hearts

MDPI

Article

Effect of Neutrophil–Platelet Interactions on Cytokine-Modulated Expression of Neutrophil CD11b/CD18 (Mac-1) Integrin Complex and CCR5 Chemokine Receptor in Stable Coronary Artery Disease: A Sub-Study of SMARTool H2020 European Project

Silverio Sbrana [1,*], Stefano Salvadori [1], Rosetta Ragusa [1], Elisa Ceccherini [1], Adrian Florentin Suman [1], Antonella Cecchettini [1,2], Chiara Caselli [1], Danilo Neglia [3], Gualtiero Pelosi [1] and Silvia Rocchiccioli [1]

[1] Institute of Clinical Physiology, National Research Council, 56124 Pisa, Italy; stefano.salvadori@ifc.cnr.it (S.S.); rosettaragusa@cnr.it (R.R.); ceccherini@ifc.cnr.it (E.C.); adrianflorentinsuman@cnr.it (A.F.S.); antonella.cecchettini@unipi.it (A.C.); chiara.caselli@ifc.cnr.it (C.C.); gualtieropelosi@gmail.com (G.P.); silvia.rocchiccioli@ifc.cnr.it (S.R.)
[2] Department of Clinical and Experimental Medicine, University of Pisa, 56126 Pisa, Italy
[3] Fondazione Toscana Gabriele Monasterio, Via Moruzzi 1, 56124 Pisa, Italy; dneglia@ftgm.it
* Correspondence: silverio.sbrana@ifc.cnr.it; Tel.: +39-050-315-2688

Abstract: Atherosclerosis is an inflammatory disease wherein neutrophils play a key role in plaque evolution. We observed that neutrophil CD11b was associated with a higher necrotic core volume in coronary plaques. Since platelets modulate neutrophil function, we explored the influence of neutrophil–platelet conjugates on the cytokine-modulated neutrophil complex CD11b/CD18 and CCR5 receptor expression. In 55 patients [68.53 ± 7.95 years old (mean ± SD); 71% male], neutrophil positivity for CD11b, CD18 and CCR5 was expressed as Relative Fluorescence Intensity (RFI) and taken as a dependent variable. Cytokines and chemokines were assessed by ELISA. Following log-10-based logarithmic transformation, they were used as independent variables in Model 1 of multiple regression together with Body Mass Index and albumin. Model 1 was expanded with the RFI of neutrophil CD41a+ (model 2). The RFI of neutrophil CD41a+ correlated positively and significantly with CD11b, CD18, and CCR5. In Model 2, CCR5 correlated positively only with the RFI of neutrophil CD41a+. Albumin maintained its positive effect on CD11b in both models. These observations indicate the complexity of neutrophil phenotypic modulation in stable CAD. Despite limitations, these findings suggest there is a role played by neutrophil–platelet interaction on the neutrophil cytokine-modulated expression of adhesive and chemotactic receptors.

Keywords: coronary artery disease; neutrophil–platelet conjugates; neutrophil phenotype; integrin molecules; chemokine receptors; cytokines; multiple regression statistics; flow cytometry

Citation: Sbrana, S.; Salvadori, S.; Ragusa, R.; Ceccherini, E.; Suman, A.F.; Cecchettini, A.; Caselli, C.; Neglia, D.; Pelosi, G.; Rocchiccioli, S. Effect of Neutrophil–Platelet Interactions on Cytokine-Modulated Expression of Neutrophil CD11b/CD18 (Mac-1) Integrin Complex and CCR5 Chemokine Receptor in Stable Coronary Artery Disease: A Sub-Study of SMARTool H2020 European Project. *Hearts* **2024**, *5*, 410–420. https://doi.org/10.3390/hearts5030029

Academic Editor: Matthias Thielmann

Received: 27 June 2024
Revised: 7 September 2024
Accepted: 10 September 2024
Published: 16 September 2024

1. Introduction

Blood neutrophils play a key role in atherosclerotic plaque formation and evolution [1,2]. Recent observations indicate that neutrophils actively sense and adapt to subtle and multiple environmental stimuli, both under healthy states and inflammatory conditions, thus exhibiting a differently modulated context-dependent phenotype [3]. In particular, the neutrophil interaction with circulating platelets represents an important amplification mechanism for neutrophil function [4]. In a recent cross-sectional study involving stable coronary artery disease (CAD) patients recruited as part of the European Project SMARTool [5], we observed that the blood neutrophil expression of the integrin activation molecule CD11b (α-chain of the Mac-1 complex) was positively associated with a higher relative volume of the lipid-rich necrotic core. These data suggested a greater coronary plaque vulnerability in CAD patients. Furthermore, we also observed that the neutrophil–platelet interaction, assessed as the number of platelets bound per neutrophil

and quantified by flow cytometry as the RFI of neutrophil CD41a+ cells, correlated positively with the expression of several surface receptors involved in cellular adhesion and chemotactic response. This last observation confirms the usefulness of using therapeutic inhibitors of the platelet CD41/CD61 receptor complex (gpIIb/IIIa) during coronary atherosclerotic disease, either in acute or chronic clinical settings, as demonstrated in the cited literature [6,7]. In the present study, we aimed to expand those previous findings in the same patient group by exploring further associations of circulating neutrophil–platelet interactions—quantified in terms of RFI of neutrophil positivity for CD41a—with neutrophil expression (RFI) of adhesion molecules CD11b and CD18 (associated with the surface integrin complex Mac-1) and the RANTES (Regulated upon Activation Normal T-cell Expressed and Secreted) chemokine receptor CCR5. In particular, we decided to evaluate the neutrophil expression of the chemokine receptor CCR5 not only because it represents the main receptor for chemokine CCL5 (RANTES) released by platelets following activation, but also because it is an expression of the neutrophil activation state during inflammation and is involved in tissue migration of these cell types [8,9]. These associations were assessed following an extensive statistical backward multiple regression analysis based on several immune–biochemical parameters as adjustment variables.

2. Materials and Methods

2.1. Patients

A subgroup of patients (n = 55), already recruited as part of the European Project SMARTool (Clinical-Trials.gov Identifier: NCT04448691) [5], was studied. The inclusion and exclusion criteria are listed in Table S1 in Supplementary Materials; in brief, they included patients with a history of suspected CAD and who are undergoing angiographical coronary examination. All patients received an aspirin (ASA) dose of 100 mg once daily. An additional inclusion criterion, besides those reported in [5], was the full matching of cytometry data and clinical–demographic and immuno-biochemical parameters. Sample collection was performed in accordance with Helsinki's Declaration and informed consent was obtained from each patient.

2.2. Biochemical Analyses

General biochemical parameters and enzyme-linked immunosorbent assay (ELISA) tests were performed as already described [10,11]. In brief, serum lipid profiles and general biochemical parameters were measured by routine clinical protocols, while plasma adhesion molecules and serum cytokines were quantified by ELISA tests. The following commercial ELISA kits were used: 950.035 (Diaclone SAS, Besançon Cedex, France) for a high-sensitivity IL-6 assessment; E-EL-H0108, E-EL-H0109 and E-EL-H0103 (Elabscience Bio-technology, Houston, TX, USA) for IFN-γ, TNF-α and IL-10 evaluations, respectively; BMS281, KHC0081, EHRNTS and EHCX3CL1 (Thermo Fisher Scientific, Waltham, MA, USA) for MCP-1, IL-8, RANTES and Fractalkine evaluations, respectively. All the biomarkers were determined in duplicate.

2.3. Flow Cytometry Analysis

Flow cytometry data were collected in EDTA-anticoagulated blood samples, within 1 h after collection, as previously reported [12]. No fixation procedure was performed. The CD41a antibody, recognizing the gpIIb glycoprotein of the gpIIb/IIIa surface platelet complex (CD41/CD61), was used to quantify (as RFI) the neutrophil CD41a+ (corresponding to the number of platelets bound per neutrophil). A combination of PC5-conjugated anti-human CD14 (cat. A07765, Beckman Coulter, Fullerton, CA, USA), PE-conjugated anti-human CD11b (cat. 555388, BD-Pharmingen, San Diego, CA, USA), PE-conjugated anti-human CD18 (cat. FAB1730P, R&D Systems, Abingdon, UK), PE-conjugated anti-human CCR5 (cat. FAB1802P, R&D Systems, Abingdon, UK) and PE-conjugated anti-human CD41a (cat. 555467, BD-Pharmingen, San Diego, CA, USA) was employed. A neutrophil phenotypic quantification (as RFI) of markers CD11b, CD18 and CCR5 was carried out

using an overlaid histogram subtraction procedure, as previously reported [12]. The neutrophil binding of a CD41a antibody, recognizing the gpIIb glycoprotein belonging to the gpIIb/IIIa surface platelet complex (CD41/CD61) and corresponding to the number of platelets bound per neutrophil, was quantified (as RFI) using the same analysis algorithm (Figure 1).

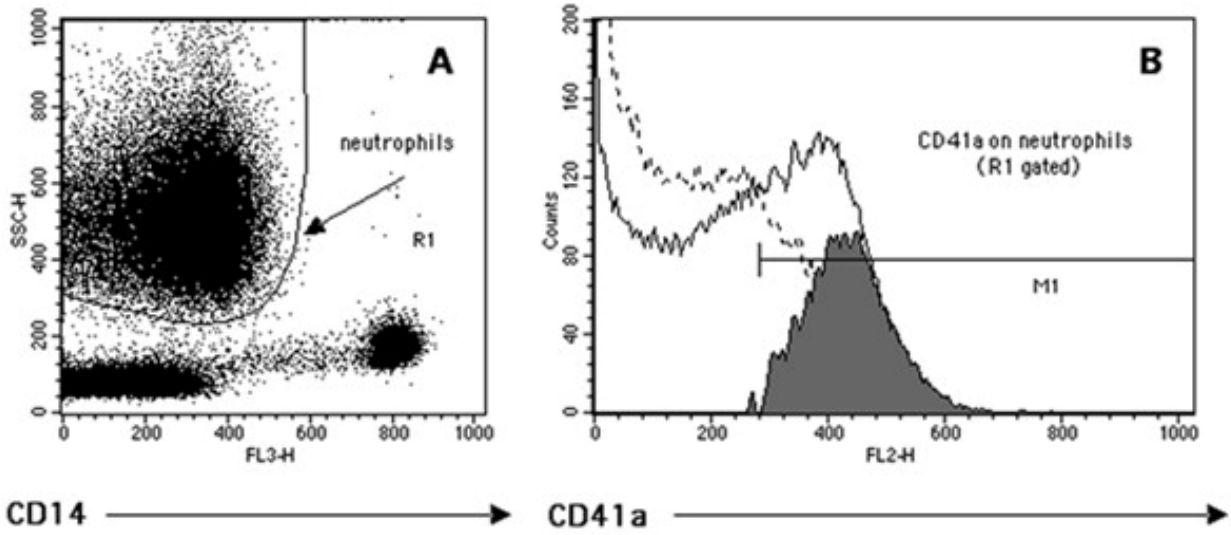

Figure 1. Representative example of flow cytometry quantification of complexes neutrophil-CD41a+ (NPAs, neutrophil–platelet aggregates). (**A**) Neutrophil cluster identification (region R1) based on its low CD14 expression (FL3) and side-scattering (SSC) morphological characteristics. (**B**) The R1-based histogram's subtraction analysis [positive events (continuous line) minus isotype control (dotted line)] was used to quantify both the percentage of complexes CD41a+ (percentage of events in M1 marker) and their RFI (median of M1 gray histogram minus median of the isotype control).

2.4. Statistical Analysis

Continuous data are presented as the mean ± standard deviation (SD). A log-10-based logarithmic transformation of cytokine data was performed in order to reduce the possible influence of outliers and to eliminate the influence of their non-normal distribution. To be log-transformed, each cytokine with zero value was assigned to the minimum non-zero cytokine-detected level. A preliminary univariate statistical selection, based on the presence of a p-value < 0.15, has been used to identify parameters to be included in the subsequent multiple regression analysis models. The RFI values of neutrophil positivity for CD11b, CD18 and CCR5 were considered dependent variables. The effects of the selected independent variables on cytokine-modulated neutrophil markers were then evaluated by two models of multiple linear regression analysis (Model 1 and Model 2 adjustments), which differ only in the inclusion of the RFI data of neutrophil CD41a+ within Model 2, and a stepwise backward selection algorithm (p < 0.1 to remove a variable) was used to determine the final statistical model. The analysis was performed using IBM SPSS Statistics, Version 29.0.1.0 (IBM SPSS, Statistics for Windows, Version 29.0.1.0 Armonk, NY, USA, IBM Corp.), and a p-value < 0.05 was considered statistically significant.

3. Results

The found percentages of neutrophil positivity (mean ± SD) for CD11b, CD18, CCR5 and CD41a were 98.37 ± 2.27, 64.84 ± 16.95, 8.29 ± 4.42 and 6.51 ± 2.63, respectively. The corresponding RFI values (a.u.) are listed in Table 1, together with the clinical, immunobiochemical, therapeutic and metabolic characteristics of the selected group of patients.

Table 1. Clinical, immuno-biochemical, therapeutic and metabolic parameters of the selected group of patients (n = 55). Data are presented as mean ± SD (standard deviation) or as number (n) and percentage (%), when appropriate. a.u. = arbitrary units.

Parameters	Study Population (n = 55)
Age (years)	68.53 ± 7.95
Gender (M/F, n)	39/16
BMI (Body Mass Index)	27.26 ± 3.63
Framingham Risk Score (a.u.) (FRS)	15.40 ± 3.38
Diabetes, n (%)	17 (30.91)
Oral antidiabetics, n (%)	15 (27.28)
Statin use, n (%)	41 (74.54)
Statin dosage, (mg/die)	13.27 ± 11.02
Creatinine (mg/dL)	0.85 ± 0.21
Hs-CRP (mg/dL)	0.44 ± 0.72
ICAM-1 (ng/mL)	225.53 ± 101.00
VCAM-1 (ng/mL)	635.15 ± 166.06
LDL cholesterol (mg/dL)	104.61 ± 34.05
HDL cholesterol (mg/dL)	54.29 ± 12.44
HDL/LDL Ratio	0.569 ± 0.216
Triglycerides (mg/dL)	133.47 ± 48.90
Albumin (gr/dL)	4.27 ± 0.51
IL-6 (pg/mL)	1.01 ± 0.93
IFN-γ (pg/mL)	32.04 ± 12.53
IL-10 (pg/mL)	27.26 ± 13.04
TNF-α (pg/mL)	69.74 ± 23.04
MCP-1 (pg/mL)	173.14 ± 65.20
IL-8 (pg/mL)	1.97 ± 1.73
RANTES (pg/mL)	141.25 ± 105.79
Fractalkine (pg/mL)	0.98 ± 1.50
RFI of neutrophil CD41a+ (a.u.) (number of platelets bound per neutrophil)	327.92 ± 57.26
RFI of neutrophil CD11b (a.u.)	618.11 ± 65.48
RFI of neutrophil CD18 (a.u.)	371.55 ± 47.77
RFI of neutrophil CCR5 (a.u.)	283.63 ± 45.15

A list of continuous plasma cytokine data and immuno-biochemical parameters, able to influence the systemic inflammatory response and the functional state of circulating neutrophils and platelets, was then used for the univariate regression statistical analysis-based identification of parameters significantly associated (p-value < 0.15) with neutrophil marker expression, as reported in Table 2.

The presence of diabetic disease as well as the gender distinction were not significantly associated with any of the neutrophil markers studied.

Table 2. Univariate regression analyses between continuous cytokine data, immuno-biochemical parameters and neutrophil marker expression (RFI). * Unstandardized. RFI = Relative Fluorescence Intensity. a.u. = arbitrary units.

Neutrophil CD11b Expression (RFI)		
Parameter	**Regression Coefficient ***	***p*-Value**
Age (years)	−0.752	0.507
BMI (Body Mass Index)	4.886	0.046
Framingham Risk Score (a.u.) (FRS)	−1.713	0.521
Creatinine (mg/dL)	27.619	0.522
Albumin (gr/dL)	31.570	0.073
Hs-CRP (mg/dL)	16.622	*0.179*
ICAM-1 (ng/mL)	0.081	0.363
VCAM-1 (ng/mL)	0.018	0.742
Log IL-6 (pg/mL)	81.296	<0.001
Log IFN-γ (pg/mL)	−15.062	0.813
Log IL-10 (pg/mL)	26.514	0.456
Log TNF-α (pg/mL)	−56.962	0.453
Log MCP-1 (pg/mL)	4.509	0.925
Log IL-8 (pg/mL)	20.409	0.033
Log RANTES (pg/mL)	−50.605	0.069
Log Fractalkine (pg/mL)	11.681	0.285
RFI of neutrophil CD41a+ (a.u.) (number of platelets bound per neutrophil)	0.673	<0.001
Neutrophil CD18 Expression (RFI)		
Parameter	**Regression Coefficient ***	***p*-Value**
Age (years)	−0.567	0.493
BMI (Body Mass Index)	2.508	0.164
Framingham Risk Score (a.u.) (FRS)	−1.107	0.570
Creatinine (mg/dL)	30.021	0.340
Albumin (gr/dL)	16.107	0.213
Hs-CRP (mg/dL)	8.614	0.342
ICAM-1 (ng/mL)	−0.013	0.843
VCAM-1 (ng/mL)	0.034	0.394
Log IL-6 (pg/mL)	54.377	0.001
Log IFN-γ (pg/mL)	−17.364	0.709
Log IL-10 (pg/mL)	23.696	0.361
Log TNF-α (pg/mL)	−41.546	0.453
Log MCP-1 (pg/mL)	11.139	0.748
Log IL-8 (pg/mL)	14.131	0.043
Log RANTES (pg/mL)	−34.774	0.087
Log Fractalkine (pg/mL)	8.708	0.274
RFI of neutrophil CD41a+ (a.u.) (number of platelets bound per neutrophil)	0.458	<0.001

Table 2. *Cont.*

Neutrophil CCR5 Expression (RFI)		
Parameter	**Regression Coefficient ***	***p*-Value**
Age (years)	0.128	0.871
BMI (Body Mass Index)	1.337	0.435
Framingham Risk Score (a.u.) (FRS)	1.051	0.568
Creatinine (mg/dL)	5.502	0.854
Albumin (gr/dL)	6.327	0.607
Hs-CRP (mg/dL)	11.628	0.173
ICAM-1 (ng/mL)	−0.035	0.570
VCAM-1 (ng/mL)	0.052	0.165
Log IL-6 (pg/mL)	63.018	<0.001
Log IFN-γ (pg/mL)	3.598	0.935
Log IL-10 (pg/mL)	20.200	0.410
Log TNF-α (pg/mL)	−36.236	0.488
Log MCP-1 (pg/mL)	15.458	0.637
Log IL-8 (pg/mL)	10.792	0.104
Log RANTES (pg/mL)	−56.939	0.002
Log Fractalkine (pg/mL)	4.231	0.576
RFI of neutrophil CD41a+ (a.u.) (number of platelets bound per neutrophil)	0.558	<0.001

Based on the univariate analysis results, the immuno-biochemical parameters positively, or negatively, associated with the expression level (RFI) of neutrophil markers, were assessed for their final statistically independent correlation with CD11b, CD18 and CCR5 expression (RFI) in Model 1 and Model 2 of the multiple regression analysis adjustment, as reported in Tables 3–5, respectively.

Table 3. Final statistical effects of blood immuno-biochemical adjustment parameters on CD11b neutrophil expression (RFI), in the two models of multiple regression analysis. * Unstandardized. RFI = Relative Fluorescence Intensity. a.u. = arbitrary units.

Neutrophil CD11b Expression (RFI)		
Model 1 Adjustment (starting from 5 independent variables)		
	Regression Coefficient *	***p*-Value**
Albumin	38.325	0.011
Log IL-6	74.693	<0.001
Log IL-8	13.547	0.088
Log RANTES	−64.395	0.006
Model 2 Adjustment (starting from 6 independent variables)		
	Regression Coefficient *	***p*-Value**
Albumin	35.248	0.013
RFI of neutrophil CD41a+ (a.u.) (number of platelets bound per neutrophil)	0.688	<0.001

Table 4. Final statistical effects of blood immuno-biochemical adjustment parameters on CD18 neutrophil expression (RFI) in the two models of multiple regression analysis. * Unstandardized. RFI = Relative Fluorescence Intensity. a.u. = arbitrary units.

Neutrophil CD18 Expression (RFI)		
Model 1 Adjustment (starting from 3 independent variables)		
	Regression Coefficient *	***p*-Value**
Log IL-6	49.181	0.002
Log IL-8	11.075	0.079
Log RANTES	−34.909	0.053
Model 2 Adjustment (starting from 4 independent variables)		
	Regression Coefficient *	***p*-Value**
RFI of neutrophil CD41a+ (a.u.) (number of platelets bound per neutrophil)	0.458	<0.001

Table 5. Final statistical effects of blood immuno-biochemical adjustment parameters on CCR5 neutrophil expression (RFI), in the two models of multiple regression analysis. * Unstandardized. RFI = Relative Fluorescence Intensity. a.u. = arbitrary units.

Neutrophil CCR5 Expression (RFI)		
Model 1 Adjustment (starting from 3 independent variables)		
	Regression Coefficient *	***p*-Value**
Log IL-6	62.040	<0.001
Log RANTES	−55.317	<0.001
Model 2 Adjustment (starting from 4 independent variables)		
	Regression Coefficient *	***p*-Value**
RFI of neutrophil CD41a+ (a.u.) (number of platelets bound per neutrophil)	0.558	<0.001

The results reported in Table 3 suggest a strong effect of IL-6 and RANTES in inducing the opposite phenotypic modulation of neutrophil CD11b expression. The strength and orientation of this modulatory capacity are indicated by the numerical value and sign, respectively, of the regression coefficients reported in multiple regression Model 1 adjustments. These opposite effects of IL-6 and RANTES seem to be completely absorbed, together with the marginally significant up-regulation induced by IL-8, into the strongly positive modulating action of the RFI of neutrophil CD41a+ parameters (Model 2 adjustment). Interestingly, the positive modulating effect of plasma albumin on neutrophil CD11b expression appears to be only marginally down-regulated by the presence of neutrophil–platelet aggregates in the Model 2 adjustment.

Considerations similar to what is referred to in Table 3 can also be applied to Table 4. On the other hand, the neutrophil expression of CD18 appears to be independent of the activating/pro-inflammatory stimulus represented by plasma albumin. The statistical results of Table 5 seem to confirm the ability of the RFI of neutrophil CD41a+ parameters (Model 2 adjustment) to replace the strongly opposite modulatory actions of IL-6 and RANTES on neutrophil phenotypes.

4. Discussion

In a recent paper [5], we observed that the neutrophil–platelet interaction in a group of stable CAD patients, and, in particular, the number of platelets bound per neutrophil correlated positively with the expression of several neutrophil surface receptors. These

receptors are involved in cellular adhesion and chemotactic response and are well known for playing a crucial role in atherosclerotic plaque formation.

In the present study, performed in the same patient group, we used a backward multiple regression analysis algorithm in order to improve our statistical approach and define more accurately the influence of the number of platelets bound per neutrophil on the cytokine-modulated neutrophil expression of integrin and chemokine receptors. In particular, we focused our interest on neutrophil integrin molecules CD11b and CD18 (associated with the pro-adhesive heterodimeric complex Mac-1) and the chemokine receptor CCR5 (cellular receptor for RANTES). All these molecules exert a key role in the functional response of circulating neutrophils to activating stimuli, leading to their adhesion to activated endothelium and migration into inflamed tissues [13]. Platelet–leukocyte interactions and platelet-released chemokines, RANTES in particular, play a crucial role in this process [14–16]. The preliminary observations of our study, in particular those resulting from the final comparison between multiple regression Models 1 and 2 reported in Table 5, seem to indicate that the number of platelets bound per neutrophil (RFI of neutrophil CD41a+) is able to completely encompass the functional combination of positive and negative independent associations of IL-6 and RANTES with CCR5 neutrophil expressions, respectively. The IL-6, in particular, shows a strong up-regulatory effect on all neutrophil activation markers evaluated in our study (Model 1 adjustment). This may be due to the dual cellular signaling mechanism of IL-6: the classic one, in which the cytokine first binds the specific membrane receptor (mIL-6R), which in turn, recruits two molecules of mgp130 that start the signaling, and the alternative one (trans-signaling), which the IL-6 binds first to its soluble receptor (sIL-6R) and then to the membrane mgp130 [17]. The statistically significant effect of up-regulation of the neutrophilic phenotype by platelets bound per neutrophil is also evident in the expression of CD11b (Table 3) and CD18 (Table 4), including also the marginally significant phenotypic up-regulatory action exerted by IL-8. The evidence of IL-6 and RANTES opposite associations with neutrophil CCR5 expressions reported in Table 5 (Model 1 adjustment) suggests that the independent positive relationship observed between the RFI of neutrophil CD41a+ cells and CCR5 expression (Model 2 adjustment) might be the result of a cellular heterotypic membrane interaction. This physical crosstalk, in fact, might lead to either a more sustained cellular activation depending on a higher concentration of activating stimuli (including cytokines/chemokines) within the peri-neutrophilic microenvironment—and related to canonical outside-in and inside-out signaling mechanisms [4]—or to the inhibition of CCR5 receptor-mediated lipid raft internalization upon binding with the specific chemokine (RANTES) released by locally activated bound platelets [18]. Both these effects might depend on the formation of a "cloud model" of oligomerized/retained platelet-mediated ligand–receptor interactions on the outer membrane of blood neutrophils [19], as schematically depicted in Figure 2.

On this basis, the presence of CD11b and CD18 integrin molecules within the aforementioned CCR5-modulated lipid rafts could also partly explain their up-regulation following the formation of neutrophil–platelet heterotypic conjugates [20]. Further pathophysiological studies, mainly focused on the mechanisms of neutrophil intracellular signaling, associations between integrin/chemokine receptors and membrane lipid rafts, as well as on integrin/chemokine receptor internalization following neutrophil binding with platelets, could help clarify this hypothesis. Finally, an interesting finding of our study is the persistence in Model 2 adjustment, which remained almost statistically unchanged when compared to Model 1, of the positive association of circulating albumin levels with neutrophil CD11b expression. In this case, albumin appears to have a pro-inflammatory effect [21]. This could indicate a unique dependence of the phenotypic modulation of this integrin molecule on the state of neutrophil activation deriving from the interaction, probably TLR-4-mediated [21], with circulating oxidized albumin [22]. Several limitations of this study must be acknowledged: First, the small number of patients studied and the lack of metabolic parameters not included in our statistical multiple regression analysis models. Second, the flow cytometry assessment of other neutrophil activation markers

(CD66b, CD62L and CD16) and chemokine receptors (CXCR1, CXCR2 and CXCR4), as well as other counter–receptor pairs involved in neutrophil–platelet heterotypic aggregation [platelet P-selectin (CD62P), platelet GPIbα (CD42b) and neutrophil P-selectin glycoprotein ligand-1 (PSGL-1, CD162)], is also missing. Therefore, further studies on larger patient populations are necessary to confirm these preliminary observations and define their role in the chronic coronary syndrome of stable CAD patients.

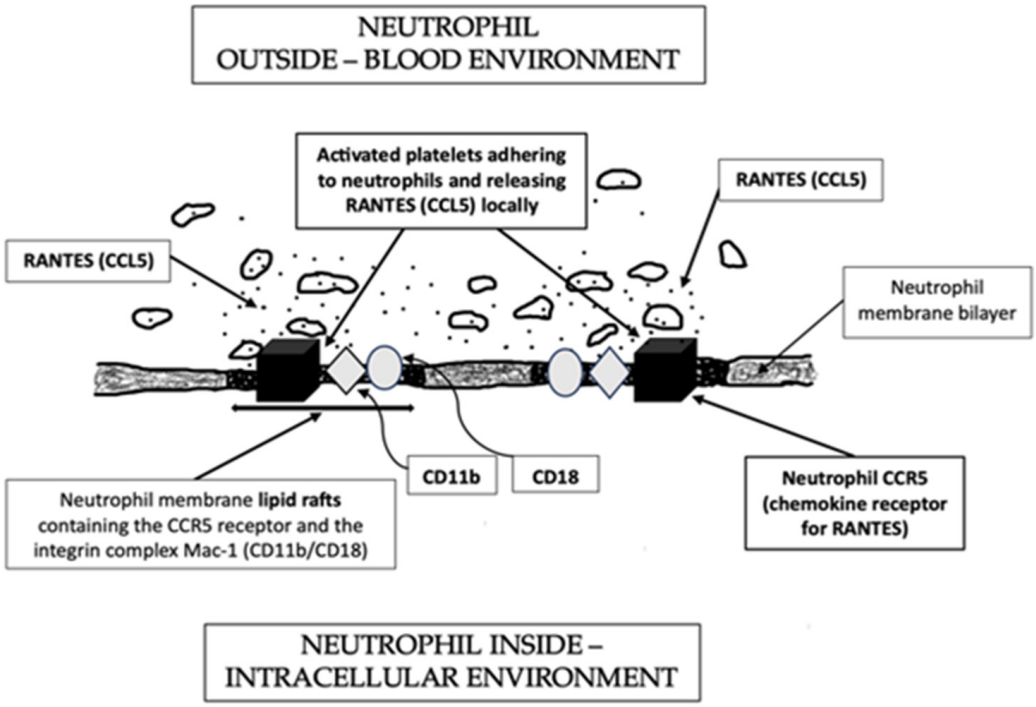

Figure 2. Schematic representation of the main soluble effector molecules involved in the platelet-mediated modulation model of circulating neutrophil phenotypes proposed in our study.

5. Conclusions

In conclusion, our study highlights the relevance of assessing neutrophil–platelet interactions for a better understanding of the environment-induced modulation of phenotypes and functions of these circulating phagocytes. Moreover, the broad, largely unknown complexity of neutrophil activation in pro-inflammatory conditions, such as chronic atherosclerosis, also emerges.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/hearts5030029/s1, Table S1: Full list of inclusion, exclusion and exit criteria of SMARTool clinical study.

Author Contributions: Conceptualization, S.S. (Silverio Sbrana); methodology, S.S. (Silverio Sbrana), S.S. (Stefano Salvadori), R.R., E.C., A.F.S., A.C. and C.C.; resources, G.P. and S.R.; data curation and statistical analysis, S.S. (Silverio Sbrana) and S.S. (Stefano Salvadori); writing—original draft preparation, S.S. (Silverio Sbrana); writing—review and editing, S.S.(Silverio Sbrana), D.N., G.P. and S.R.; project administration, S.R.; funding acquisition, S.R. All authors have read and agreed to the published version of this manuscript.

Funding: This research was funded by the European Commission: Project SMARTool, "Simulation Modeling of coronary ARTery disease: A tool for clinical decision support—SMARTool" (GA number 689068).

Institutional Review Board Statement: This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethical Committee of the Area Vasta Nord-Ovest of Tuscany Region (Italy, prot number 23534, 14 April 2016), (ClinicalTrials.gov Identifier: NCT04448691, 14 April 2016).

Informed Consent Statement: Informed consent was obtained from all subjects involved in this study.

Data Availability Statement: The data are contained within the article or Supplementary Materials.

Acknowledgments: We would like to thank all the researchers involved in the SMARTool clinical trial, who made it possible to enroll patients, perform clinical and imaging exams, and carry out laboratory tests.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Soehnlein, O. Multiple roles of neutrophils in atherosclerosis. *Circ. Res.* **2012**, *110*, 875–888. [CrossRef] [PubMed]
2. Silvestre-Roig, C.; Braster, Q.; Ortega-Gomez, A.; Soehnlein, O. Neutrophils as regulators of cardiovascular inflammation. *Nat. Rev. Cardiol.* **2020**, *17*, 327–340. [CrossRef] [PubMed]
3. Soehnlein, O. Neutrophil research, quo vadis? *Trends Immunol.* **2019**, *40*, 561–564. [CrossRef] [PubMed]
4. Németh, T.; Mócsai, A. Feedback amplification of neutrophil function. *Trends Immunol.* **2016**, *37*, 412–424. [CrossRef] [PubMed]
5. Sbrana, S.; Cecchettini, A.; Bastiani, L.; Mazzone, A.; Vozzi, F.; Caselli, C.; Neglia, D.; Clemente, A.; Scholte, A.J.H.H.; Parodi, O.; et al. Association of circulating neutrophils with relative volume of lipid-rich necrotic core of coronary plaques in stable patients: A substudy of SMARTool European project. *Life* **2023**, *13*, 428. [CrossRef]
6. Faraday, N.; Braunstein, J.B.; Heldman, A.W.; Bolton, E.D.; Chiles, K.A.; Gerstenblith, G.; Schulman, S.P. Prospective evaluation of the relationship between platelet-leukocyte conjugate formation and recurrent myocardial ischemia in patients with acute coronary syndromes. *Platelets* **2004**, *15*, 9–14. [CrossRef]
7. Hézard, N.; Metz, D.; Garnotel, R.; Droullé, C.; Potron, G.; Nguyen, P. Platelet-leukocyte interactions in coronary heart disease: Pathophysiology, clinical relevance, pharmacological modulation. *J. Mal. Vasc.* **2000**, *25*, 343–348. [PubMed]
8. Holmannova, D.; Kolackova, M.; Mandak, J.; Kunes, P.; Holubcova, Z.; Holubec, T.; Krejsek, J. Effects of conventional CPB and mini-CPB on neutrophil CD162, CD166 and CD195 expression. *Perfusion* **2017**, *32*, 141–150. [CrossRef]
9. Hartl, D.; Krauss-Etschmann, S.; Koller, B.; Hordijk, P.L.; Kuijpers, T.W.; Hoffmann, F.; Hector, A.; Eber, E.; Marcos, V.; Bittmann, I.; et al. Infiltrated neutrophils acquire novel chemokine receptor expression and chemokine responsiveness in chronic inflammatory lung diseases. *J. Immunol.* **2008**, *181*, 8053–8067. [CrossRef]
10. Sbrana, S.; Campolo, J.; Clemente, A.; Bastiani, L.; Cecchettini, A.; Ceccherini, E.; Caselli, C.; Neglia, D.; Parodi, O.; Chiappino, D.; et al. Blood monocyte phenotype fingerprint of stable coronary artery disease: A cross-sectional substudy of SMARTool clinical trial. *BioMed Res. Int.* **2020**, *2020*, 8748934. [CrossRef]
11. Sbrana, S.; Cecchettini, A.; Bastiani, L.; Di Giorgi, N.; Mazzone, A.; Ceccherini, E.; Vozzi, F.; Caselli, C.; Neglia, D.; Clemente, A.; et al. Blood M2-like polarization is associated with calcific plaque phenotype in stable coronary artery disease: A sub-study of SMARTool clinical trial. *Biomedicine* **2022**, *10*, 565. [CrossRef] [PubMed]
12. Sbrana, S.; Bevilacqua, S.; Buffa, M.; Spiller, D.; Parri, M.S.; Gianetti, J.; De Filippis, R.; Clerico, A. Post-reperfusion changes of monocyte function in coronary blood after extracorporeal circulation. *Cytom. Part B Clin. Cytom.* **2005**, *65*, 14–21. [CrossRef] [PubMed]
13. Nourshargh, S.; Alon, R. Leukocyte migration into inflamed tissues. *Immunity* **2014**, *41*, 694–707. [CrossRef] [PubMed]
14. Bakogiannis, C.; Sachse, M.; Stamatelopoulos, K.; Stellos, K. Platelet-derived chemokines in inflammation and atherosclerosis. *Cytokine* **2019**, *122*, 154157. [CrossRef] [PubMed]
15. Soehnlein, O. Decision shaping neutrophil-platelet interplay in inflammation: From physiology to intervention. *Eur. J. Clin. Investig.* **2018**, *48*, e12871. [CrossRef]
16. Pircher, J.; Engelmann, B.; Massberg, S.; Schulz, C. Platelet-neutrophil crosstalk in atherothrombosis. *Thromb. Haemost.* **2019**, *119*, 1274–1282. [CrossRef]
17. Aliyu, M.; Zohora, F.T.; Anka, A.U.; Ali, K.; Maleknia, S.; Saffarioun, M.; Azizi, G. Interleukin-6 cytokine: An overview of the immune regulation, immune dysregulation, and therapeutic approach. *Int. Immunopharmacol.* **2022**, *111*, 109130. [CrossRef]
18. Cardaba, C.M.; Kerr, J.S.; Mueller, A. CCR5 internalization and signaling have different dependence on mem-brane lipid raft integrity. *Cell. Signal.* **2008**, *20*, 1687–1694. [CrossRef]
19. Graham, G.J.; Handel, T.M.; Proudfoot, A.E.I. Leukocyte adhesion: Reconceptualizing chemokine presentation by glycosamino-glycans. *Trends Immunol.* **2019**, *40*, 472–481. [CrossRef]
20. Murphy, A.J.; Woollard, K.J.; Suhartoyo, A.; Stirzaker, R.A.; Shaw, J.; Sviridov, D.; Chin-Dusting, J.P.F. Neutrophil activation is attenuated by high-density lipoprotein and apolipoprotein A-I in in vitro and in vivo models of inflammation. *Arterioscler. Thromb. Vasc. Biol.* **2011**, *31*, 1333–1341. [CrossRef]

21. Wheeler, D.S.; Giuliano Jr, J.S.; Lahni, P.M.; Denenberg, A.; Wong, H.R.; Zingarelli, B. The immunomodulatory effects of albumin in vitro and in vivo. *Adv. Pharmacol. Sci.* **2011**, *2011*, 691928. [CrossRef] [PubMed]
22. Michelis, R.; Kristal, B.; Zeitun, T.; Shapiro, G.; Fridman, Y.; Geron, R.; Sela, S. Albumin oxidation leads to neutrophil activation in vitro and inaccurate measurement of serum albumin in patients with diabetic nephropathy. *Free Radic. Biol. Med.* **2013**, *60*, 49–55. [CrossRef] [PubMed]