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RESEARCH COMMUNICATION

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Neutrophil to lymphocyte ratio is not related to carotid atherosclerosis progression and cardiovascular events in the primary prevention of cardiovascular disease: Results from the IMPROVE study

Massimo R. Mannarino[1] | Vanessa Bianconi[1] | Bruna Gigante[2] | Rona J. Strawbridge[2,3,4] | Kai Savonen[5,6] | Sudhir Kurl[7] | Philippe Giral[8,9] | Andries Smit[10,11] | Per Eriksson[2] | Elena Tremoli[12] | Fabrizio Veglia[13] | Damiano Baldassarre[13,14] | Matteo Pirro[1] | IMPROVE study group

[1]Unit of Internal Medicine, Department of Medicine and Surgery, University of Perugia, Perugia, Italy

[2]Division of Cardiovascular Medicine, Department of Medicine, Solna, Karolinska Institutet, Stockholm, Sweden

[3]Institute of Health and Wellbeing, University of Glasgow, Glasgow, UK

[4]Health Data Research UK, Glasgow, UK

[5]Foundation for Research in Health Exercise and Nutrition, Kuopio & Research Institute of Exercise Medicine, Kuopio, Finland

[6]Department of Clinical Physiology and Nuclear Medicine, Kuopio University Hospital, Kuopio, Finland

[7]Institute of Public Health and Clinical Nutrition, University of Eastern Finland, Kuopio, Finland

[8]Assistance Publique - Hopitaux de Paris, Paris, France

[9]Service Endocrinologie-Metabolisme, Groupe Hôspitalier Pitie-Salpetriere, Unités de Prévention Cardiovasculaire, Paris, France

[10]Department of Medicine, University Medical Center Groningen, Groningen, the Netherlands

[11]Department of Medicine, Isala Clinics Zwolle, Zwolle, The Netherlands

[12]Maria Cecilia Hospital, Cotignola, (RA), Italy

[13]Centro Cardiologico Monzino, IRCCS, Milan, Italy

[14]Department of Medical Biotechnology and Translational Medicine, Università degli Studi di Milano, Milan, Italy

Correspondence
Massimo R. Mannarino, Unit of Internal Medicine, University of Perugia, Perugia, Italy, Hospital "Santa Maria della Misericordia", Piazzale Menghini, 1 –

Abstract

Inflammation is a component of the pathogenesis of atherosclerosis and is associated with an increased risk of atherosclerotic cardiovascular disease (ASCVD). The neutrophil to lymphocyte ratio (NLR) is a possible

Abbreviations: ASCVD, atherosclerotic cardiovascular disease; BIF, carotid artery bifurcation; BMI, body mass index; CANTOS, Canakinumab Anti-Inflammatory Thrombosis Outcomes Study; CC, common carotid artery; CHD, coronary heart disease; cIMT, carotid intima-media thickness; CIRT, Cardiovascular Inflammation Reduction Trial; CRP, C-reactive protein; DBP, diastolic blood pressure; GFR, glomerular filtration rate; HDL-C, high density lipoprotein cholesterol; HRs, hazard ratios; ICA, internal carotid artery; IMPROVE, IMT-Progression as Predictors of VEs; LDL-C, low density lipoprotein cholesterol; LEAD, lower extremity artery disease; MI, myocardial infarction; NLR, neutrophil to lymphocyte ratio; SBP, systolic blood pressure; SD, standard deviation; SPIRE, Studies of PCSK9 Inhibition and the Reduction of vascular Events; VEs, vascular events; WHR, waist-hip ratio.

Massimo R. Mannarino & Vanessa Bianconi equally contributed as co-first authors; Damiano Baldassarre & Matteo Pirro equally contributed as co-last authors.

06129, Perugia, Italy.
Email: massimo.mannarino@unipg.it

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inflammation metric for the detection of ASCVD risk, although results of prospective studies are highly inconsistent on this topic. We investigated the cross-sectional relationship between NLR and carotid intima-media thickness (cIMT) in subjects at moderate-to-high ASCVD risk. The prospective association between NLR, cIMT progression, and incident vascular events (VEs) was also explored. In 3341 subjects from the IMT-Progression as Predictors of VEs (IMPROVE) study, we analyzed the association between NLR, cIMT, and its 15-month progression. The association between NLR and incident VEs was also investigated. NLR was positively associated with cross-sectional measures of cIMT, but not with cIMT progression. The association between NLR and cross-sectional cIMT measures was abolished when adjusted for confounders. No association was found between NRL and incident VEs. Similarly, there were no significant differences in the hazard ratios (HRs) of VEs across NLR quartiles. NLR was neither associated with the presence and progression of carotid atherosclerosis, nor with the risk of VEs. Our findings do not support the role of NLR as a predictor of the risk of atherosclerosis progression and ASCVD events in subjects at moderate-to-high ASCVD risk, in primary prevention. However, the usefulness of NLR for patients at a different level of ASCVD risk cannot be inferred from this study.

KEYWORDS
cardiovascular, carotid, IMT, lymphocyte, neutrophil, NLR, prospective

1 | INTRODUCTION

Inflammation is considered as an integral part of the pathophysiology of atherosclerosis and the associated ischemic cardiovascular complications.[1] Accordingly, elevations of plasma levels of several markers of systemic inflammation have been found in patients with documented atherosclerotic deposition at different arterial territories,[2–5] including at the extracranial carotid district.[6] Further support for the association between systemic inflammation and the risk of atherosclerotic cardiovascular disease (ASCVD) is provided by the results of large longitudinal studies[7–9] and recent clinical trials with anti-inflammatory drugs[10,11] in patients with ASCVD.

Neutrophils are the predominant circulating leucocytes; they are key participants in innate immune response and also influence effector cells of adaptive immunity.[12,13] In addition to being observed in atherosclerotic plaques, the participation of neutrophils during various stages of atherosclerosis has been documented.[14] As the effector cells of the adaptive immune system, lymphocytes are closely associated with atherosclerosis, with some subsets possessing pro-inflammatory and pro-atherogenic properties and some others exerting anti-inflammatory and anti-atherogenic effects.[15]

Both elevated neutrophil and reduced lymphocyte counts have been associated with impaired ASCVD prognosis.[16,17] Thus, the neutrophil to lymphocyte ratio (NLR) has been explored either as a possible inflammation metric for the detection of the presence of carotid atherosclerosis in case–control and cross-sectional studies[18–20] or as a predictor of ASCVD events in prospective studies.[21]

A direct cross-sectional association between NLR and carotid intima-media thickness (cIMT) has been found in different studies.[22–26] However, this relationship has not been always confirmed.[23,27–29] In addition, the prospective association between NLR and cIMT progression has not been investigated so far. Hence, further cross-sectional, and prospective data from large cohorts exploring the link between NLR and carotid atherosclerosis are warranted.

Similarly, the longitudinal investigation of the association between NLR and ASCVD events has produced variable results. In particular, most studies reported a positive association,[30–32] whereas others failed to replicate the same result in patients without overt ASCVD.[33–36] In addition, NLR cut-offs, multivariable adjustment and time to ASCVD events appeared to strongly influence the prospective relationship between

NLR and ASCVD risk.[30,31,33–36] Hence, the independent impact of NLR, both as a continuous and categorical variable, on the dynamics of carotid atherosclerosis and ASCVD risk requires further evaluation especially in patients in the primary prevention of ASCVD.

Current guidelines suggest the use of risk-enhancing factors, including inflammation biomarkers, in order to improve ASCVD risk estimates particularly in subjects at moderate-intermediate risk.[37,38] The IMPROVE study is a prospective multicenter longitudinal study exploring determinants of the presence and progression of carotid atherosclerosis and predictors of ASCVD events after correction for a consistent number of potential confounders.[39,40] Importantly, this cohort study includes subjects at moderate-to-high baseline ASCVD risk, which makes it particularly suitable for searching potential ASCVD risk-enhancing factors. Given the clinical need of robust predictors of ASCVD risk,[37,38] the role of some inflammation biomarkers in improving ASCVD risk reclassification[37,38] and the controversial impact of NLR on both atherosclerosis and ASCVD events in the different clinical settings (e.g., primary vs. secondary cardiovascular prevention),[30–32] the association of NLR with atherosclerosis presence and progression, as well as with ASCVD events occurrence was explored in the large cohort of the IMPROVE study.

2 | METHODS

2.1 | Subjects

Design and methods of the IMPROVE study have been previously reported.[39] In brief, 3703 subjects were recruited, aged 54–79 years, with at least three CVD risk factors, but without overt cardio- or cerebrovascular event at baseline. Seven centers in five European countries – Finland (Kuopio, two centers), France (Paris), Italy (Milan and Perugia), The Netherlands (Groningen) and Sweden (Stockholm), participated in the enrollment.

Methods for laboratory analyses have been previously reported.[40] The NLR was calculated as the neutrophil count divided by the lymphocyte count. Subjects with white blood cells count of 10,000/mm^3 or more, with overt inflammatory diseases or on corticosteroid treatment were excluded. Glomerular filtration rate (GFR) was estimated by Cockcroft–Gault formula.

The occurrence of combined VEs, including coronary VEs (myocardial infarction [MI], sudden cardiac death, angina pectoris, any revascularization or surgical intervention of the coronary arteries), cerebro-VEs (ischemic stroke, transient ischemic attack, any revascularization or surgical intervention of the carotid arteries) and lower extremity artery disease (LEAD) events (new diagnosis of intermittent claudication, any revascularization or surgical intervention of lower limb arteries) were recorded at months 15 and 30 by regular visits, and at the end of follow-up (average 36.2 months) by phone interview. The occurrence of VEs and death of all participants was validated by local specialists, and adjudicated by a designated specialist, who was unaware of the relevant clinical history and cIMT data. The sample size considered for this report is 3341, since white blood cells measurement was not available in 51 subjects, 82 subjects had white blood cells levels of 10,000/mm^3 or above, 85 had current inflammatory disease, 144 were on corticosteroid treatment.

2.2 | Ultrasonographic assessment

The ultrasonographic assessment of carotid arteries of the IMPROVE study was performed as previously described.[39,40] The ultrasonographic variables were measured centrally by trained readers at the ultrasound reading center in Milan (Italy) at baseline and measurements were repeated 15 months later. In each carotid segment (1 cm length), both mean ($_{mean}$) and maximal ($_{max}$) IMT were evaluated. Composite variables cIMT$_{mean}$, cIMT$_{max}$ and cIMT$_{mean\text{-}max}$ refer to the whole carotid tree. cIMT$_{mean}$ is the average of 1st cm of common carotid artery (1st CC) proximal to the bifurcation IMT$_{mean}$, common carotid artery (CC) IMT$_{mean}$, carotid artery bifurcation (BIF) IMT$_{mean}$ and internal carotid artery (ICA) IMT$_{mean}$. cIMT$_{max}$ is the greatest value among 1st CC-IMT$_{max}$, CC-IMT$_{max}$, BIF-IMT$_{max}$ and ICA-IMT$_{max}$. cIMT$_{mean\text{-}max}$ is the average of 1st CC-IMT$_{max}$, CC-IMT$_{max}$, BIF-IMT$_{max}$, and ICA-IMT$_{max}$.

To evaluate changes of cIMT over time, ultrasonographic measurements were repeated at 15 months using the same ultrasonographic protocol (positions and angles of ultrasound transducer with respect to the neck) used at baseline. Carotid IMT change for each ultrasonographic variable, expressed in mm/year, was calculated as the difference between the 15-month measurement and the corresponding baseline value divided by the length of the intervening time period. The Fastest-cIMT$_{max\text{-}progr}$, that is, the greatest value chosen among the progressions of cIMT$_{max}$, was also assessed, as a measure of the maximal focal progression of cIMT.

2.3 | Ethical considerations

The Ethics Committees of all participating institutions approved the IMPROVE study, which complied with the

Declaration of Helsinki. Written informed consent was obtained from all participants.

2.4 | Statistical analysis

NLR was calculated and participants were grouped according to NLR quartiles (division points: 1.34, 1.73, 2.24). Descriptive and comparative statistical analyses have been performed. In two-tailed tests, probability values less than 0.05 have been considered statistically significant. Logarithmic transformation was applied to skewed variables.

Multiple linear regression analyses have been performed with each ultrasonographic measure as the dependent variable, in the entire population. In model 1 covariates were NLR and latitude. In Model 2 covariates were age, sex, and latitude. In model 3, also smoking status (current vs. former or never), body mass index (BMI), systolic blood pressure (SBP), glucose, low-density-lipoprotein cholesterol (LDL-C), and C-reactive protein (CRP) were added as covariates. Model 4 included as covariates also waist-hip ratio (WHR), diastolic blood pressure (DBP), high-density lipoprotein cholesterol (HDL-C), triglyceride, GFR, lipid-lowering treatment, and anti-hypertensive treatment. Also, adjusted association between neutrophil and lymphocyte count and cIMT measures were calculated by linear regression analyses. Furthermore, the associations between NLR and cIMT measures were analyzed in subgroups divided by median age, sex, BMI, smoking status, history of diabetes and hypertension, and median CRP levels. Since the different measures of cIMT are not independent of each other, no correction was made for multiple tests.

Cox regression analyses have been used to estimate adjusted hazard ratios (HRs). In model 1, adjusted for latitude, HRs for combined VEs, coronary VEs, peripheral VEs (i.e., lower extremities atherosclerotic disease [LEAD] events plus cerebrovascular events) and cerebro-VEs have been calculated for each NLR quartile and for NLR as continuous log-transformed variable. In model 2, age and sex were added as covariates. In model 3, also smoking status (current vs. former or never), BMI, SBP, DBP, glucose, LDL-C, HDL-C, triglyceride and CRP were added as covariates. Model 4 included WHR, CRP, GFR, lipid-lowering treatment, and anti-hypertensive treatment as covariates. A sample of about 190 VEs was 80% power to deem as significant, with alpha = 0.05, an adjusted HR of 1.23 for one SD of NLR, assuming a total R^2 of 0.25 among the covariates included in the model. HRs of combined VEs for each NLR quartile were also calculated in subgroups divided by median age, sex, BMI, smoking status, history of diabetes and hypertension, and median CRP levels. In addition, adjusted HRs for combined VEs have been calculated for neutrophil and lymphocyte count quartiles and for neutrophil and lymphocyte count as continuous Log-transformed variables. Survival functions, over a 36.2 month follow-up, have been generated to compare event-free survival between NLR quartiles. All the analyses have been performed using the SPSS statistical package v. 22.0 (IBM statistics).

3 | RESULTS

The baseline characteristics of 3341 subjects, categorized according to NLR quartiles, are described in Table 1.

Increasing age and a higher percentage of male subjects, current smokers and hypertensive subjects and lower prevalence of diabetes were observed with increasing NLR quartiles. Also, in higher NLR quartiles, increased CRP levels and reduced levels of total and LDL cholesterol and triglycerides were observed (Table 1). The use of lipid lowering drugs was higher among subjects in the first quartile of NLR compared to those in the higher quartiles.

3.1 | NLR and carotid atherosclerosis

With increasing NLR quartiles, higher values of cross-sectional measures of carotid atherosclerosis ($cIMT_{mean}$, $cIMT_{max}$ and $cIMT_{mean\text{-}max}$) were observed (Table 1). No differences were found in fastest-$cIMT_{max\text{-}progr}$ across NLR quartiles.

When used as a continuous variable, NLR was positively associated with all measures of baseline cIMT but not with Fastest-$cIMT_{max\text{-}progr}$ (Table 2, Model 1), after correction for latitude. NLR was not associated with baseline cIMT variables or changes in cIMT in multiple linear regression analyses after further adjustment for age and sex (Table 2, Model 2) and after further adjustment for other covariates (Table 2, Models 3 and 4). The analysis was repeated for neutrophil and lymphocyte counts separately. Neutrophil count was positively associated with cross-sectional cIMT measures, but such an association was lost after full adjustment for covariates (Table S1). A positive association of lymphocyte count with $cIMT_{mean}$, and $cIMT_{mean\text{-}max}$ emerged after adjustment for age, sex, and latitude (Table S2, Model 2). However, such an association was not confirmed after further adjustment (Table S2, Models 3 and 4).

Associations between NLR and cIMT measures were also analyzed in subgroups defined by sex, median age, BMI, smoking status, history of diabetes and

TABLE 1 Characteristics of IMPROVE study participants according to NLR quartiles

	NLR quartiles			
	1st	**2nd**	**3rd**	**4th**
NLR range	≤1.33	>1.33, ≤1.73	>1.73, ≤2.24	>2.24
Number of subjects	838	835	838	830
Age, years	63.1 (58.7, 66.9)	64.2 (59.5, 67.3)	64.8 (59.8, 67.3)	65.8 (60.5, 67.5)
Sex, males %	42.2%	46.8%	48.3%	54.9%
Current smoke, %	36.4	37.6	35.7	37.3
Diabetes, %	25.7	24.4	22.9	22.0
Hypertension, %	68.1	69.3	74.8	74.1
Lipid-lowering, %	45.0	53.3	52.3	52.1
Anti-hypertensive, %	49.2	56.7	60.5	61.2
Anti-inflammatory, %	18.1	18.3	17.1	21.1
Hypoglycemic, %	17.5	16.8	15.8	15.8
Body Mass Index, kg/m^2	27.0 (24.4, 29.8)	26.9 (24.7, 29.4)	26.2 (23.8, 28.7)	26.2 (23.8, 28.7)
Waist/Hip ratio	0.91 (0.85, 0.97)	0.92 (0.86, 0.97)	0.92 (0.86, 0.97)	0.93 (0.87, 0.98)
Systolic Blood Pressure, mmHg	140 (130–154)	140 (130, 152)	140 (130, 153)	140 (130, 152)
Diastolic Blood Pressure, mmHg	82 (75, 90)	81 (75, 88)	81 (75, 88)	80 (75, 88)
White blood cells, n/mm^3	5600 (4760, 6400)	5700 (4800, 6600	5900 (5100, 6900)	6300 (5400, 7290)
Neutrophil count, n/mm^3	2580 (2163, 3001)	3068 (2610, 3600)	3583 (3023, 4127)	4222 (3578, 4970)
Lymphocyte count, n/mm^3	2381 (2017, 2843)	2002 (1711, 2356)	1808 (1550, 2115)	1459 (1242, 1701)
Blood glucose, mmol/L	5.56 (4.95, 6.40)	5.50 (4.89, 6.30)	5.50 (4.90, 6.17)	5.50 (5.00, 6.23)
Total Cholesterol, mg/dl	216 (186, 245)	212 (183, 243)	208 (184, 239)	207 (178, 236)
Triglyceride, mg/dl	118 (84, 177)	116 (81, 165)	115 (83, 165)	110 (79, 158)
HDL-Cholesterol, mg/dl	47 (40, 58)	46 (39, 56)	46 (39, 56)	47 (39, 56)
LDL-Cholesterol, mg/dl	139 (112, 165)	137 (111, 165)	135 (110, 162)	132 (106, 159)
CRP, mg/dl	1.51 (0.57, 2.97)	1.74 (0.71, 3.35)	2.05 (0.91, 3.83)	1.98 (0.78, 4.03)
GFR, ml/min	81 (67, 95)	81 (68, 97)	81 (68, 95)	79 (67, 94)
Latitude, degrees	53 (45, 62)	53 (45, 62)	53 (45, 59)	53 (45, 62)
$cIMT_{mean}$, mm	0.834 (0.728, 0.975)	0.851 (0.733, 0.998)	0.851 (0.745, 0.998)	0.867 (0.760, 1.023)
$cIMT_{max}$, mm	1.76 (1.35, 2.32)	1.84 (1.39, 2.50)	1.85 (1.45, 2.50)	2.03 (1.48, 2.61)
$cIMT_{mean\text{-}max}$, mm	1.30 (1.10, 1.59)	1.34 (1.11, 1.63)	1.35 (1.14, 1.64)	1.39 (1.15, 1.69)
PF CC- IMT_{mean}, mm	0.700 (0.639, 0.756)	0.698 (0.645, 0.763)	0.707 (0.646, 0.757)	0.704 (0.651, 0.770)
Fastest-$cIMT_{max\text{-}progr}$, mm/year	0.201 (0.104, 0.332)	0.176 (0.094, 0.324)	0.215 (0.109, 0.357)	0.190 (0.100, 0.366)

Note: Values are median (25th, 75th percentile) or percentage.

Abbreviations: CC, common carotid; CRP, C-reactive protein; GFR, glomerular filtration rate; HDL, high density lipoproteins; cIMT, intima media thickness; LDL, low density lipoprotein; NLR, neutrophil-lymphocyte ratio; PF, plaque-free.

TABLE 2 Associations between NLR and measures of cIMT

	Multivariable linear regression							
	Model 1		Model 2		Model 3		Model 4	
	β	*p* Value	β	*p* Value	β	*p* Value	β	*p* Value
$cIMT_{mean}$	0.044	0.007	0.003	0.849	0.003	0.856	−0.007	0.673
$cIMT_{max}$	0.054	0.001	0.023	0.154	0.019	0.242	0.007	0.670
$cIMT_{mean\text{-}max}$	0.045	0.006	0.008	0.607	0.007	0.680	−0.005	0.749
Fastest-$cIMT_{max\text{-}progr}$	0.003	0.865	−0.011	0.529	−0.020	0.276	−0.019	0.313

Notes: Model 1: adjusted for latitude. Model 2: adjusted for age, sex, and latitude. Model 3: adjusted for covariates in Model 2 plus body mass index, systolic blood pressure, glucose, smoking status, LDL-cholesterol and C-reactive protein. Model 4: adjusted for covariates in Model 3 plus waist-hip ratio, diastolic blood pressure, HDL-cholesterol, triglyceride, glomerular filtration rate, lipid-lowering treatment and antihypertensive treatment.
Abbreviations: cIMT, carotid intima media thickness; NLR, neutrophil-to- lymphocyte ratio.

TABLE 3 Hazard ratios (95% confidence interval) for VEs for one SD increase of log-transformed NLR

	Model 1	Model 2	Model 3	Model 4
NLR				
Combined VEs	1.01 (0.87, 1.16)	0.99 (0.85, 1.14)	0.95 (0.82, 1.10)	0.98 (0.84, 1.14)
Coronary VEs	0.93 (0.77, 1.13)	0.91 (0.76, 1.10)	0.91 (0.74, 1.11)	0.94 (0.76, 1.14)
Peripheral VEs	1.12 (0.90, 1.39)	1.10 (0.88, 1.37)	1.00 (0.80, 1.26)	1.03 (0. 82, 1.31)
Cerebro-VEs	1.13 (0.89, 1.44)	1.10 (0.86, 1.41)	1.02 (0.79, 1.31)	1.04 (0.81, 1.34)

Notes: Model 1: adjusted for latitude. Model 2: adjusted for age, sex, and latitude. Model 3: adjusted for covariates in Model 2 plus body mass index, systolic blood pressure, glucose, smoking status, LDL-cholesterol and C-reactive protein. Model 4: adjusted for covariates in Model 3 plus waist-hip ratio, diastolic blood pressure, HDL-cholesterol, triglyceride, glomerular filtration rate, lipid-lowering treatment and antihypertensive treatment.
Abbreviations: NLR, neutrophil to lymphocyte ratio; VEs, vascular events.

hypertension, and median CRP levels (Figures S1–S4). No significant association between NLR and cross-sectional cIMT measures, after adjustment for confounders, was observed in any of the subgroups.

3.2 | NLR and ASCVD events

During the median 36.2-month follow-up period of the 3341 subjects considered in this study, a total of 190 combined VEs were recorded, of these 110 were coronary VEs, 66 were cerebro-VEs and 14 LEAD events.

Table 3 shows HRs and 95% confidence interval for VEs for one SD increase of log-transformed NLR. There was no significant association between NLR and the risk of combined, coronary, peripheral, and cerebro-VEs. When using NLR as a categorical variable, no significant differences in terms HRs for VEs were found across NLR quartiles (Table 4). Combined event-free survival curves among NLR quartiles are presented in Figure 1.

Even when analyzing the neutrophil and lymphocyte counts separately, either as continuous log-transformed variables or divided into quartiles, no significant associations with the risk of VEs emerged (Tables S3 and S4), except for an increase in HR in the fourth quartile of neutrophils, in the minimally adjusted model (Table S4 – Model 1), which was lost after further adjustment for other covariates. Furthermore, the analyses in subgroups defined by sex, age, BMI, smoking status, history of diabetes and hypertension and CRP levels confirmed the lack of association between NLR and combined VEs (Figure 2).

4 | DISCUSSION

In this large multicenter cohort of subjects at moderate-to-high ASCVD risk, the results did not show a cross-sectional and prospective association between NLR, indices of carotid atherosclerosis presence and progression and ASCVD risk, after adjustment for confounders.

The results of our study contrast with previous observations of an association between increased NLR and the presence of atherosclerosis. NLR was found to be associated with the presence and severity of coronary artery disease,[41,42] and peripheral artery disease.[43] Moreover, previous studies have documented an independent association between NLR and carotid atherosclerosis in

TABLE 4 Hazard ratios of VEs according to NLR quartiles

	NLR quartiles	Number of subjects with/without events	Model 1	Model 2	Model 3	Model 4
Combined VEs	1st	45/793	1.00	1.00	1.00	1.00
	2nd	51/784	1.16 (0.77, 1.72)	1.09 (0.73, 1.63)	1.06 (0.70, 1.59)	1.22 (0.80, 1.85)
	3rd	42/796	0.94 (0.62, 1.43)	0.86 (0.57, 1.31)	0.81 (0.52, 1.24)	1.00 (0.64, 1.56)
	4th	52/778	1.16 (0.78, 1.72)	1.01 (0.67, 1.51)	1.94 (0.62, 1.42)	1.30 (0.84, 2.04)
Coronary VEs	1st	30/808	1.00	1.00	1.00	1.00
	2nd	30/805	1.02 (0.61, 1.70)	0.96 (0.57, 1.60)	0.93 (0.55, 1.56)	1.07 (0.63, 1.82)
	3rd	21/817	0.71 (0.40, 1.23)	0.63 (0.37, 1.12)	0.67 (0.38, 1.17)	0.82 (0.46, 1.47)
	4th	29/801	0.97 (0.58, 1.61)	0.83 (0.49, 1.39)	0.87 (0.51, 1.49)	1.19 (0.67, 2.11)
Peripheral VEs	1st	15/823	1.00	1.00	1.00	1.00
	2nd	21/814	1.42 (0.73, 2.76)	1.36 (0.70, 2.63)	1.32 (0.68, 2.56)	1.50 (0.76, 2.98)
	3rd	21/817	1.41 (0.73, 2.73)	1.31 (0.68, 2.55)	1.05 (0.53, 2.08)	1.33 (0.66, 2.69)
	4th	23/807	1.53 (0.80, 2.93)	1.37 (0.71, 2.64)	1.06 (0.54, 2.08)	1.47 (0.72, 3.01)
Cerebro-VEs	1st	12/826	1.00	1.00	1.00	1.00
	2nd	19/816	1.60 (0.77, 3.29)	1.52 (0.74, 3.13)	1.48 (0.72, 3.05)	1.69 (0.81, 3.52)
	3rd	15/823	1.25 (0.59, 2.67)	1.16 (0.54, 2.49)	0.91 (0.42, 2.00)	1.08 (0.48, 2.41)
	4th	20/810	1.66 (0.81, 3.40)	1.48 (0.72, 3.04)	1.21 (0.58, 2.52)	1.50 (0.69, 3.26)

Note: Models and abbreviations as in Table 3.

different clinical settings[18–20]; Massiot et al.[18] found that NLR was associated with symptomatic internal carotid stenosis in patients undergoing endarterectomy. However, in the same study, NLR was not associated with the degree of carotid stenosis. In 324 patients admitted to an Internal Medicine ward, Corriere et al.[19] found that NLR predicted the presence and the number of carotid atherosclerotic plaques. Furthermore, in a large retrospective study, NLR and was positively associated with the prevalence of carotid atherosclerosis. These two latter studies did not investigate the extent of stenosis or the carotid wall thickness. The few studies exploring the association between NLR and cIMT reported conflicting results.[22–29] Accordingly, some of them found an independent association between NLR and cIMT,[22–26] whereas this association was not observed in other studies.[27–29] Also, in a retrospective study, in patients with ischemic stroke, NRL was found to be a significant predictor of the cIMT in men but not in women.[23] These studies were conducted on relatively small patient groups and involved very heterogeneous populations in terms of cardiovascular risk (ranging from apparently lower cardiovascular risk,[27] up to higher risk populations[22,23,25]), type of IMT measure, baseline IMT and NLR values (Table S5). In evaluating the differences between ours and previous studies, it must be also considered that NLR can be influenced by a series of concomitant diseases (e.g., cancer, inflammatory diseases, chronic kidney disease), which are associated with the development of atherosclerosis. Furthermore, it has been observed that many pharmacological therapies used for the prevention of ASCVD (e.g., lipid-lowering, antihypertensive therapies) can affect NLR. The confounding effect of these variables has not always been fully considered in previous studies, and this could partly motivate the inconsistency of the data in the literature. To limit the confounding effect of these variables, in our work we excluded patients with concomitant inflammatory diseases, and we considered variables such as CRP levels, GFR, as well as concomitant lipid-lowering and antihypertensive therapies. Although the reasons for the inconsistency of findings across ours and other studies may lie in the differences in the characteristics of the study populations and concomitant confounding factors, our findings are consistent across multiple indices of carotid atherosclerosis. Indeed, multiple cross-sectional indicators of cIMT were considered (i.e., $cIMT_{mean}$, $cIMT_{max}$, and $cIMT_{mean\text{-}max}$), but none of these were independently associated with NLR. In addition, our finding of the lack of a significant prospective association between NLR and cIMT progression, an issue that has never been evaluated in previous studies, further supports our conclusion of a neutral effect of NLR on carotid atherosclerosis.

A prospective association between NLR and ASCVD risk has been reported in different clinical settings and confirmed in meta-analyses of studies including patients

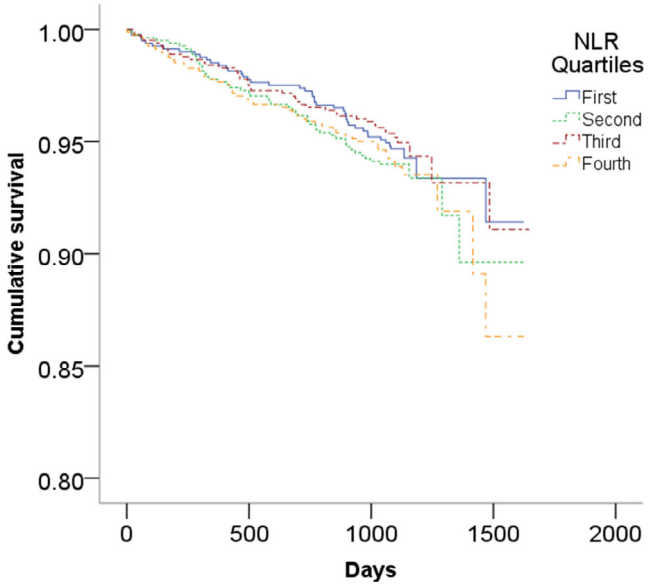

FIGURE 1 Combined vascular event-free survival across NLR quartiles (log-rank $p = 0.632$). NLR, neutrophil-to-lymphocyte ratio

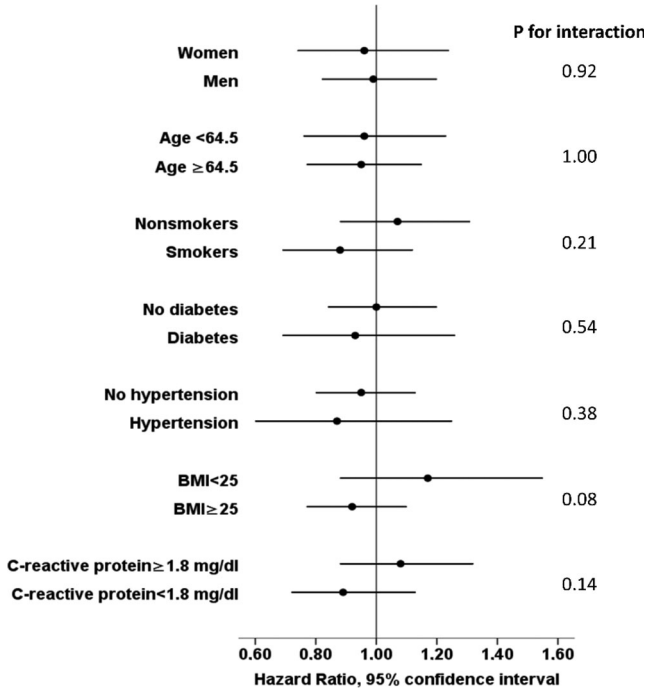

FIGURE 2 Hazard ratios for combined vascular events associated with one SD increase of log-transformed NLR: Subgroup analysis. Dots represent hazard ratios and horizontal lines represent 95% confidence interval, adjusted for covariates in model 4 of the cox regression (see Section 2), excluding the respective stratification variables. BMI, body mass index; cIMT, carotid intima media thickness; NLR, neutrophil to lymphocyte ratio; SD, standard deviation

at different degree of baseline ASCVD risk.[30,31,44] Most studies included in these meta-analyses included patients with significant comorbidities at baseline, including ASCVD[30,44,45] or advanced chronic kidney disease.[46] More studies, conducted in the general population and cumulatively analyzed in an additional meta-analysis,[31] had either a cross-sectional or a case–control design.[31] Hence, the prospective impact of NLR on ASCVD risk has been less investigated in the primary CV prevention. In this regard, NLR predicted an increased risk of ASCVD-related deaths in the healthy primary prevention US cohort of the National Health and Nutrition Examination Survey-III.[47] However, in this survey,[47] only the minority of participants with a very high NLR (>4.5), but not subjects with a lower NLR (≥1.5 to <3.0, 3.0–4.5), were exposed at a significant increased risk of ASCVD-related death. In our cohort, five ASCVD events were counted during follow-up among the only 51 patients with an NLR >4.5 (results not shown), thus compromising any prospective statistics in this specific patients' subgroup. In the cohort of African–American participants of the Jackson Heart Study, including also patients with ASCVD events at baseline, high NLR was associated with an increased risk for all-cause mortality and coronary heart disease (CHD) events.[21] However, in the same article reporting the results of the Jackson Heart Study, the analysis of the data of the Normative Aging Study in white men free of chronic disease at baseline, did not find a significant association between NLR and overall mortality.[21] Importantly, among apparently healthy men and women recruited in the prospective matched, nested case–control analysis of the Copenhagen City Heart Study and the Copenhagen General Population Study, NLR failed to predict the risk of myocardial infarction within 4 years of NLR measurement.[33]

Therefore, our findings and those from other prospective studies lend support to the lack of an association between NLR and ASCVD events in the primary CV prevention. Accordingly, in a very recent study, which analyzed data from five randomized trials, NLR predicted cardiovascular events in the secondary prevention setting.[32] However, in the primary prevention Jupiter cohort, adjusted HRs for incident VEs did not differ across NLR quartiles.[32]

A speculative hypothesis might be proposed in order to explaining such an apparent disagreement of findings. In this regard, it should be reported that, unlike subjects without overt ASCVD (i.e., primary CV prevention), those with overt ASCVD are typically characterized by higher degree of systemic and arterial inflammation,[48,49] which may contribute to atherosclerotic plaque instability and promote the early occurrence/recurrence of ischemic events in more vulnerable patients.[50] In agreement

with this hypothesis, the 4th quartile of baseline NLR was much higher in the CANTOS (NLR > 3.09), CIRT (NLR > 2.96), SPIRE-1 (NLR > 2.93), and SPIRE-2 (NLR > 2.83),[32] than in our study. In addition, in the Jackson Heart Study, the prognostic ability of NLR was strongly attenuated for those ASCVD events occurring later during the follow-up; in the same study,[21] NLR predictivity was abolished for prediction of late stroke events and overall mortality. Moreover, the prospective association between NLR and late ASCVD events was no more significant when the NLR predictive cut-off was reduced from 2.15 to 1.77.[21]

A pathophysiological hypothesis explaining the lack of an independent association between NLR, carotid atherosclerosis and the risk of VEs can be proposed. Although pro-inflammatory and atherogenic roles have been commonly attributed to neutrophils,[14] a possible protective action in the context of cardiovascular inflammation has been also proposed for these cells.[14,51,52] Accordingly, neutrophils may have vascular protective effects by attenuating inflammation and promoting angiogenesis.[14] Moreover, neutrophils have been found to participate in repairing the injured endothelium,[53] which is believed as an anti-inflammatory and anti-atherogenic event. On the other hand, the different subsets of lymphocytes (e.g., T-helper vs. regulatory T-cells) have been associated with either proinflammatory and proatherogenic activities, or immunomodulatory and athero-protective effects.[15] Hence, the variable impact of the individual components of NLR (i.e., neutrophil and lymphocyte counts, lymphocyte subtypes, etc.) on inflammation and atherosclerosis might undermine the overall predictive impact of their ratio. In agreement with this conclusion our results show the lack of an independent association between both the components of NLR (i.e., neutrophil and lymphocyte counts) with carotid atherosclerosis and VEs.

Several strengths of this study should be acknowledged. First, a large population of subjects at moderate-to-high CVD risk, recruited in five European countries, has been studied, and numerous cardiovascular confounders, including drug treatments, were considered. Second, carotid image acquisition and cIMT measurements were highly standardized across centers. Furthermore, subjects with extremely high neutrophil count were excluded from the analysis to minimize the confounding effect of any unrecognized infectious or hematological conditions.

As a limitation of this study, only subjects at moderate-to-high risk of ASCVD have been included, so the interpretation of our results cannot be directly applicable to populations at different risk levels. Moreover, racial differences, which might affect the relationship between NLR and incident VEs,[54] were not considered in this analysis. However, the lack of an association between NLR, carotid atherosclerosis and VEs was consistent across the five European countries involved in this multicenter study.

In conclusion, in subjects at moderate-to-high ASCVD risk, without prior VEs at baseline, NLR was neither associated with the presence and progression of carotid atherosclerosis, nor with the risk of future VEs.

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CONFLICT OF INTEREST

None.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Massimo R. Mannarino https://orcid.org/0000-0003-4694-5504
Damiano Baldassarre https://orcid.org/0000-0002-2766-8882
Matteo Pirro https://orcid.org/0000-0002-5527-4821

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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Supplemental material

[§]The IMPROVE Study Group

- Centro Cardiologico Monzino, IRCCS, Milan, Italy: Daniela Coggi, Beatrice Frigerio, Daniela Sansaro, Alessio Ravani, Palma Ferrante, Manuela Damigella, Fabrizio Veglia, Alice Bonomi, Nicolò Capra, Calogero Tedesco, Mauro Amato, Damiano Baldassarre.
- Department of Medical Biotechnology and Translational Medicine, Università degli Studi di Milano, Milan, Italy: Damiano Baldassarre.
- Maria Cecilia Hospital, Cotignola (RA), Italy: Elena Tremoli
- Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano, Milan, Italy: Laura Calabresi, Cesare R. Sirtori.
- Division of Cardiovascular Medicine, Department of Medicine Solna, Karolinska Institutet, Stockholm, Sweden: Per Eriksson, Rona J. Strawbridge, Bruna Gigante, Angela Silveira, Anders Hamsten.
- Karolinska University Hospital, Solna, Stockholm, Sweden: Angela Silveira, Per Eriksson, Anders Hamsten.
- Institute of Health and Wellbeing, University of Glasgow, Glasgow, United Kingdom: Rona J. Strawbridge.
- Health Data Research UK: Rona J. Strawbridge.
- Division of Cardiovascular and Nutritional Epidemiology, Institute of Environmental Medicine, Karolinska Institutet: Karin Leander, Federica Laguzzi, Ulf de Faire
- Cardiovascular Genetics, Institute Cardiovascular Science, University College of London, Rayne Building, University Street, London, United Kingdom: Steve E. Humphries, Jackie A. Cooper, Jayshree Acharya.
- Foundation for Research in Health Exercise and Nutrition, Kuopio Research Institute of Exercise Medicine, Kuopio, Finland: Kai Savonen, Kirsi Huttunen, Eva Rauramaa, Ilkka M. Penttila, Jukka Törrönen.

- Department of Clinical Physiology and Nuclear Medicine, Kuopio University Hospital, Kuopio, Finland: Kai Savonen.
- Department of Medicine, University Medical Center Groningen, Groningen & Isala Clinics Zwolle, Department of Medicine; the Netherlands: Andries J. Smit, A.I. van Gessel, A.M van Roon, A. Nicolai, D.J. Mulder, G.H. Smeets.
- Assistance Publique - Hopitaux de Paris; Service Endocrinologie-Metabolisme, Groupe Hôspitalier Pitié-Salpetriere, Unités de Prévention Cardiovasculaire, Paris, France: Philippe Giral, Anatole Kontush, Alain Carrié, Antonio Gallo.
- Institute of Public Health and Clinical Nutrition, University of Eastern Finland, Kuopio Campus: Sudhir Kurl, J. Karppi, T. Nurmi, K. Nyyssönen, T.P. Tuomainen, J.Tuomainen, J. Kauhanen.
- Internal Medicine, Angiology and Arteriosclerosis Diseases, Department of Medicine and Surgery, University of Perugia, Perugia, Italy: Matteo Pirro, M.R. Mannarino, G. Vaudo, V. Bianconi, E. Marini, F. Figorilli.

Supplementary tables

Supplementary table 1. Association between neutrophil count and measures of cIMT.

	Multivariable linear regression							
	Model 1		Model 2		Model 3		Model 4	
	β	P value	β	P value	B	P value	β	P value
$cIMT_{mean}$	0.093	<0.001	0.062	<0.001	0.027	0.090	0.012	0.471
$cIMT_{max}$	0.081	<0.001	0.059	<0.001	0.031	0.069	0.015	0.393
$cIMT_{mean-max}$	0.088	<0.001	0.061	<0.001	0.028	0.087	0.012	0.476
Fastest-$cIMT_{max-progr}$	0.026	0.150	0.017	0.351	-0.007	0.707	0.012	0.526

Model 1: adjusted for latitude. Model 2: adjusted for age, sex, and latitude. Model 3: adjusted for covariates in Model 2 plus body mass index, systolic blood pressure, glucose, smoking status, LDL-cholesterol and C-reactive protein. Model 4: adjusted for covariates in Model 3 plus waist-hip

ratio, diastolic blood pressure, HDL-cholesterol, triglyceride, glomerular filtration rate, lipid-lowering treatment and antihypertensive treatment. cIMT, carotid intima-media thickness.

Supplementary table 2. Associations between lymphocyte count and measures of cIMT.

	Multivariable linear regression							
	Model 1		Model 2		Model 3		Model 4	
	β	P value	β	P value	β	P value	β	P value
$cIMT_{mean}$	0.033	0.044	0.058	<0.001	0.022	0.158	0.020	0.204
$cIMT_{max}$	0.009	0.602	0.028	0.090	0.003	0.834	0.004	0.792
$cIMT_{mean-max}$	0.028	0.091	0.049	0.002	0.018	0.263	0.018	0.260
Fastest-$cIMT_{max-progr}$	0.022	0.226	0.032	0.078	0.020	0.277	0.014	0.464

Model 1: adjusted for latitude. Model 2: adjusted for age, sex, and latitude. Model 3: adjusted for covariates in Model 2 plus body mass index, systolic blood pressure, glucose, smoking status, LDL-cholesterol and C-reactive protein. Model 4: adjusted for covariates in Model 3 plus waist-hip ratio, diastolic blood pressure, HDL-cholesterol, triglyceride, glomerular filtration rate, lipid-lowering treatment and antihypertensive treatment. cIMT, carotid intima-media thickness.

Supplementary table 3. Hazard ratios (95% confidence interval) for VEs for one SD increase of Log-transormed neutrophyl and lymphocyte count.

		Model 1	Model 2	Model 3	Model 4
Neutrophil count	Combined VEs	1.13 (0.98, 1,31)	1.10(0.95, 1,27)	0.98(0.84, 1.14)	0.97 (0.83, 1.13)
	Coronary VEs	1.07 (0.89, 1.30)	1.03 (0.85, 1.25)	0.95 (0.77, 1.17)	0.95 (0.77, 1.17)
	Peripheral VEs	1.22 (0.97, 1.53)	1.19 (0.95, 1.49)	1.00 (0.79, 1.27)	0.98 (0.77, 1.24)
	Cerebro-VEs	1.16 (0.90, 1.47)	1.13 (0.88, 1.45)	0.96 (0.74, 1.25)	0.94 (0.72, 1.22)
Lymphocyte count	Combined VEs	1.08 (0.94, 1.25)	1.11 (0.97, 1.29)	1.05 (0.91, 1,22)	0.99 (0.85, 1.16)
	Coronary VEs	1.13 (0.94, 1.36)	1.17 (0.97, 1.41)	1.09 (0.89, 1.33)	1.04 (0.85, 1.27)
	Peripheral VEs	1.02 (0.82, 1.27)	1.04 (0.84, 1.30)	1.00 (0.79, 1.26)	0.93 (0.74, 1.18)
	Cerebro-VEs	0.95 (0.75, 1.21)	0.98 (0.77, 1.25)	0. 94 (0.73, 1.20)	0.89 (0.69, 1.14)

Model 1: adjusted for latitude. Model 2: adjusted for age, sex, and latitude. Model 3: adjusted for covariates in Model 2 plus body mass index, systolic blood pressure, glucose, smoking status, LDL-cholesterol and C-reactive protein. Model 4: adjusted for covariates in Model 3 plus waist-hip ratio, diastolic blood pressure, HDL-cholesterol, triglyceride, glomerular filtration rate, lipid-lowering treatment and antihypertensive treatment. VEs, vascular events

Supplementary table 4. Hazard ratios of combined VEs across neutrophil and lymphocyte count quartiles.

		Number of subjects with/without events	Model 1	Model 2	Model 3	Model 4
Neutrophil quartiles	1st	40/796	1.00	1.00	1.00	1.00
	2nd	45/790	1.14 (0.74, 1.74)	1.11 (0.73, 1.70)	1.01 (0.65, 1.55)	1.02 (0.66, 1.57)
	3rd	46/789	1.16 (0.76, 1.77)	1.07 (0.70, 1.64)	0.94 (0.61, 1.45)	0.90 (0.58, 1.40)
	4th	59/776	1.50 (1.00, 2.24)*	1.39 (0.93, 2.08)	1.07 (0.70, 1.64)	1.04 (0.68, 1.60)
Lymphocyte quartiles	1st	52/783	1.00	1.00	1.00	1.00
	2nd	39/797	0.76 (0.50, 1.15)	0.75 (0.49, 1.13)	0.69 (0.45, 1.05)	0.67 (0.44, 1.03)
	3rd	39/796	0.76 (0.50, 1.15)	0.77 (0.50, 1.17)	0.73 (0.48, 1.11)	0.68 (0.45, 1.05)
	4th	60/775	1.18 (0.81, 1.71)	1.27 (0.88, 1.85)	1.09 (0.74, 1.60)	0.95 (0.63, 1.41)

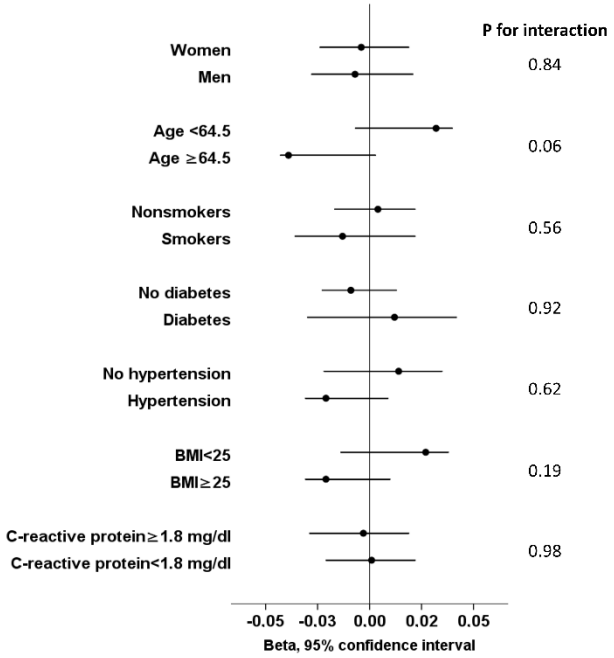
Model 1: adjusted for latitude. Model 2: adjusted for age, sex, and latitude. Model 3: adjusted for covariates in Model 2 plus body mass index, systolic blood pressure, glucose, smoking status, LDL-cholesterol and C-reactive protein. Model 4: adjusted for covariates in Model 3 plus waist-hip ratio, diastolic blood pressure, HDL-cholesterol, triglyceride, glomerular filtration rate, lipid-lowering treatment and antihypertensive treatment. VEs, vascular events.

*P=0.049

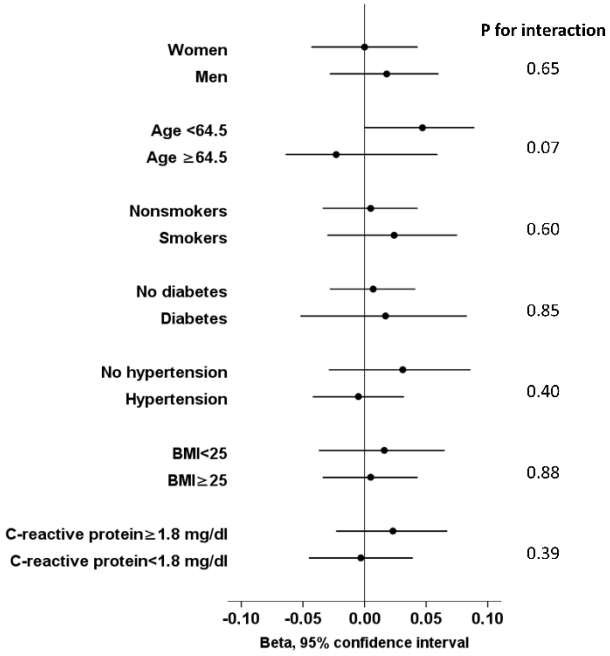
Supplementary table 5. Studies investigating the association between NLR and IMT.

Author	Reference	n. of subjects	Clinical setting	Evaluated parameter	Mean or median IMT values (mm)	IMT cut-off (mm)	Mean or Median N values
Li X et al.	25	320 diabetic patients 250 age- and sex matched controls	Uncomplicated type 2 diabetes	Carotid IMT	Diabetic patients 1.18±0.30 Controls 0.71±0.13	1	Diabetes g 2.68±0.70 Controls 2.08±0.76
Hyun S et al.	26	252	Ischemic stroke	Maximum IMT of both internal carotid arteries	Men 1.12±0.43 mm Women 1.14±0.52.	1	Men 3.27± Women 3.52±3.60
Sonaglioni A et al.	27	73	Healthy pregnant women	Common carotid artery IMT	0.57 ± 0.08	0.55	2.10±0.55
Çırakoğlu OF et al.	28	215	Hypertensive patients	Common carotid artery IMT	n.a.	0.9	2.23 (1.71
Li H et al.	29	268	Hemodialisis patients	Common carotid artery IMT	1.15 ± 0.16	1.2	3.36 ± 1.6
Carranza-Lira S. et al.	30	82	Pre- and postmenopausal women	Common carotid artery IMT	Premenopausal 0.08 (0.01-1.07) Postmenopausal 0.07 (0.01-0.7)	1	Premenop 1.53 (0.59 Postmeno 1.59 (0.76
Suárez-Cuenca JA et al.,	31	31	Obese subjects candidates for bariatric surgery	Common carotid artery IMT	1 (0.86-1.09)	0.9	1.68 (1.45
Tarantino et al.	32	100	Obese patients with NAFLD	Common carotid artery IMT	0.08 (0.07–0.10)	n.a.	2.06 (1.62

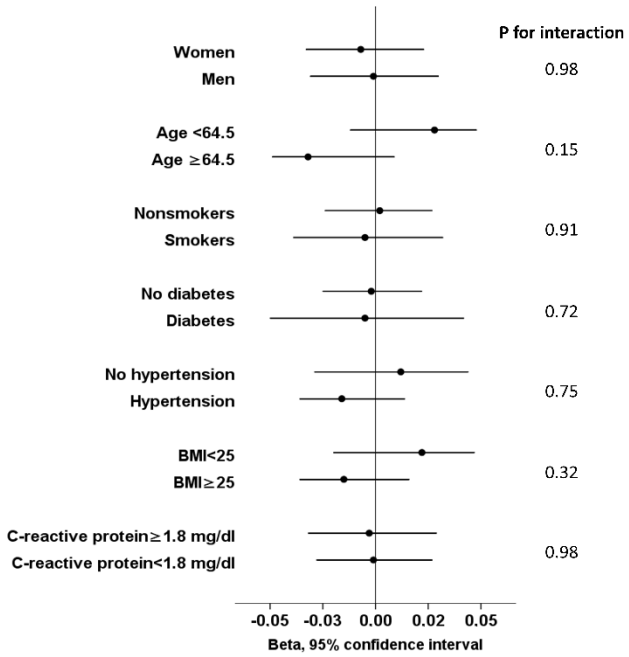
IMT, intima-media thickness; n.a, not available; NLR, neutrophil to lymphocyte ratio.



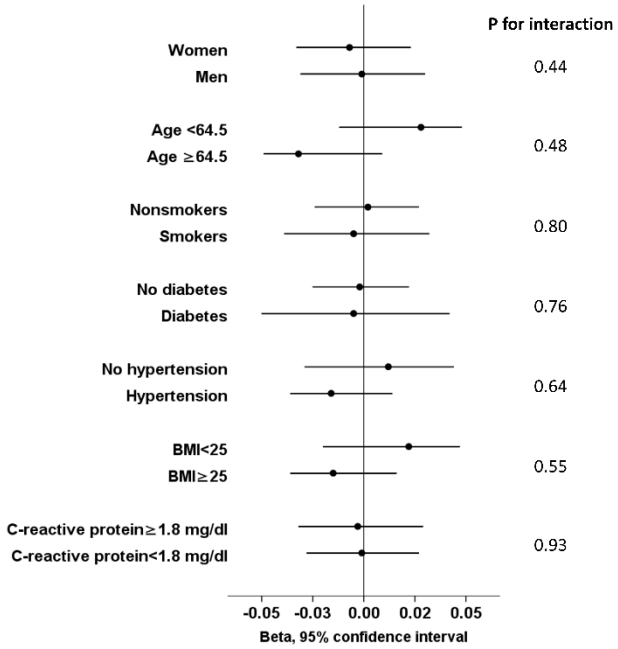
Supplementary figure 1. Adjusted association between Log-transformed NLR and $cIMT_{mean}$: subgroup analysis. Dots represent Beta coefficient and horizontal lines represent 95%conficence interval, adjusted for covariates in Model 4 (see Methods), excluding the respective stratification variables. BMI, body mass index; cIMT, carotid intima media thickness; NLR, neutrophil to lymphocyte ratio.



Supplementary figure 2. Adjusted association between Log-transformed NLR and $cIMT_{max}$: subgroup analysis. Dots represent Beta coefficient and horizontal lines represent 95%confidence interval, adjusted for covariates in Model 4 (see Methods), excluding the respective stratification variables. BMI, body mass index; cIMT, carotid intima media thickness; NLR, neutrophil to lymphocyte ratio.



Supplementary figure 3. Adjusted association between Log-transformed NLR and $cIMT_{mean\text{-}max}$: subgroup analysis. Dots represent Beta coefficient and horizontal lines represent 95%confidence interval, adjusted for covariates in Model 4 (see Methods), excluding the respective stratification variables. BMI, body mass index; cIMT, carotid intima media thickness; NLR, neutrophil to lymphocyte ratio.



Supplementary figure 4. Adjusted association between Log-transformed NLR and Fastest-cIMT$_{max\text{-}progr}$: subgroup analysis. Dots represent Beta coefficient and horizontal lines represent 95%confidence interval, adjusted for covariates in Model 4 (see Methods), excluding the respective stratification variables. BMI, body mass index; cIMT, carotid intima media thickness; NLR, neutrophil to lymphocyte ratio.