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Interdisciplinary CardioVascular and Thoracic Surgery 2023, 36(5), ivad062
https://doi.org/10.1093/icvts/ivad062 Advance Access publication 26 April 2023

ORIGINAL ARTICLE

Cite this article as: Van Paassen J, De Graaf-Dijkstra A, Brunsveld-Reinders AH, De Jonge E, Klautz RJ., Tsonaka R *et al.* Leucocyte and platelet activation in cardiac surgery patients with and without lung injury: A prospective cohort study. Interdiscip CardioVasc Thorac Surg 2023; doi:10.1093/icvts/ivad062.

THORACIC NON-ONCOLOGY

Leucocyte and platelet activation in cardiac surgery patients with and without lung injury: A prospective cohort study

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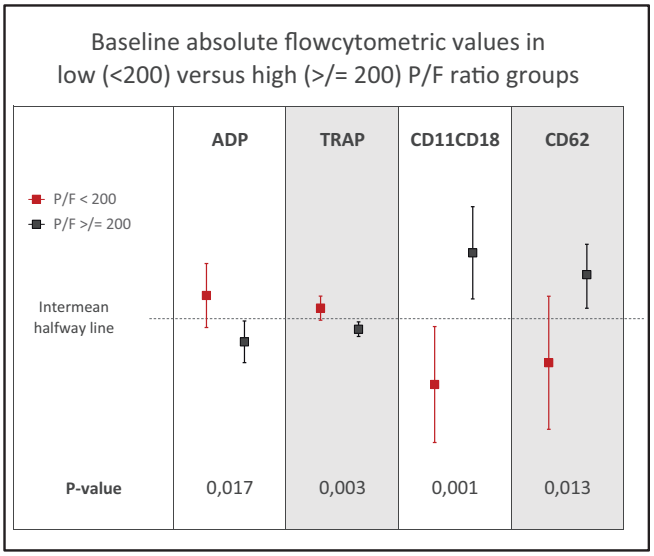
Received 16 November 2022; received in revised form 16 April 2023; accepted 25 April 2023

Leucocyte and platelet activation in cardiac surgery patients with and without lung injury

Summary

In this prospective cohort study in cardiac surgery patients, preoperatively, platelet activatability was higher, and neutrophil activation was lower in patients that developed lung injury postoperatively.
Furthermore, after correction for these baseline differences, the perioperative thrombocyte activatability was decreased, and the neutrophil activation pattern was different.



P/F ratio - PaO2/FiO2 ratio; ADP - Adenosine Di-Phosphate; TRAP - Trombine Receptor Activator Peptide; CD11CD18 - neutrophil activator ligand expression; CD62 - Neutrophil selectine expression

[†]Both authors contributed equally to this study.

Abstract

OBJECTIONS: Development of acute lung injury after cardiac surgery is associated with an unfavourable outcome. Acute respiratory distress syndrome in general is, besides cytokine and interleukin activation, associated with activation of platelets, monocytes and neutrophils. In relation to pulmonary outcome after cardiac surgery, leucocyte and platelet activation is described in animal studies only. Therefore, we explored the perioperative time course of platelet and leucocyte activation in cardiac surgery and related these findings to acute lung injury assessed via PaO2/FiO2 (P/F) ratio measurements.

METHODS: A prospective cohort study was performed, including 80 cardiac surgery patients. At five time points, blood samples were directly assessed by flow cytometry. For time course analyses in low (< 200) versus high (≥200) P/F ratio groups, repeated measurement techniques with linear mixed models were used.

RESULTS: Already before the start of the operation, platelet activatability ($P = 0.003$ for thrombin receptor-activator peptide and $P = 0.017$ for adenosine diphosphate) was higher, and the expression of neutrophil activation markers was lower (CD18/CD11; $P = 0.001$, CD62L; $P = 0.013$) in the low P/F group. After correction for these baseline differences, the peri- and postoperative thrombin receptor-activator peptide-induced thrombocyte activatability was decreased in the low P/F ratio group ($P = 0.008$), and a changed pattern of neutrophil activation markers was observed.

CONCLUSIONS: Prior to surgery, an upregulated inflammatory state with higher platelet activatability and indications for higher neutrophil turnover were demonstrated in cardiac surgery patients who developed lung injury. It is difficult to distinguish whether these factors are mediators or are also aetiologically related to the development of lung injury after cardiac surgery. Further research is warranted.

Trial registration: Clinical Registration number: ICTRP: NTR 5314, 26-05-2015

Keywords: Cardiac surgery • Lung injury • Platelet activation • Neutrophil activation • Flowcytometry

ABBREVIATIONS

ADP	Adenosine diphosphate
ARDS	Acute respiratory distress syndrome
BMI	Body mass index
CABG	Coronary artery bypass grafting
CPB	Cardiopulmonary bypass
EPD	Electronic Patient Dossier
ICU	Intensive care unit
IL	Interleukin
P/F ratio	PaO_2/FiO_2 ratio
TRAP	Thrombin receptor-activator peptide

INTRODUCTION

Cardiac surgery induces a systemic inflammatory response syndrome with an incidence of 42% that can lead to concurrent single or multiple organ dysfunction [1]. Development of acute lung injury and even acute respiratory distress syndrome (ARDS) in this respect is reported in 0.5–20% of patients after cardiac surgery [1–3] and is associated with a complicated postoperative course [1, 3, 4], high mortality (50-90%) [2, 4–7] and significant long-term physical and psychological sequelae [7].

The pathophysiology of postoperative acute lung injury is thought to lie in inflammation-induced disruption and increased permeability of the alveolar–capillary membrane and development of pulmonary oedema [8]. Furthermore, it is thought to be evoked by multiple successive factors, such as pre-existent impaired left ventricular function, the use and duration of cardiopulmonary bypass (CPB), lung ischaemia–reperfusion injury, transfusion of blood products and complexity of the operation [2, 5]. Complement activation through both the classical and alternate pathways, the subsequently or concurrently released proinflammatory cytokines [tumour necrosis factor-α, interleukin (IL)-1, IL-2, IL-6, IL-8] and anti-inflammatory cytokines (IL-10, IL-1ra, tumour necrosis factors sr1 and 2 and transforming growth factor) influence the magnitude and severity of the inflammatory response after cardiac surgery and the development of ARDS [5, 9]. A prominent role in the development of ARDS in general has been assigned to activated platelets, monocytes and neutrophils [10, 11]. Studies in cardiac surgery demonstrated that platelet and leucocyte activation and platelet–leucocyte aggregation were increased [8, 12, 13] and suggested endothelial adhesion and entrapment of platelets and polymorphonuclear neutrophils in the lungs [5, 14]. However, these studies have described flow cytometric patterns of platelets and leucocytes after cardiac surgery in general, but relating them to postoperative development of acute lung injury and ARDS has only been done to a limited extent in combination with other biomarkers [15] or in animal studies [14, 16].

It was our hypothesis that increased platelet and leucocyte activation patterns, as can be measured by flow cytometry, would be present in cardiac surgery patients who developed acute lung injury compared to the situation in cardiac surgery patients who did not develop acute lung injury. Therefore, in this study, our goal was to further explore the intra- and postoperative time courses of platelet activation and activatability, leucocyte activation and the platelet–leucocyte interactions in adult cardiac surgery patients and relate these findings to the occurrence of acute lung injury. Further insight into platelet- and leucocyte-associated biomarkers related to acute lung injury after cardiac surgery is important for understanding the pathophysiology and to bring personalized preventive measures a step closer to clinical practice.

PATIENTS AND METHODS

Ethical statement

The study was approved by the Medical Ethical Committee (27-09-2011; protocol P11-117) of the Leiden University Medical Center and was conducted in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments [17]. The trial was registered at the International

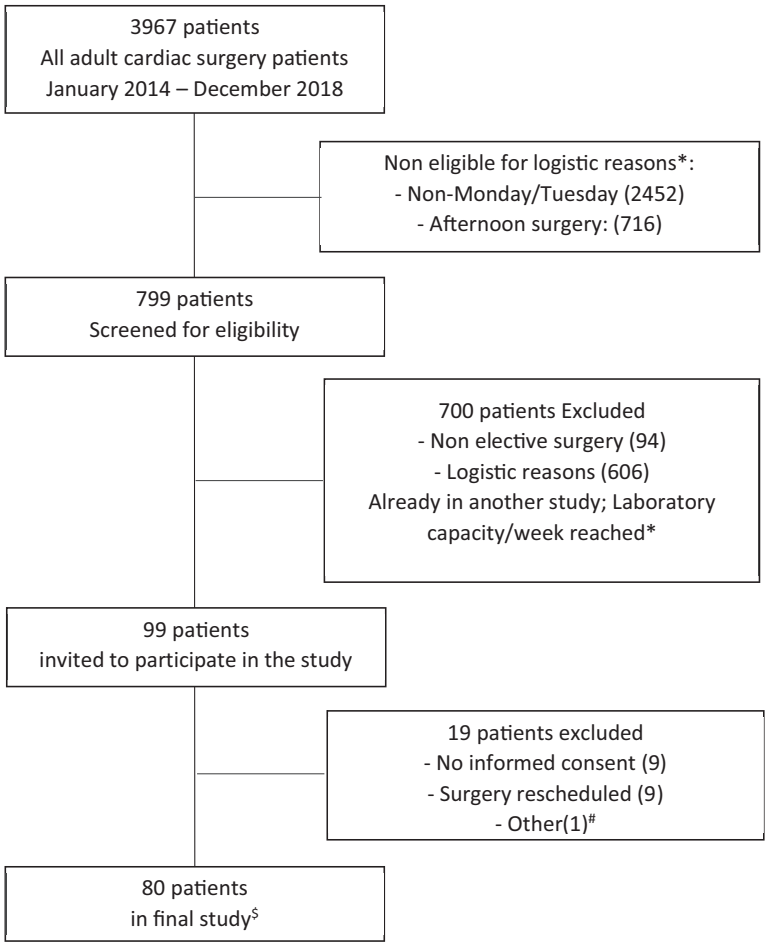


Figure 1: Flow chart inclusion process. *A precise logistic network, consisting of a dedicated researcher and laboratory employee, was set up to ensure immediate transportation to the laboratory and direct analyses of blood samples on a readily available flow cytometer in the laboratory on all consecutive study days. Therefore, patients could only be considered for inclusion when surgery was planned early in the morning on a Monday or Tuesday. Maximal capacity was limited to 1 patient a week. # IV access difficulties (history of chemotherapy) prohibited drawing blood from a peripheral vein. Preoperative blood sampling would mean additional invasiveness and was judged too burdensome for the patient. $ Four patients were excluded from analyses because they were extubated in the operation room and no postoperative P/F ratios were available.

Clinical Trials Registry Platform (NTR 5314, 26–05–2015). The Strobe statement checklist [18] is available in Supplement 1. Written informed consent was obtained from all included patients on the day before surgery.

Study design and study population

A prospective cohort study was performed in the intensive care unit (ICU) of a tertiary referral hospital, the Leiden University Medical Center in the Netherlands. Eligible patients were adults undergoing elective cardiac surgery. A dedicated researcher and a laboratory technician were available to ensure immediate transportation of samples to the laboratory and direct analyses on a readily available flow cytometer in the laboratory on all consecutive study days. Therefore, patients could only be considered for inclusion when surgery was planned for early morning on a Monday or Tuesday. The maximal capacity was limited to 1 patient a week and then only when critical laboratory and research personnel were available. The selection of Monday or Tuesday did not introduce a bias of specific types of surgery because in our hospital all types of cardiac operations are performed equally over the weekdays. The exclusion criteria were inability to sign an informed consent form, being younger than 18 years old, the need for an emergency operation, participation in another study and preoperative use of corticosteroids. The follow-up period was until discharge from the hospital.

Sample size

A formal sample size estimation was not possible because only a few earlier studies were available [12, 19–23], and these studies were small (all included fewer than 20 patients); research questions varied; and tests for platelet or white blood cell activity were different from the methods we intended to use. Therefore, we applied a pragmatic approach to include as many patients as possible during the years that were assigned to conduct this study.

Perioperative care

All patients visited the preoperative outpatient clinic for preoperative screening. All patients were admitted 1 day before surgery.

Table 1: Patient characteristics

Characteristics	P/F ratio < 200 n = 23		P/F ratio ≥ 200 n = 53		*P*-value*
Demographic parameters					
Age (year) (mean, SEM)	66.6	2.1	65.0	1.2	0.583
Gender (male) (n, %)	12	55	26	49	0.147
BMI (kg/m^2) (mean, SEM)	25.8	1,9	21.7	0.7	0.217
Co-morbidity (n, %)					
Myocardial infarction in history	4	18	7	13	0.032
History of PCI	4	18	1	2	0.021
History of thoracic surgery	0	0	2	4	0.491
Hypertension	9	41	14	26	0.147
Malignancy	0	0	1	2	0.707
Chronic kidney insufficiency	1	5	0	0	0.293
Chronic liver disease	2	9	0	0	0.083
Diabetes	4	18	0	0	0.006
COPD	1	5	8	15	0.478
Smoking	12	55	21	40	0.205
Pack-years (median, IQR)	12	50	20	25	0.493
Forced vital capacity (L) (median, IQR)	3,4	1.8	3.8	1.5	0.516
FEV_1/VC (median, IQR)	69.5	8.6	74.4	9.6	0.328
Preoperative medication use (n, %)					
Diuretics	11	50	20	38	0,284
ACE blockers	13	56	25	47	0.195
Beta-blockers	15	65	29	55	0.276
Calcium antagonist	6	26	8	15	0.205
Statins	13	56	25	47	0.309
Thrombocyte aggregation blockers[a]	10	43	20	38	0.412
Preoperative performance state (n, %)					
ASAI	0	0	0	0	
II	4	18	5	9	
III	16	73	41	77	
IV	1	5	0	0	0.203
LVEF Good LVEF > 55%	9	41	23	43	
Reasonable LVEF 40-55%	6	27	11	21	
Moderate LVEF 25-40%	5	23	2	4	
Poor LVEF < 25%	2	9	2	4	0.048
EuroSCORE 2 (logistic) (median, IQR)	3.0	7.7	2.2	2.3	0.014
Surgical parameters					
Surgical procedure (n, %)					
CABG	3	14	11	21	
CABG + single valve	4	18	6	11	
CABG + multiple valves	1	5	1	2	
Single valve	5	23	13	25	
Multiple valves	1	5	6	11	
Thoracic aorta surgery (+/- valve +/- CABG)	4	18	13	25	
Heart failure surgery	3	14	4	8	
Other	2	9	4	8	0.415
Surgical duration					
Operation (min) (median, IQR)	360	126	345	90	0.361
Cardiopulmonary bypass (min) (median, IQR)	115	106	110	71	0.704
Corticosteroid therapy[b]					
Intraoperative use, overall (n, %)	8	36	11	21	0.450
Dexamethasone 0.1-0.5 mg/kg	6	27	8	15	
Hydrocortison 100 mg	2	9	2	4	
Prednisolone 0.5 mg/kg	0	0	1	2	
ICU admittance risk score					
APACHE IV (medIan, IQR)	58	17.7	44	19	0.012

*χ^2 test for categorial parameters. Means and *t*-test for normally distributed parameters. Median and Mann-Whitney test for skewed distributed parameters.

[a]Antiplatelet therapy is discontinued 5–10 days before surgery unless patients underwent recent coronary artery stenting or suffer from instable angina pectoris and had a semi-acute indication for surgery.

[b]Patients who had an indication for corticosteroids preoperatively were excluded from this study. Indications for intraoperative corticosteroid use are systemic inflammatory response with high vasopressor demand, allergic reaction and bronchospasm.

ACE: angiotensin-converting enzyme; APACHE: Acute Physiology and Chronic Health Evaluation; ASA: American Standards Association; BMI: body mass index; CABG: coronary artery bypass graft; COPD: chronic obstructive pulmonary disease; QR: interquartile range; FEV_1: forced expiratory volume in 1 s; LVEF: left ventricular ejection fraction; P/F ratio: PaO2/FiO2 ratio; PCI: percutaneous coronary intervention; SEM: standard error of means; VC: vital capacity.

Table 2. Patient Outcome

Characteristics	P/F ratio < 200 n = 23		P/F ratio ≥ 200 n = 53		*P*-value*
Outcome					
Ventilation time, min (median, IQR)	840.0	2704	675.0	405	0.005
Length of ICU stay, h (median, IQR)	43.5	161	22.5	3	0.047
Length of hospital stay, days (median, IQR)	11.0	18	8.0	4	0.064
ARDS within 7 days after surgery (n, %)	14	64	9	17	< 0.001
Mortality (n, %)	2	9	0	0	0.088

*$\chi^2$for categorial parameters. Means and *t*-test for normally distributed parameters. Median and Mann-Whitney test for skewed distributed paramters.
ARDS: acute respiratory distress syndrome; ICU: intensive care unit; IQR: interquartile range; P/F ratio: PaO2/FiO2 ratio.

Perioperative care for cardiac surgery patients is standardized in the Leiden University Medical Center and follows a pre-established care path. All details regarding the perioperative care are summarized in Supplement 2.

Data collection

All pre-, intra- and postoperative data and clinical parameters were obtained from the electronic patient database system of the hospital. This database is used preoperatively, in the operating room and in the ICU. In the ICU, continuous haemodynamic and ventilation monitoring is recorded. An arterial blood gas analysis is done 4 times per day and more frequently on indication.

Sample collection

At 5 different time points, blood samples were drawn [before the start of anaesthesia/surgery at the holding facility (T0), 1 h after weaning from CPB (T1), T1 + 3 h (T2), on the first postoperative day (T3) and on the second postoperative day (T4). Samples were collected in lithium heparin vacuettes. Samples were transported to the laboratory and analysed immediately after collection.

Flow cytometry analyses

The Beckman Coulter FC500MPL was used for flow cytometric analyses. A designated laboratory employee was available for our study. Before starting the actual measurements of our study samples, daily quality checks were performed to ensure the reliability and accuracy of the results. For platelet activation markers and leucocyte–platelet complexes, dilutions were created using 3% bovine serum albumin (BSA)/phosphate buffered saline (PBS).

Platelet activation: Fluorescein antibodies against CD61 [fluorescein isothiocyanate (FITC)] and CD62 (P-slectine Exposure (PE)) were used as platelet activation markers. Adenosine diphosphate (ADP) and thrombin receptor activator for peptide (TRAP) were chosen as well-known examples of, respectively, weak and strong platelet agonists that in our hands showed good day-to-day reproducibility of agonist concentration-dependent aggregation responses. Analyses were done with 8 increasing concentrations of the different agonists.

Leucocyte activation: White blood cells were adjusted to a maximum concentration of 20 x 10^6/ml in PBS, fluorescein antibodies against neutrophil CD66b (FITC) and activation markers CD11b/18 (PE), CD62L (PE) were used; OptiLyse-C for lysis of the erythrocytes and stem cell count beads for calibration.

Leucocyte-platelet complex formation: White blood cells were adjusted to a maximum concentration of 20 x 10^6/ml in PBS. Fluorescein antibodies against monocyte CD14 (FITC), neutrophil CD66b (FITC) and platelets CD42b (PE), for calibration beads stem count were used. OptiLyse-C was used for lysis of the erythrocytes, and stem cell count beads were used for calibration.

The laboratory studies yielded dose-response activation curves from which the maximum, the minimum and the mean percentage, which is an equivalent of the area under the activation curve [24], of responsive cells can be derived. Because the mean percentage of responsiveness is an aggregate measure, reflecting both the maximum number of responses and the minimum agonist concentration at which cells respond [24], it was decided to use the mean percentage. Results were expressed as mean percentage responsive cells in the dose-response activation curve.

End points

The primary goals of this study were to monitor the perioperative time course of leucocytes, platelets and their activation status and complex formation in adult cardiac surgery patients with and without acute lung injury. We used the PaO_2/FiO_2 (P/F) ratio to measure lung injury. It is an objective tool to identify acute hypoxemic respiratory failure when supplemental oxygen and positive pressure ventilation are being administered and, as such, it is a measure of acute lung injury and an important item in the definition of ARDS according to the Berlin Criteria [25].

In patients having thoracic surgery, a P/F ratio < 200 is associated with an unfavourable outcome, and each increase in the ARDS severity category, which is per definition only determined by a decline of the P/F ratio, is reported with a significant increase in morbidity and mortality [4]. Hence, to distinguish patients with mild from those with more severe and clinically more relevant lung injury, we dichotomized the P/F ratio into 2 subgroups of lung injuries: a moderate to severe impaired respiratory state (lowest postoperative P/F ratio < 200 mmHg) versus a mildly impaired and normal respiratory state (lowest postoperative P/F ratio ≥ 200 mmHg).

To determine surgery-related lung injury, we used, analogous to these Berlin criteria, a maximum time period of 1 week after surgery. The FiO_2 is notoriously unreliable from the moment low-flow oxygen supply is initiated [27, 28], and the PaO_2 is only measured when clinically indicated when the patient is in the general thoracic surgery ward. Therefore, the P/F ratio was not available for extubated patients. It is unlikely that we missed patients who developed a low P/F ratio and lung injury on the ward, especially because all patients are transferred back to the ICU once (non-) invasive ventilation becomes mandatory.

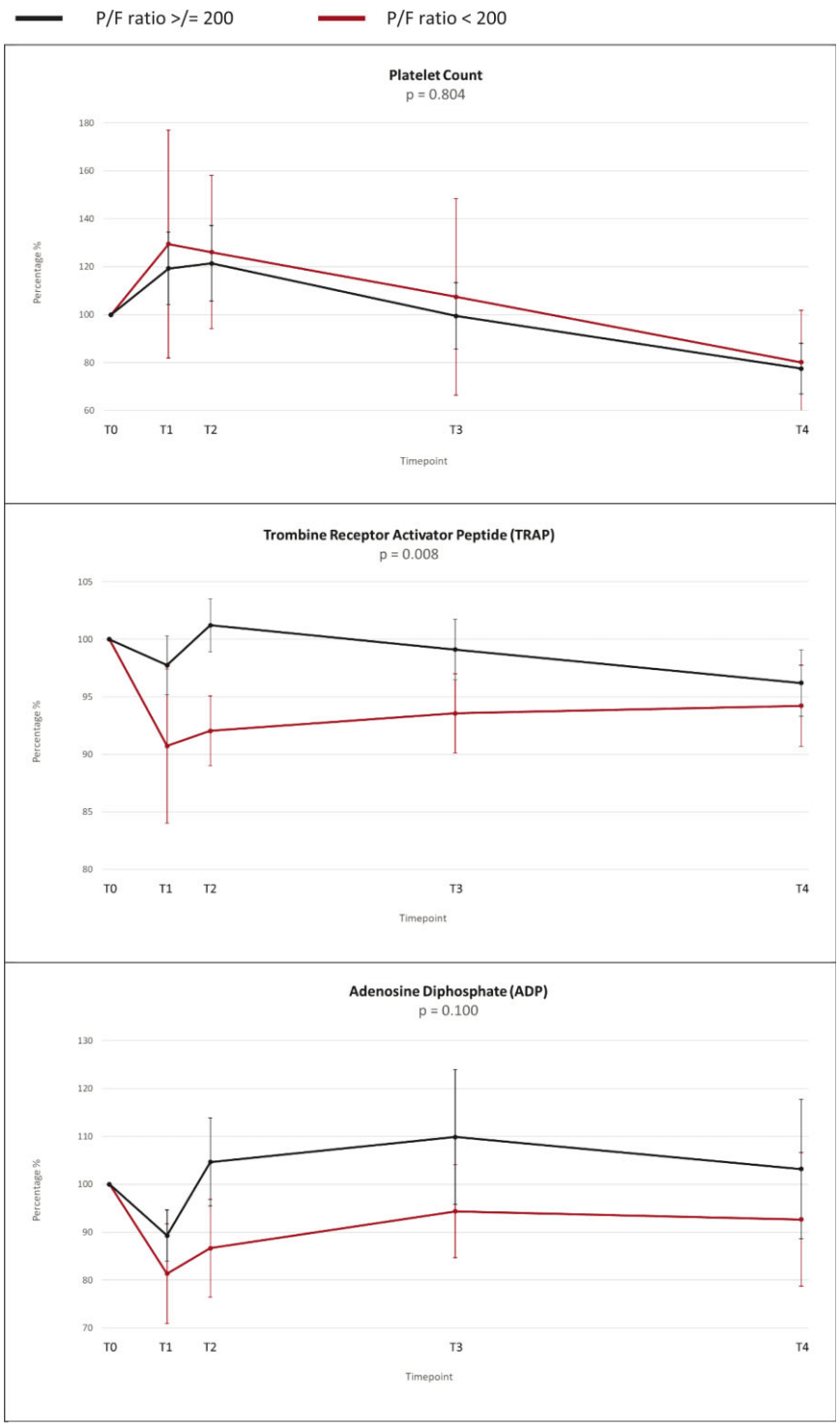


Figure 2: Platelets and platelet activatability in time for low (<200) and high (≥200) P/F ratio *(*adjusted for T0 difference*)*. P-values are given for the differences between low and high P/F ratio groups by linear mixed modelling with T0 as covariate. T0: preoperative; T1: end CPB + 1 h; T2: T1 + 3 h; T3: T1 + 18 h; T4: postoperative day 2.

Statistical analyses

In consultation with the Departments of Biomedical Data Sciences and Clinical Epidemiology of the Leiden University Medical Centre, a statistical analysis plan was defined in advance, before disclosure of the data. The statistical analysis was carried out according to the plan.

Demographic and clinical characteristics are presented as mean (SD) or median (interquartile range) for continuous variables and as absolute values and percentages for categorical variables. For the normal/mildly impaired versus moderate/severe respiratory groups, baseline characteristics were compared with a Fischer's exact test for categorical variables and the Mann–Whitney *U* test for continuous variables.

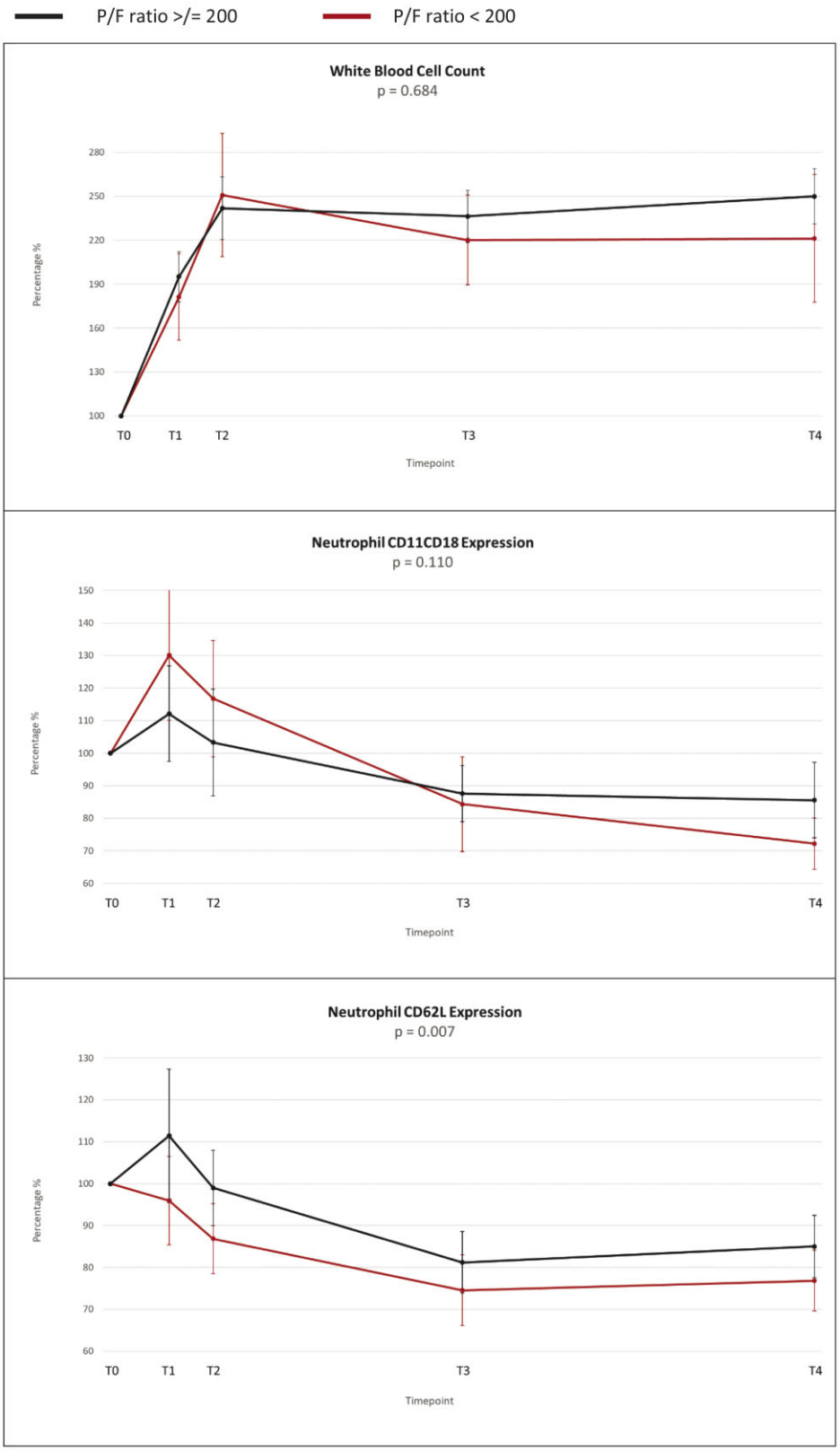


Figure 3: Leucocytes and leucocyte activation over time for low (<200) and high (≥200) P/F ratio groups (adjusted for T0 difference). P-values are given for the differences between low and high P/F ratio groups by linear mixed modelling with T0 as covariate. T0: preoperative; T1: end CPB + 1 h; T2: T1 + 3 h; T3: T1 + 18 h; T4: postoperative day 2.

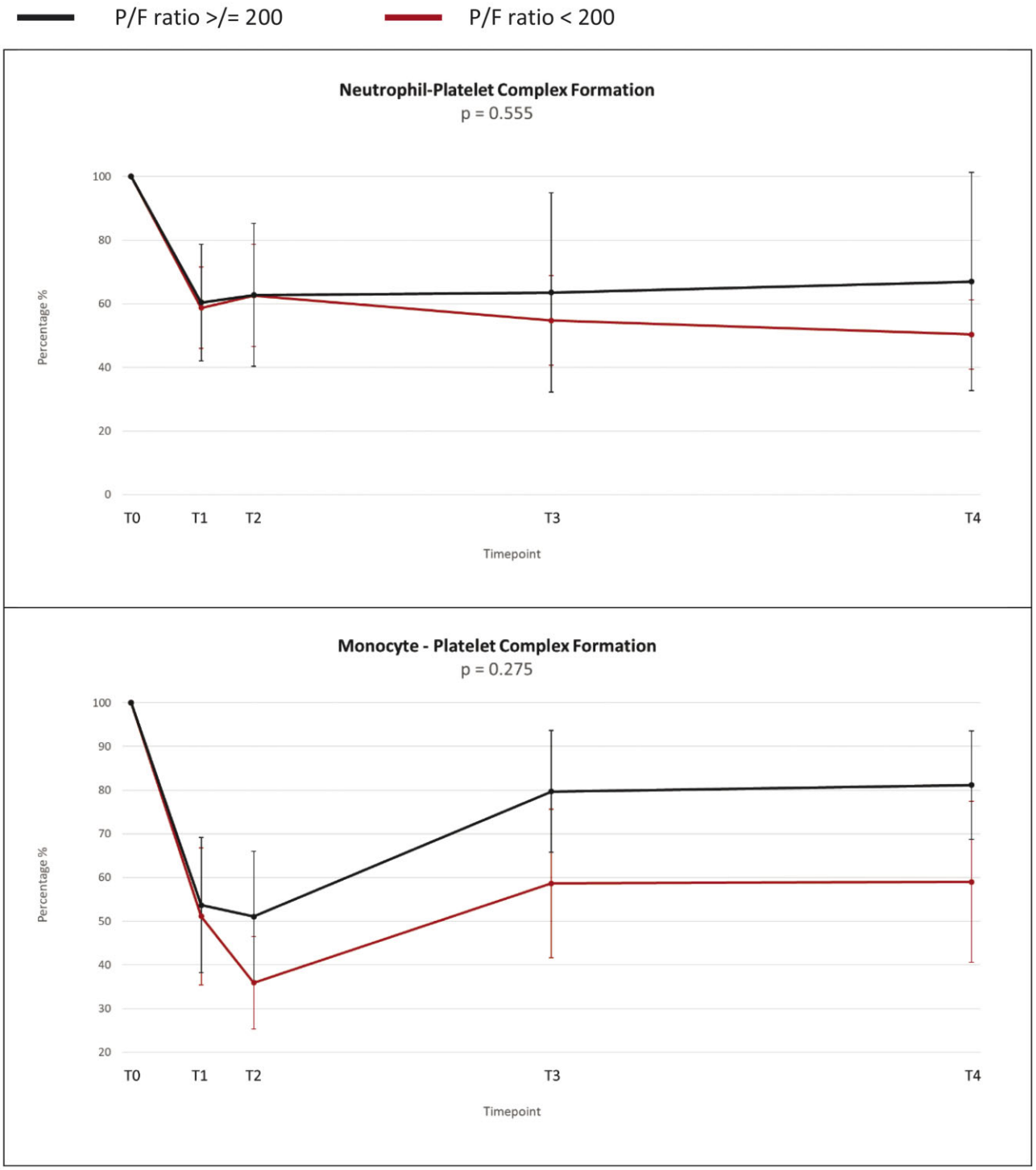


Figure 4: Complex formation over time for low (<200) and high (≥200) P/F ratio groups (adjusted for T0 difference). *P*-values are given for the differences between low and high P/F ratio groups by linear mixed modelling with T0 as covariate. T0: preoperative; T1: end CPB + 1 h; T2: T1 + 3 h; T3: T1 + 18 h; T4: postoperative day 2.

Furthermore, the perioperative time courses of white blood cells, platelets, platelet and leucocyte activation and platelet-leucocyte complex formation were tested for significance. Baseline differences in the P/F ratio groups were tested with a *t*-test, and the further perioperative time course was tested with repeated measurements techniques, i.e. linear mixed models. Of particular interest was the (i) effect of P/F ratio groups, i.e. high (≥200) versus low (<200) P/F ratio and (ii) the effect of time in the different P/F ratio groups. To distinguish baseline differences (i.e. at T0) from postoperative effects, the baseline value was compared in both P/F ratio groups using a *t*-test, and introduced as a variable in the repeated measurement analysis.

The statistical analyses were conducted using SPSS (Statistical Package for the Social Sciences), release 25.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

A total of 80 patients were included in this study from January 2014 to January 2018. In Fig. 1, a flow chart of the inclusion process is shown. Four patients were excluded from the analyses because they had already been extubated when they arrived in the ICU, and no P/F ratios could be derived. The patient characteristics are summarized in Table 1. Patients were predominantly middle-aged males: their body mass indexes were higher in the low-versus-high P/F ratio groups; their left ventricular function was worse in the low compared to the high P/F ratio group; their EuroSCOREs were higher in the low P/F ratio group; and heart failure surgery was performed more frequently in the low P/F ratio group. Development of ARDS was more common in the low (P/F < 200) versus the high (P/F >/= 200) P/F ratio group [14/23 (64%) versus 9/53 (17%) ($P < 0.001$)]. Furthermore, patients in the low P/F ratio group spent a longer time on the mechanical ventilator, had a longer ICU stay and died more often (Table 2).

Before the start of the operation (Fig. 2), platelet activatability, as measured after adding ADP and TRAP, was higher in the low compared to the high P/F ratio group ($P = 0.003$ for TRAP and $P = 0.017$ for ADP). Expression of neutrophil activation markers was lower for both CD18/CD11 ($P = 0.001$) and CD62L ($P < 0.013$) in the low compared to the high P/F group.

The perioperative time course of platelets, platelet activatability, WBC and neutrophil activation markers and leucocyte–platelet complex formation are shown in Figs. 2–4. To visually correct for the T0 differences, T0 is preset for both groups at 100%; the subsequent values are then shown in relation to this T0. In the time course analyses, only TRAP-induced thrombocyte activatability decreased more in the low P/F ratio group ($P = 0.029$). Additionally, a clear neutrophil activation pattern was observed in the latter group with increasing CD11CD18 and lowering of CD62L.

DISCUSSION

In this prospective cohort, we measured platelet- and leucocyte-associated biomarkers in the pre-, intra- and postoperative phases of 80 cardiac surgery patients. We related these biomarkers to the development of acute lung injury, defined by the P/F ratio. We demonstrated that before the start of the operation, the patient group that developed acute lung injury as defined by P/F ratio < 200 had a significantly higher capacity of platelets to be activated by ADP and the presence of TRAP. Furthermore, lower expression of both CD11/CD18 and CD62L was present in the low P/F ratio group. After correction of T0 differences, the postoperative course for the low P/F ratio group showed a significant decrease in TRAP-induced platelet reactivity and a typical pattern of the 2 neutrophil activation markers: Though not significantly different, neutrophil CD11/CD18 and CD62L expression showed an inverse change, i.e. increasing CD11/CD18 expression and decreasing CD62L expression, that could be interpreted as a higher turnover of granulocytes in the lower P/F ratio group [29, 30].

Some limitations of our study must be addressed, the most important of which is the sample size. Although, to our knowledge, this is the largest study of platelet and neutrophil activation in cardiac surgery patients and the first to relate these data to an acute lung reaction after cardiac surgery, still the sample size, based on pragmatic grounds, is not large enough to allow correction for or in-depth subgroup analyses of the different types of operations, steroid use, antiplatelet therapy and other factors that might confound the outcomes in this study.

Secondly, the preoperative flow cytometric differences in the 2 P/F ratio groups are not necessarily aetiologic factors in the development of pulmonary complications. They could also be mediators associated with other preoperatively present comorbidities, such as impaired left ventricular function, previous coronary artery disease, chronic heart failure, arteriosclerosis and atrial fibrillation, all of which are well-known risk factors for the development of acute lung injury and unfavourable outcomes after cardiac surgery [26] and all of which are associated with increased inflammatory status, platelet activation and endothelial-driven hypercoagulability [27–30]. In our study, these risk factors were more common in patients in the low P/F group. Therefore, a (partly) non-causal or indirect relation of our biomarkers with this outcome cannot be excluded.

Furthermore, the local role of platelets and neutrophils (and their complexes) in the lung itself cannot automatically be translated from measurements in circulating blood. In this respect, it would have been interesting to study platelet and neutrophil activation status in blood and alveolar fluid concomitantly. Likewise, to clarify the role of CPB, sequential sampling of blood in both the inlet and outlet of CPB would have been informative.

Notwithstanding all the aforementioned limitations, clear differences in platelet activatability and neutrophil counts were demonstrated at baseline. Although the low P/F group showed a more typical neutrophil activation pattern in the postoperative time course with clear inverse reacting CD11CD18 (up) and CD62L (down), other parameters, when percentualized at the baseline level, showed mostly not significant but discrete postoperative time courses in both outcome groups. From this result, it has to be realized that differences in absolute values during the first postoperative days remain.

Although a non-aetiologic effect between flow cytometric results and outcome is uncertain and extrapolating whole blood results to pathophysiological processes in the lungs has important limitations, the results of our study cannot entirely be brushed off. Our findings suggest that the preoperative presence of increased activatability of platelets and lower neutrophil activation marker expression (with possibly also a higher neutrophil turnover) may have an aetiologic role in the development of lung injury after heart surgery. The differences in biomarker baseline values between both study P/F ratio groups fit with the well-known pathophysiology of acute lung injury in other patient groups. In previous studies in patients with ARDS, neutrophils indeed are shown to migrate to the inflamed tissue site, where the multiple steps of neutrophil-endothelial tethering, rolling, adhesion, crawling and transmigration take place. Subsequently, neutrophil extracellular traps enable them to elicit their immunological action locally [4, 31, 32]. In accordance with these results, earlier observational studies in cardiac surgery reported the activation of monocytes, neutrophils and platelets [12, 13, 16, 20, 33], endothelial transmigration and the influx of these complexes in several organ systems as well [14, 16, 33]. Such inflammatory processes could well be augmented if presurgery

platelets and neutrophils are already activated, as was the case in our low P/F group.

Despite the limitations of this study, especially the limited sample size, some important additional research questions arise when considering the signals that emerged from this study. Initially, it would be worthwhile to further explore thrombocyte and neutrophil activation patterns in predefined subgroups, such as the various surgical subtypes and in patients with and without antiplatelet therapy. Also it would be interesting to further explore the activation patterns in patients who, according to prevailing practice, received steroids postoperatively versus those who did not. Furthermore, in this study, due to sample size, we were unable to perform reliable prognostic studies, and we refrained from making receiver operating characteristic curves and calculating sensitivity and specificity. However, in future studies, it would be valuable to explore the prediction capacity of flow cytometric markers in addition to already known and applied prediction models. Finally, the perioperative signals of thrombocyte and neutrophil activation in this specific study population of thoracic surgery patients and the association with pulmonary outcomes might also be extended to other patient groups. It is conceivable that similar processes play a role in other major surgery types, such as vascular/aortic surgery, extensive gastrointestinal surgery and transplant surgery. Future studies are necessary to elucidate inflammatory responses and specifically the role of platelet and white cell activation after other types of major operations.

CONCLUSION

We showed that, prior to commencement of cardiac surgery, an upregulated inflammatory state is present in patients who develop (acute) lung injury. The observed higher platelet activatability, the signs of higher neutrophil turnover before surgery and a typical neutrophil activation pattern later could well contribute to more severe acute lung injury in this respect. Further research, however, is needed and should at least involve (i) predefined subgroups (various types of operations, steroid use and antiplatelet therapy); (b) the effect of the CPB circuit on platelets and neutrophils; (iii) the influence of transfused blood products and (iv) the neutrophil-platelet interactions at the level of the alveolar-capillary lung. If biomarkers on platelets and neutrophils and lung vasculature interactions can eventually be causally linked to acute lung injury, we would be a step closer to personalized medicine with patient-specific considerations with regard to interventions, expected outcomes and specific preventive measures.

SUPPLEMENTARY MATERIAL

Supplementary material is available at ICVTS online.

FUNDING STATEMENT

This study was funded by grant PPOC-13-RvB-02 from Sanquin Research, Plesmanlaan 125, 1066 CX Amsterdam, The Netherlands.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

DATA AVAILABILITY

The data underlying this article cannot be shared publicly due to privacy regulations to protect the privacy of the individuals who participated in the study. The data will be shared on reasonable request to the corresponding author.

Author contributions

Judith Van Paassen: Conception and design; Analysis and interpretation; Fieldwork/laboratory work; Drafting the manuscript for important intellectual content. **Alice De Graaf-Dijkstra:** Fieldwork/laboratory work: Drafting the manuscript for important intellectual content. **Anja H. Brunsveld-Reinders:** Fieldwork/laboratory work: Drafting the manuscript for important intellectual content. **Evert De Jonge:** Conception and design; Analysis and interpretation; Drafting the manuscript for important intellectual content. **Robert J.M. Klautz:** Conception and design; Drafting the manuscript for important intellectual content. **Roula Tsonaka:** Analysis and interpretation; Drafting the manuscript for important intellectual content. **Jaap Jan Zwaginga:** Conception and design; Analysis and interpretation; Drafting the manuscript for important intellectual content. **M. Sesmu Arbous:** Conception and design; Analysis and interpretation; Drafting the manuscript for important intellectual content.

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