undergoing major non-cardiac surgery, increased pre-operative pRP is related to 30 day mortality and PMI.

Keywords: Blood platelets, Platelet activation, Platelet count, Mortality, Myocardial infarction, Myocardial ischaemia
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INTRODUCTION

Post-operative care following non-cardiac surgery is hampered by the occurrence of adverse events, including myocardial infarction and death. According to the latest universal definition,[1] myocardial infarction occurs in 3–6% of patients,[2–5] while post-operative myocardial injury (PMI) defined as troponin elevation above the clinical cut off level of 0.06 μg/L with or without clinical symptoms, occurs in approximately 20% of patients.[6,7] Increased peak troponin values during the first three days after non-cardiac surgery are strongly associated with 30 day and one year mortality.[5–8]

The first step in preventing PMI is to identify patients at risk, so that preventive measures can be taken. Current guidelines[9,10] recommend the revised cardiac risk index (RCRI)[11] and the National Surgery Quality Improvement Program (NSQIP) Myocardial Infarction and Cardiac Arrest (MICA) risk prediction rules[12] as tools to identify patients at

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risk of post-operative cardiac complications. These models are constructed from a patient's history, pre-operative creatinine, and procedure characteristics. The RCRI has a moderate predictive value in discriminating patients at high risk of post-operative cardiac complications.[13] In vascular surgery patients, its performance is limited.[13]

Monitoring of troponin levels during the first three days after surgery substantially improved 30 day mortality risk stratification compared with stratification before surgery based on clinical risk factors only.[5,7] Although implementing standard troponin surveillance improved prediction of all cause mortality, it did not improve clinical outcome.[6] In contrast, by being able to predict PMI and 30 day mortality prior to surgery, such clinical outcome improvement would be more likely to be realised.

A potential pre-operative marker might be the newly released platelets, also termed reticulated platelets. Atherosclerosis reduces platelet survival and thereby increases platelet turnover. To compensate for this increased turnover, platelet release from the bone marrow is stimulated, thus increasing the percentage of young, reticulated platelets in the circulation. Elevated levels of circulating reticulated platelets have been extensively associated with a pro-thrombotic phenotype and participate most actively in thrombosis.[14–17] Moreover, young platelets are more prone to participate in thrombus formation compared with older platelets.[18] They tend to be larger, contain more dense granules and display a greater *ex vivo* reactivity profile in response to agonists compared with mature platelets.[15,19] Elevated levels of reticulated platelets have been observed in the setting of acute coronary syndromes and stroke.[20–22] Therefore, it was hypothesised that a high percentage of reticulated platelets (pRP) might be predictive of PMI and 30 day mortality.

The purpose of this study was to determine the predictive value of the pre-operative pRP on PMI and 30 day mortality, in patients aged ≥60 years undergoing moderate to high risk non-cardiac surgery.

METHODS

Patients

This observational cohort study included consecutive patients undergoing major non-cardiac surgery between 1 January 2011 and 31 December 2012 at the University Medical Centre Utrecht, the Netherlands.[6] Patients were eligible if they were aged ≥60 years, were undergoing moderate to high risk non-cardiac surgery with an expected post-operative hospital stay of ≥24 h and if haematological measurements within one month prior to surgery were available. For patients who underwent surgery more than once during this period, only the first operation was included in the analysis.

Medical ethical approval

The local medical ethics committee waived the need for informed consent because only routinely collected patient data were used and data were anonymised before analysis (UMC Utrecht Medical Research Ethics committee 14-189/C).

The Utrecht Patient Orientated Database (UPOD)[23] containing haematology data of automated blood cell analysis, was set up in accordance with the guidance of the Institutional Review Board (IRB) and privacy board of the UMC Utrecht, which allows the use of clinical data from patients who did not object to the use of their data for scientific purposes, provided the patients cannot be identified directly from the data.

Data collection

All pre- and post-operative data were collected from electronic medical and administrative records. Data collected for all patients were patient characteristics, pre-operative physical status for the American Society of Anaesthesiologists (ASA) classification, comorbidities, post-operative troponin I measurements, and death within 30 days after surgery. Mortality data were obtained from the municipal personal records database.

Platelet measurements were obtained from UPOD, which contains haematology data of all automated blood cell analyses at the UMC Utrecht.

Reticulated platelets

The blood cell analyses were performed with the CELL-DYN Sapphire (Abbott Diagnostics, Lake Forest, IL, USA). A feature of this type of blood cell analyser is that it not only reports the parameters requested by the physician, but all haematological parameters that it is capable of measuring can be extracted for further analysis.[24] All data captured by the blood cell analysers were downloaded into the UPOD and anonymised for further research. From this database, the pRP, mean platelet volume, and platelet count were obtained in blood samples that were ordered for pre-operative blood typing and cross matching, a maximum of 28 days prior to surgery. Analyses were performed within 4 h after blood samples were taken. The cut off for a high pRP was defined at 2.82%, according to the standard hospital protocol.[25] This threshold has been determined on the 2.5th and 97.5th percentile of repeated measurements in 151 healthy volunteers, according to the CLSI guidelines.[26]

Outcomes

The primary outcome was death within 30 days. The secondary outcome was PMI, defined as a troponin I > 0.06 μg/L. This is the lowest value measurable with a 10% coefficient of variation above the 99th percentile of 0.04 μg/L of the assay used. Troponin was analysed using the third generation enhanced AccuTnI assay (Beckman Coulter, Brea, CA, USA).[27] According to the hospital protocol, cardiac troponin I measurements were ordered for the first three days after surgery. For each patient the highest troponin value was used in the analysis. As the goal of this primary research question was to investigate whether there was a relationship/association between pRP and PMI and

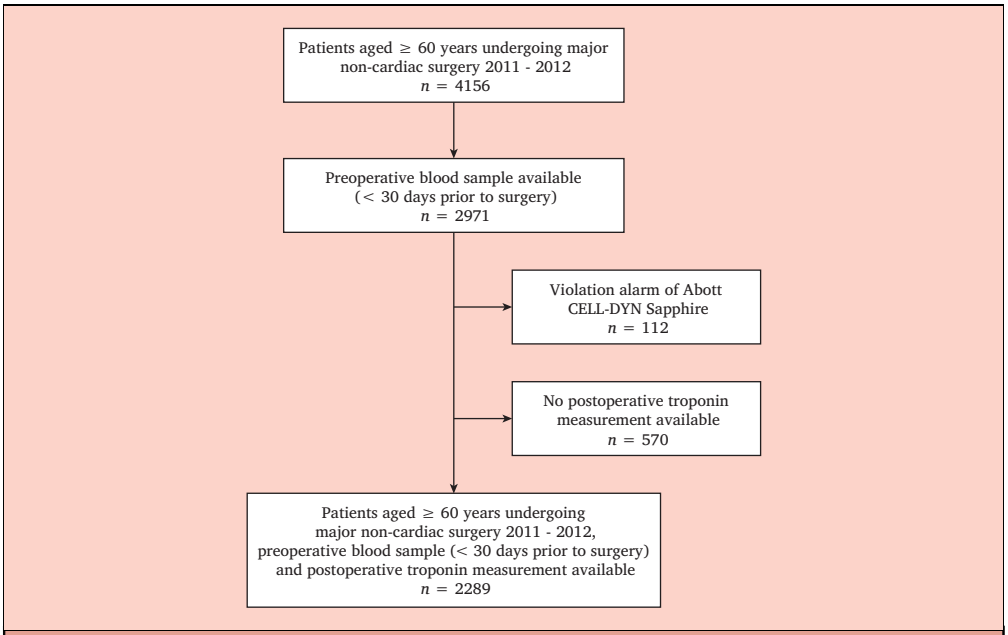

Figure 1. Flowchart for patients undergoing major non-cardiac surgery, aged ≥60 years.

30 day mortality in all patients who underwent non-cardiac surgery, no separate analyses were performed for the different operative procedure groups.

Statistical analysis

The analysis was performed with SPSS (IBM SPSS Statistics 21 for Windows). In advance of data analysis, it was known that the pRP would not be available for all patients because not all patients had pre-operative blood typing and cross matching. Patients with missing platelet data <1 month prior to intervention were excluded. Next, patients with registered violation alarms from the data obtained by the Abbott CELL-DYN Sapphire were excluded to ensure the reliability of the data. To check whether the missing data and alarms could be considered "at random," the baseline characteristics were compared for included (all data available) and excluded (incomplete data) patients.

Statistics

To investigate the abovementioned aims, the following were studied:

1. Differences in baseline characteristics using the chi-square test or Fisher's Exact test for categorical variables; and *t* test or Mann—Whitney *U* test for normally distributed or non-normally distributed continuous variables, respectively. A double sided *p* value < .05 was considered to be statistically significant.
2. The incidence of PMI and 30 day mortality for patients with and without increased pRP using the Fisher's Exact test.
3. The median pRP for patients with or without PMI and 30 day mortality by Mann—Whitney *U* test.
4. The predictive value of pRP on PMI and 30 day mortality using univariable and multivariable logistic regression analysis with clinical baseline and procedural characteristics as predictor variables. Results of the logistic regression were displayed as odds ratios (OR) with 95% confidence intervals (CI).
5. The survival of patients with and without increased pRP using Kaplan—Meier curves (differences with log-rank analysis).
6. The predictive value of a model with known predictors of PMI and 30 day mortality, to a model in which the pRP was added using the area under the receiving operator characteristics curve.

RESULTS

In total, 2971 patients fulfilled the inclusion criteria. Of these patients, 682 patients were excluded because of missing troponin values ($n = 570$) or errors of the CELL-DYN Sapphire ($n = 112$). In total, 2289 (77%) patients were included in the final analysis (Fig. 1).

Baseline characteristics of included and excluded patients resulting from errors and missing troponin values are given in Table 1. The urgency and duration of the procedure, and surgical specialty differed for included and excluded patients: 27.5% *vs.* 35.5% emergency surgery ($p < .001$), 199 *vs.* 153 min ($p < .001$), respectively. More general and vascular surgery was reported in the included patients. The overall myocardial injury and 30 day mortality were 30.1% (716/2371) and 4.9% (145/2943).

Table 1. Baseline characteristics of 2971 patients aged ≥60 years undergoing major non-cardiac surgery between 2011 and 2012, stratified by study inclusion

	Included patients (n = 2289)	Excluded patients (n = 682)	p
Age − years	71.2 ± 7.8	70.7 ± 7.5	.11
Male gender	1263 (55.2)	351 (52.5)	.09
ASA classification			
I	261 (11.4)	82 (12.0)	.63
II	1409 (61.6)	406 (59.5)	
III	587 (25.6)	187 (27.4)	
IV	32 (1.4)	7 (1.0)	
Diabetes			
No	1870 (81.7)	557 (81.7)	1.0
Yes	419 (18.3)	125 (18.3)	
Renal failure	308 (13.5)	100 (14.7)	.45
Emergency surgery	629 (27.5)	242 (35.5)	<.001
Elective surgery	1660 (72.5)	440 (64.5)	
General surgery	531 (23.2)	145 (21.3)	.02
Vascular surgery	402 (17.6)	90 (13.2)	
Gynaecology/urology	146 (6.4)	46 (6.4)	
Other surgery	1210 (52.9)	401 (58.8)	
MPV − fL	7.67 ± 1.02	7.71 ± 1.14	.92
Platelet count − × 10^9/L	269.44 ± 101.52	268.11 ± 113.07	.26
Reticulated platelets − %	2.46 ± 1.44	4.50 ± 7.41	.06
Surgery duration − min	199.2 ± 130.9	152.79 ± 97.46	<.001
Time in hospital − d	12.06 ± 14.90	12.16 ± 16.35	.83
Myocardial injury	695/2289 (30.4)	21/82 (25.6)	.39
30 day mortality	112/2271 (4.9)	33/672 (4.9)	1.0

Data are presented as n (%) or mean ± standard deviation. ASA = American Society of Anesthesiologists; MPV = mean platelet volume; n = number.

Baseline characteristics

Of the 2289 patients, 607 patients (26.5%) showed pre-operative high pRP. Patients with high pRP were more often male, had a higher ASA classification, more type II diabetes and renal failure, underwent more emergency surgery, and had longer hospital stays, compared with patients with normal pRP levels (Table 2).

30 day mortality

Patients with high pRP died more often within 30 days compared with patients with a normal pRP (8.6% *vs.* 3.6%, $p < .001$, Fig. 2). During the first five days post-operatively the survival of patients with and without elevated pRP was equal. After 10 days the survival decreased for patients with a raised pre-operative pRP, resulting in significantly different 30 day survival (Fig. 4). Equally, patients with PMI died more often within 30 days compared with patients without PMI (9.6% *vs.* 3.0%, $p < .001$).

Patients who died within 30 days more often displayed high pRP and PMI (46.4% *vs.* 25.6%, $p < .001$ and 58.9% *vs.* 30.7%, $p < .001$).

Table 2. Baseline characteristics of 2289 analysed patients aged ≥60 years undergoing major non-cardiac surgery between 2011 and 2012, stratified by presence of reticulated (young) platelets

	Reticulated platelets <2.82% n = 1682	Reticulated platelets ≥2.82% n = 607	p
Age − years	71.2 ± 7.8	71.5 ± 7.9	.25
Male gender	906 (53.9)	357 (58.8)	.04
ASA classification			
I	200 (11.9)	61 (10.1)	.03
II	1052 (62.5)	357 (58.8)	
III	411 (24.4)	176 (29.0)	
IV	19 (1.1)	13 (2.1)	
History of MI	169 (10.0)	57 (9.4)	.62
Prior coronary revascularisation	217 (12.9)	62 (10.2)	.06
Diabetes			
No	1376 (81.8)	494 (81.4)	.81
Yes	306 (18.2)	113 (18.6)	
Smoking			
Current	300/1520 (19.7)	98/524 (18.7)	.61
Ever smoker	458 (30.1)	150 (28.6)	
Never smoker	762 (50.1)	276 (52.7)	
Hypertension	892 (53.0)	299 (49.3)	.12
Chronic heart failure	58 (3.5)	22 (3.6)	.80
Peripheral arterial disease	184 (10.9)	78 (12.9)	.21
Hypertension	892 (53.0)	299 (49.3)	.12
Renal failure (GFR <50 mL/min)	210 (12.5)	97 (16.0)	.03
Type of surgery			
Emergency surgery	429 (25.5)	200 (33.0)	.001
Elective surgery	1253 (74.5)	407 (67.1)	
General surgery	542 (32.2)	240 (39.5)	.004
Vascular surgery	292 (17.4)	110 (18.1)	
Gynaecology/urology	181 (10.8)	52 (8.6)	
Other surgery	666 (39.6)	205 (33.8)	
Surgery duration (min)	199.2 ± 131.2	198.23 ± 130.3	.82
Time in hospital (days)	10.9 ± 12.8	15.37 ± 19.1	<.001
MPV (fL)	7.7 ± 1.0	7.5 ± 1.02	<.001
Platelet count (× 10^9/L)	268.6 ± 94.7	271.2 ± 117.29	.70
Reticulated platelets − %	1.8 ± 0.5	4.3 ± 1.59	<.001
Myocardial injury (troponin I ≥ 0.06 µg/L)	476 (28.3)	219 (36.1)	<.001
30 day mortality	60/1666 (3.6)	52/605 (8.6)	<.001

Data are presented as n (%) or mean ± standard deviation. ASA = American Society of Anaesthesiologists; GFR = glomerular filtration rate; MI = myocardial infarction; MPV = mean platelet volume; n = number.

Of the high risk patients who had an increased pRP and PMI, 12.0% (26/217) died within 30 days, compared with 8.5% (40/471) of patients with normal pRP but elevated post-operative troponin ($p = .16$).

The median pRP was higher in patients with 30 day mortality, 2.63% (IQR 1.76—4.15) compared with no 30 day mortality, 2.09% (IQR 1.52—2.89), $p < .001$ (Appendix 1, Table A1).

After multivariable logistic regression (Table 3), pRP was associated with 30 day mortality (OR = 2.35 [95% CI: 1.56—

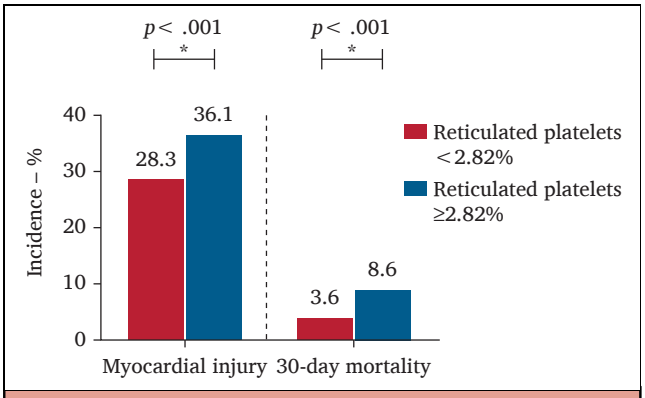

Figure 2. Incidence of myocardial injury and 30 day mortality, stratified by percentage reticulated platelets.

3.55], $p < .001$). Myocardial injury was strongly related to 30 day mortality (OR = 2.33 [95% CI: 1.52—3.57], $p < .001$). The strongest correlation was found for ASA IV classification (OR = 4.60 [95% CI: 1.23—17.16], $p = .23$) and emergency surgery (OR = 3.39 [95% CI: 2.14—5.37], $p < .001$).

The AUC of the model with only the known pre-operative predictors was 0.80 (95% CI: 0.78—0.82) with a sensitivity of 74.8% and specificity of 75.5%. Adding pRP to the model resulted in an AUC of 0.81 (95% CI: 0.67—0.76) with a sensitivity of 67.6% and specificity of 84.7%. This was not a significant increase ($p = .07$).

Post-operative myocardial injury

Patients with high pRP more often developed PMI compared with patients with normal pRP (36.1% *vs.* 28.3%, $p < .001$, Fig. 2). The median pRP was higher in patients with PMI, 2.21% (IQR 1.57—3.11) compared with no PMI, 2.07% (IQR 1.52—2.78), $p = .002$ (Appendix 1, Table A1).

After multivariable logistic regression (Table 4), pRP was an independent predictor of PMI (OR = 1.28 [95% CI: 1.04—1.59], $p = .02$). The strongest association was found for emergency surgery (OR = 2.59 [95% CI: 2.07—3.25], $p < .001$), ASA IV (OR = 2.32 [95% CI: 0.97—5.55], $p = .06$), ASA III (OR = 1.28 [95% CI: 0.85—1.92], $p = .23$) and renal failure (OR = 1.70 [95% CI: 1.28—2.25], $p < .001$).

The area under the receiver operating characteristics curve (AUC) of the model with only the known pre-operative predictors was 0.71 (95% CI: 0.67—0.76), with a sensitivity of 59.4% and specificity of 73.4%. Adding pRP to the model resulted in an AUC of 0.72 (95% CI: 0.67—0.76) with a sensitivity of 63.6% and specificity of 68.5% ($p = .30$, Fig. 3).

Table 3. Univariable and multivariable associations with 30 day mortality after moderate to high-risk non-cardiac surgery

	30 d mortality (univariable)		30 d mortality (multivariable)*	
	OR (95% CI)	*p*	OR (95% CI)	*p*
Reticulated platelets — per % increment	2.5 (1.7—3.7)	<.001	2.3 (1.6—3.5)	<.001
Platelet count (× 10^9/L)	1.0 (1.0—1.0)	.02	1.0 (1.0—1.0)	.09
Mean platelet volume, fL	1.2 (1.0—1.4)	.04	1.1 (0.9—1.3)	.45
Age — per y increment	1.0 (1.0—1.0)	<.001	1.0 (1.0—1.0)	.11
Male sex	1.1 (0.8—1.7)	.47	—	—
ASA classification				
ASA I	1.0 (ref.)		1.0 (ref.)	
ASA II	1.5 (0.6—3.5)	.40	—	—
ASA III	4.1 (1.7—9.7)	<.001	2.1 (0.8—5.3)	.12
ASA IV	10.1 (3.0—33.6)	<.001	4.6 (1.2—17.2)	.02
History myocardial infarction	1.7 (1.0—2.9)	.06	—	—
History coronary intervention	1.0 (0.6—1.8)	.94	—	—
Renal failure (GFR <50 mL/min)	2.5 (1.6—3.9)	<.001	1.5 (0.9—2.5)	.14
Hypertension	1.1 (0.8—1.7)	.50	—	—
Diabetes	1.6 (1.0—2.5)	.04	—	—
Chronic heart failure	1.9 (0.9—4.3)	.12	—	—
Peripheral arterial disease	0.9 (0.5—1.7)	.78	—	—
Smoking				
Current	0.9 (0.5—1.7)	.93	—	—
Ever	0.7 (0.4—1.3)	.72	—	—
Emergency surgery	6.0 (4.0—9.0)	<.001	3.4 (2.1—5.4)	<.001
Surgery duration — per min increment	1.0 (1.0—1.0)	.04	1.0 (1.0—1.0)	.78
Time in hospital — per day increment	1.0 (1.0—1.0)	.19	—	—
Surgical specialty				
Other surgery	1.0 (ref.)		1.0 (ref.)	
General	0.9 (0.6—1.4)	.77	0.6 (0.4—1.0)	.03
Gynaecology/urology	0.1 (0.0—0.5)	.005	0.2 (0.1—1.0)	.05
Vascular	0.4 (0.2—0.8)	.006	0.3 (0.1—0.6)	.001
Post-operative myocardial injury	3.5 (2.4—5.2)	<.001	2.3 (1.5—3.6)	<.001

* Multivariable logistic regression was performed, adjusted for age, ASA classification, renal failure, platelet count, mean platelet volume, increased pRP, emergency surgery, surgical specialty, surgery duration, and post-operative myocardial infarction. ASA = American Society of Anaesthesiologists; CI = confidence intervals; GFR = glomerular filtration rate; *n* = number; OR = odds ratio.

Table 4. Univariable and multivariable associations with post-operative myocardial injury after moderate to high-risk non-cardiac surgery

	Post-operative myocardial injury (univariable)		Post-operative myocardial injury (multivariable)*	
	OR (95% CI)	*p*	OR (95% CI)	*p*
Reticulated platelets − per % increment	1.4 (1.2–1.7)	<.001	1.3 (1.0–1.6)	.02
Platelet count (× 10^9/L)	1.0 (1.0–1.0)	.83	–	–
Mean platelet volume, fL	1.0 (0.9–1.1)	.45	–	–
Age − per y increment	1.0 (1.0–1.0)	<.001	1.0 (1.0–1.0)	<.001
Male sex	1.4 (1.2–1.7)	<.001	1.2 (1.0–1.5)	.05
ASA classification				
ASA I	1.0 (ref.)		1.0 (ref.)	
ASA II	1.3 (1.0–1.8)	.07	–	–
ASA III	2.5 (1.8–3.5)	<.001	1.3 (0.8–1.9)	.23
ASA IV	6.1 (2.8–13.2)	<.001	2.3 (1.0–5.5)	.06
History myocardial infarction	2.2 (1.6–2.9)	<.001	0.6 (0.4–1.0)	.06
History coronary intervention	1.9 (1.5–2.4)	<.001	0.8 (0.6–1.1)	.20
Renal failure, GFR <50 mL/min	2.5 (2.0–3.2)	<.001	1.7 (1.3–2.2)	<.001
Hypertension	1.3 (1.1–7.6)	.002	1.1 (0.9–1.1)	.24
Diabetes	1.2 (1.0–1.6)	.05	–	–
Chronic heart failure	2.0 (1.3–3.2)	.002	1.4 (0.8–2.4)	.19
Peripheral arterial disease	1.6 (1.2–2.0)	.001	1.1 (0.8–1.5)	.48
Smoking				
Current	1.1 (0.8–1.4)	.62	–	–
Ever	0.8 (0.7–1.1)	.17	–	–
Emergency surgery	2.4 (2.0–3.0)	<.001	2.6 (2.1–3.2)	<.001
Surgery duration − per min increment	1.0 (1.0–1.0)	<.001	1.0 (1.0–1.0)	<.001
Surgical specialty				
Other surgery	1.0 (ref.)		1.0 (ref.)	
General	1.9 (1.5–2.3)	<.001	1.8 (1.5–2.3)	<.001
Gynaecology/Urology	0.4 (0.3–0.7)	<.001	0.6 (0.4–1.0)	.04
Vascular	1.5 (1.2–2.0)	.001	1.4 (1.0–1.8)	.05

* Multivariable logistic regression was performed with adjustment for age, male sex, ASA classification, history of myocardial infarction and coronary intervention, renal failure, hypertension, chronic heart failure, peripheral arterial disease, increased pRP, emergency surgery, surgical duration, and surgical specialty. ASA = American Society of Anaesthesiologists; CI = confidence intervals; GFR = glomerular filtration rate; *n* = number; OR = odds ratio.

DISCUSSION

This study shows that an increased percentage of RP before surgery is related to 30 day mortality and is associated with post-operative myocardial injury for patients undergoing major non-cardiac surgery, aged ≥60 years. Patients with an increased pRP have more cardiovascular risk factors (male sex, diabetes, and renal failure) compared with patients with normal pRP.

This study confirms the presence of an increased pRP in patients with multiple cardiovascular risk factors, such as high age, male sex, renal failure, diabetes, and higher ASA score. Reduced glomerular filtration rate, as in chronic kidney disease, independently predicts mortality and accelerates the overall progression of cardiovascular disease.[28]

It has been proven that elevated pRP is associated with a pro-thrombotic phenotype.[15,29] Consequently, researchers in the field of cardiovascular disease have also gained interest in measuring the pRP. So far, increased pRP has been associated in non-surgical patients with acute coronary syndrome[20,30] and cardio-embolic stroke,[21,31] and non-response to antiplatelet therapy in patients with coronary artery disease.[19,29,32] In addition, the HaEmostasis In carotid Stenosis (HEIST) study showed a higher percentage of reticulated platelets in patients with a symptomatic carotid artery stenosis compared with patients with an asymptomatic carotid artery stenosis.[22] Therefore, elevation of immature platelets may be used as a marker for patients with extensive cardiovascular risk and consequent increased risk of major adverse cardiovascular events.[33,34] These patients could be monitored more frequently. Future prospective studies are needed to determine the clinical relevance of careful peri-operative cardiovascular risk management in patients with elevated immature platelets.

Enhanced platelet turnover, as seen in patients with diabetes or acute coronary syndrome, may interfere with the anti-aggregatory effect of antiplatelet therapy. Patients with enhanced platelet turnover might even represent the subgroup of patients that are not sufficiently protected by standard antiplatelet therapy.[35] A single study compared the outcome of specific agonist dependent platelet function with platelet turnover in patients with antiplatelet drugs Measuring platelet turnover, by mean platelet volume, resulted in a higher predictive value for cardiac death than platelet function testing with VerifyNow.[36] Platelet turnover might thus represent a major predictive factor for cardiovascular risk, while platelet reactivity testing only allows specific identification of

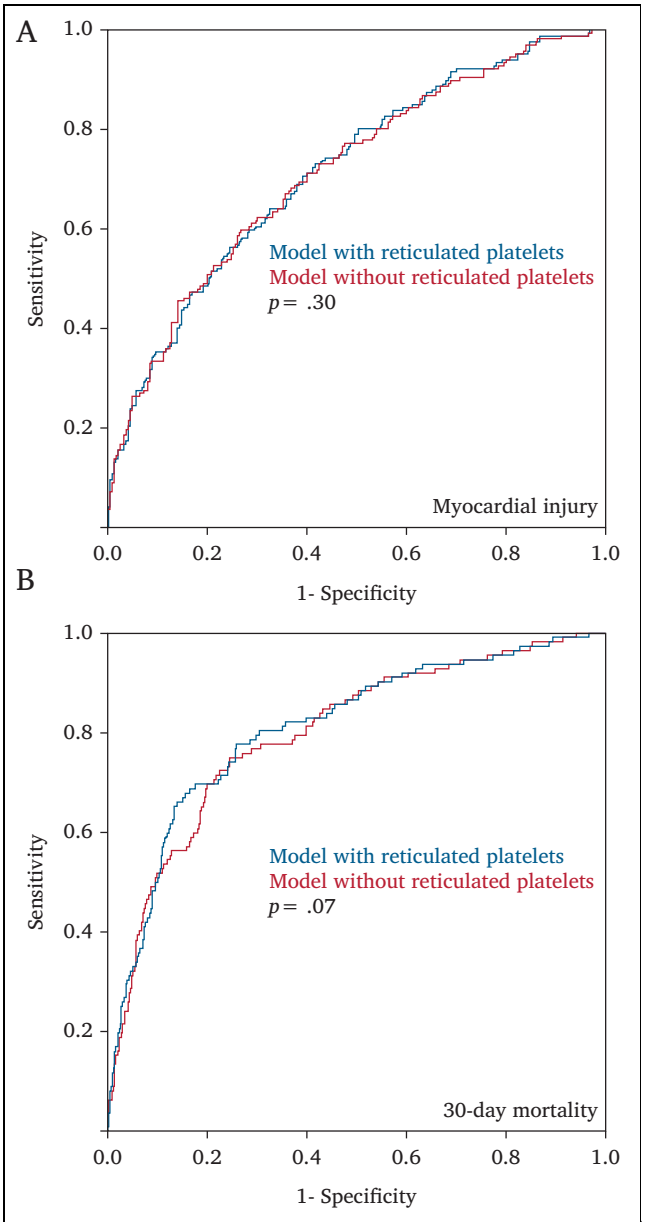

Figure 3. (A) The area under the receiver operating characteristics curve for myocardial injury. (B) The area under the receiver operating characteristics curve for 30 day mortality. We used the chi-square test of Fisher's Exact test for categorical variable, and the T test or Mann-Whitney U test for normally distributed or non-normally distributed continuous variables. A p value <0.05 was considered to be statistically significant.

non-responders to antiplatelet therapy.[37] The pRP can be assessed on two commercially available, haematology analysers; Sysmex (XE- and XN- series) and CELL-DYN Sapphire, applying different reference ranges. Direct comparison between the two analysers showed low to moderate correlation, with a higher sensitivity of the CELL-DYN for identification of patients with high platelet turnover.[38,39] The CELL-DYN Sapphire is an easy to use, standardised, flow cytometry based analyser that is available in most laboratories, and therefore is suitable for use in standard care. Because the pRP was obtained by the CELL-DYN Sapphire in this study, the results cannot be generalised for the Sysmex.

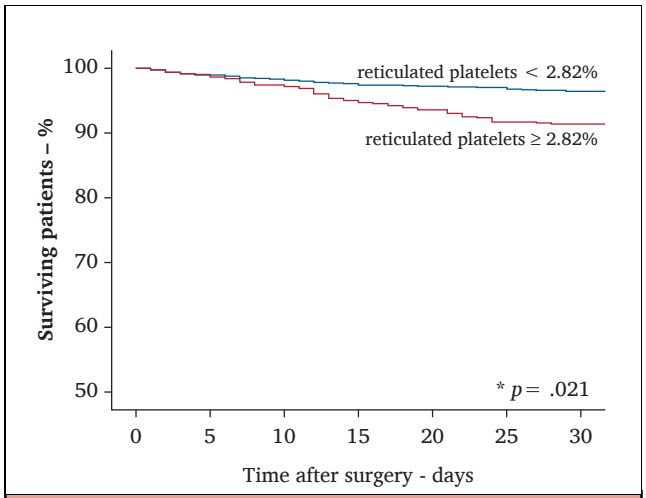

Figure 4. Cumulative Kaplan Meier survival estimate over 30 postoperative days after major non-cardiac surgery, stratified by preoperative proportion of reticulated platelets.

Multiple pre-operative markers have been studied as predictors in cardiovascular risk stratification including N-terminal pro-brain natriuretic peptide (NT-BNP), cystatin C, C reactive protein (CRP), mean platelet volume, neutrophil to lymphocyte ratio (NLR), mean platelet volume/platelet count (MPV/PC) ratio, and coronary artery calcium (CAC).[40–42] Mean platelet volume and NLR are independent predictors of in hospital mortality.[41] The MPV/PC ratio is an independent predictor of an adverse long term prognosis in patients with ST elevation MI undergoing percutaneous coronary intervention.[42] NT-BNP shows a 75-88% sensitivity and 62-100% specificity, studied in vascular surgery patients.[43] It is well known that CRP is related to the inflammatory mechanism of atherosclerotic disease; however, no sensitivity and specificity have been determined.[44,45] The evidence concerning these biomarkers is modest, therefore routine pre-operative measurement is not recommended. Based on the results of the current study, it cannot be concluded that pRP is a better/more sensitive marker than those mentioned previously. In the present authors' opinion, these markers are complementary to each other. Future research must be performed to investigate which one is the most sensitive.

Some study limitations have to be recognised. First, although the reference range of 2.82% for reticulated platelets is used for standard clinical care in the study hospital, different reference ranges are applied in other studies, rising up to 6.0%.[46] The use of a more strict cut off value for elevation of pRP, may have resulted in different outcomes. Second, although an association has been found between pre-operative pRP with PMI and 30 day mortality, a causal connection cannot be concluded because the study was retrospective. Multiple other (cardiovascular) factors may contribute to PMI and 30 day mortality. Third, because troponin I was not measured before surgery, the results could not be adjusted for pre-existing chronic raised troponin I, the result of subclinical myocardial damage that may occur in patients with renal failure or other cardiovascular conditions. In addition, for the feasibility of the

study, troponin elevation was used as a surrogate marker. Including only clinical myocardial infarctions (electrocardiogram changes) would require inclusion of an enormous number of patients, which is beyond the scope of the present analysis.[47] Fourth, lab values up to one month were used while the lifetime of platelets is eight to ten days. This could lead to either an underestimation or an overestimation of the number of patients with high pRP values. Fifth, data on antiplatelet and statin use were not available, and this might have influenced the results.[48] In addition, no data on the use and outcome of platelet function tests (PFT) were available. In the study hospital, PFT were only performed on indication (e.g. recurrent peripheral occlusions despite antiplatelet therapy) or in patients who participated in a trial, because not all of the numerous platelet tests proved to be able to identify patients at increased cardiovascular risk.[49] Finally, the overall incidence of PMI is relatively high in this cohort of patients (30.4%) compared with previous studies, which can be explained by the exclusion of patients without pre-operative blood testing $<$ 1 month prior to surgery.[7,50] Low risk, healthy patients had no indication for pre-operative blood sampling and could therefore not be included in this study, leaving a high risk population for inclusion. The applicability of reticulated platelets as a pre-operative marker in low and intermediate risk patients should be determined in future studies.

In conclusion, there is an association between pre-operative increased pRP and post-operative myocardial injury and 30 day mortality in patients undergoing major non-cardiac surgery, aged $\geq$60 years. Patients with an increased pRP have more cardiovascular risk factors (male sex, diabetes, and renal failure) compared with patients with normal pRP. Future, prospective studies with consequent adjustment of cardiovascular risk management after pRP measurement should determine its clinical relevance.

FUNDING

None.

APPENDIX 1.

Table A1. Distribution of percentage reticulated platelets

	n	Median reticulated platelets − %	IQR (25−75%)
Myocardial injury			
No (troponin I < 0.06 μg/L)	1594	2.07	1.52−2.78
Yes (troponin I 0.06−0.6 μg/L)	695	2.21	1.57−3.11
30 day mortality			
No	2159	2.09	1.52−3.98
Yes	112	2.63	1.76−4.15

IQR = interquartile range; *n* = number.

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COUP D'OEIL

Isolated Mycotic Iliac Artery Aneurysm due to *Candida Albicans* Infection

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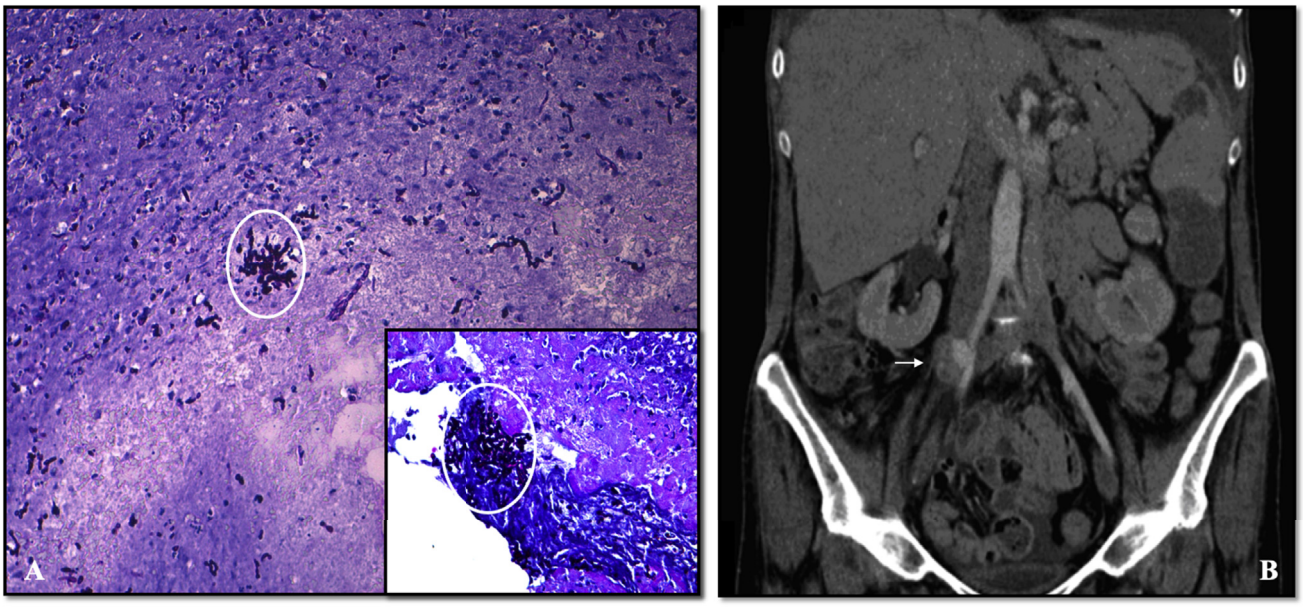

A 37 year old with a history of Fallot's Tetralogy and valvular replacement secondary to infective endocarditis was admitted with acute ischemia of the right lower limb and was treated promptly by femoral embolectomy. On histological analysis, fungal hyphae were isolated in the thrombus (**A**). As the cardiac study was normal, computed tomography angiography was performed, which revealed a 40mm mycotic aneurysm of the right common iliac artery (**B**). The patient was treated by open iliac reconstruction with an interposition graft using spiraled great saphenous vein. Histological examination of the vessel wall confirmed structural changes compatible with mycotic aneurysm, and Candida Albicans was isolated in culture.

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