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White cell counts in relation to mortality in a general population of cohort study in the Netherlands: a mediating effect or not?

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To cite: Abete I, Lu Y, Lassale C, *et al*. White cell counts in relation to mortality in a general population of cohort study in the Netherlands: a mediating effect or not? *BMJ Open* 2019;**9**:e030949. doi:10.1136/bmjopen-2019-030949

► Prepublication history for this paper is available online. To view these files, please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2019-030949).

Received 08 April 2019
Revised 09 July 2019
Accepted 09 August 2019

Check for updates

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ABSTRACT

Background White cell count (WCC) is a clinical marker of inflammation. Data are limited regarding the association of total and differential WCC with risk of mortality, and its role related with smoking and body mass index (BMI).

Methods A total of 14 433 participants (4150 men; 10 283 women; average age 47.3±11.8 years) from the Dutch European Prospective Investigation into Cancer and Nutrition-Netherlands cohort were included. The associations between prediagnostic total WCC and its subtypes and risk of all-cause, cancer and cardiovascular disease (CVD) mortality were assessed. The role of WCC related with smoking and BMI on mortality was further explored. Multivariate Cox regression models were performed to estimate the HR and 95% CI.

Results After an average follow-up of 15.8 years, a total of 936 death cases were identified (466 cancer; 179 CVD; 291 other causes). Statistically significant graded associations between total WCC, and counts of lymphocytes, monocytes, neutrophils and eosinophils and risk of total mortality were observed. These associations were more apparent in current smokers. Strong associations for all-cause mortality or cancer mortality were observed in subjects with BMI ≥25 kg/m^2, ever smoking and elevated WCC (HR 3.92, 95% CI 2.76 to 5.57; HR 3.93, 95% CI 2.30 to 6.72). WCC partly mediated the associations between smoking or BMI and all-cause mortality.

Conclusions Prediagnostic WCC and its subtypes are associated with all-cause, cancer and CVD mortality risk. It may play a partially mediate role on the association between smoking or obesity and mortality.

Strengths and limitations of this study

- ► It is large prospective cohort study with a long follow-up period.
- ► Blood samples were collected at baseline and measured with standardised approaches.
- ► Other information, including smoking, physical activity, educational level, alcohol consumption and anthropometric variables, was collected.
- ► Only one measurement of white cell count was performed.
- ► Multiple comparisons were used in the analyses, which might produce smaller p values by chance and exaggerate type I error.

INTRODUCTION

White cell counts (WCC) include several cell types (lymphocytes, monocytes, neutrophils, eosinophils and basophils) and are widely considered as sensitive biomarkers of systemic inflammation. Although quite a few studies have found WCC is a good predictor of survival in patients with certain clinical diagnoses, studies on the association of this inexpensive and ubiquitous test with mortality in the healthy population are insufficient and inconsistent. A number of studies have associated WCC with cancer mortality, cardiovascular diseases (CVDs) mortality and all-cause mortality especially in elderly population or female population with limited sample size,[1–6] leaving inadequate evidence from the general population.

A few studies have shown that WCC or subtype is an independent predictor of mortality after controlling for potential confounding, for example, smoking.[1 5 7–12] This conclusion, however, might conflict with the fact that the WCC is influenced by many factors, such as infection, inflammation, smoking and obesity. Cigarette smoking is a well-recognised cause of elevated WCC.[13 14] Smokers have higher WCC than non-smokers, and the extent of the increase rises with the number of cigarettes smoked.[15] Obesity, an important risk factor for the development of CVD such as hypertension, diabetes and dyslipidaemia, is also associated with substantial increases in WCC.[16 17] Therefore, the association of WCC or subtype on mortality might be mediated by smoking or obesity, however, a few studies have investigated whether WCC or subtype played a mediate role on the pathway

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from smoking to mortality, or from obesity to mortality, or whether the three factors play a joint role on mortality.

Evidence of WCC subtype as a predictor of mortality in healthy populations is limited and largely inconsistent. Higher neutrophils[2 3] or lower lymphocytes[3 18 19] were associated with increased mortality, but no associations were found in other subtype in the same study.[3] Several studies have shown that monocyte counts have been independently associated with all-cause,[2 20] CVD[2 21] or cancer-related mortality,[2 20] while other studies have reported no association.[3 22] A U-shaped association of eosinophil count with all-cause mortality was reported in a Danish study.[23] Smoking or obesity has been associated with differential WCC subtype,[24 25] while whether this impacts mortality differently is unknown. Due to the differential function of WCC subtype in the immune system, more studies on mortality of general populations are warranted.

In order to further clarify the role of WCC and its subtype on the risk of all-cause, CVD and cancer mortality, we initiated an analysis using data from the European Prospective Investigation into Cancer and Nutrition-Netherlands (EPIC-NL), a prospective cohort recruited from the generally healthy population in the Netherlands.[26] Specifically, we gauged the relation of WCC and its subtype with smoking and obesity, and further examined the joint effect of those factors on mortality.

MATERIALS AND METHODS

Study population

This prospective cohort study combines the two Dutch cohorts of the EPIC, the EPIC-Medical Ethical Committee of TNO Nutrition and Food Research (MORGEN) and EPIC-Prospect,[27] which were set up simultaneously between 1993 and 1997. The EPIC-Prospect study includes 17 357 women aged 49–70 years living in Utrecht and its vicinity who participated in the nationwide Dutch breast cancer screening programme. The EPIC-MORGEN cohort consists of 22 654 men and women aged 21–64 years selected from random samples of the Dutch population in three different towns (Doetinchem, Amsterdam, Maastricht). At baseline, all participants filled out a general questionnaire and a validated food frequency questionnaire and underwent a physical examination. All participants provided written informed consent before study inclusion.

Beginning June 1995, after 19 214 subjects had been enrolled, a blood sample was drawn of all subsequent subjects for measurement of blood cell count at entry into the cohort. For the remaining inclusion period, blood cell counts were not available in some cohort participants. Moreover, valid smoking data were missing for 156 subjects. With further removal of 253 participants who never had followed up, we initially had 15 973 participants included in this study. Participants with a baseline history of cancer (n=555), stroke and heart diseases (n=397), diabetes (n=259) and comorbidity of above two or three diseases (n=86) were excluded. Moreover, participants with missing values (n=243) were further excluded. A total of 14 433 (4150 men and 10 283 women) participants were included in the present study.

Patient and public involvement

We appreciated all patients who participated in the study and contributed their personal information to the research. All patients and the public, however, were not involved in the design or planning of the study.

Blood collection

The blood sample for measurement of the complete blood cell count was drawn in an ethylenediaminetetraacetic acid (EDTA) Monovette at entry into the cohort. The storage process as well as the analysis of blood samples was described previously.[13] Total WCC and subtypes, including absolute counts of lymphocytes, monocytes, neutrophils and eosinophils, were measured using standard automated clinical methodologies.

Covariates

Information on sex (male, female), age (years) at study entry, educational level, smoking habit, alcohol consumption and physical activity was obtained by questionnaire. Educational level was grouped into four categories: primary school, technical/professional education, secondary school and longer education (including university). Smoking was defined as never, former (quit smoking >20 years ago, quit 10–20 years ago, quit ≤10 years ago) and current smoker (1–15, 16–25, >16 cigarettes/day), therefore coding included seven categories. Smokers were also asked if they were pipe or cigar smoker. Alcohol consumption was considered as a continuous variable (gram of ethanol/day). Physical activity was categorised into four levels: inactive, moderately inactive, moderately active or active according to the validated Cambridge Physical Activity Index.[28] Body mass index (BMI, kg/m^2) was calculated dividing body weight (kg) by the square of the body height (m^2), and waist circumferences were measured by centimetres.

Outcome assessment

The outcomes of interest were all-cause, cancer and CVD mortality. EPIC-NL participants were followed for vital status and date of death by regular linkage with the municipal registries. Subsequently, causes of death for deceased persons were obtained through linkage with Statistics Netherlands. Follow-up was complete through 31 December 2012.

Statistical analyses

Descriptive statistics of baseline characteristics are presented for all participants by categories of WCC. Cox proportional hazards regression models were used to estimate the HR and 95% CI of the associations between WCC, its subtypes (lymphocytes, monocytes, neutrophils and eosinophils) and all-cause, cancer and CVD mortality. Distributions of WCC are shown by tertiles (T_1=WCC ≤5.7×10^9/L; T_2=WCC >5.7×10^9/L -<7.2×10^9/L;

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T_3=WCC ≥7.2×10^9/L). Categories based on clinical standard ranges were also considered and analysed. Due to limited number in certain categories using clinical standard range, we only reported results based on tertiles. The follow-up of all participants in the cohort started since the date of study entry, and censored at the date of death, lost to follow-up or 31 December 2012, whichever came first. We used attained age as the time scale in the Cox regression model. Proportional hazard assumption was tested based on Schoenfeld residuals. In some models, sex did not meet the assumption. We then set it as a strata variable. Cox regression models were stratified by age (set as an integer) and sex and were adjusted for potential confounders: educational level, smoking habit, alcohol consumption, physical activity, BMI and waist circumference. Linear trend across tertiles was assessed by modelling the median of each tertile as a continuous variable. To test for effect modification of WCC and subtypes on mortality risk by BMI, waist circumference or smoking, we used a likelihood ratio test to compare the model with and without interaction terms.

In order to measure the joint effect of BMI, smoking and WCC, a new variable was created by combining BMI (<25 and ≥25 kg/m^2), WCCcategories (≤10, >10×10^9 cells/L, based on clinical standard range) and smoking status (no smoking or ever smoking). The combined category of normal WCC range (≥4 to ≤10×10^9 cells/L) with BMI <25 kg/m^2 and never smoking was chosen as reference. Analyses of joint effects were controlled for age and sex in the basic model (model 1), or age, sex, alcohol consumption, physical activity and educational level in the second model.

In order to assess if counts of WCC are a mediating factor between smoking, or obesity and risk of mortality, we performed a mediation analysis based on counterfactual mediation modelling.[29–31] Proportion mediated was calculated based on indirect effects divided by total effects. This method, unfortunately, has not been well developed in application to time-to-event data. Therefore, we also calculated mediation using the traditional 'change-in-estimate' method.[32 33] We calculated the quantification of the percentage reduction in estimate (smoking) after controlling for the potential mediating factor (WCC).

Two-sided p<0.05 were considered to be statistically significant. All statistical analyses were conducted using the statistical software package STATA V.12.0 (StataCorp).

RESULTS

Baseline characteristics

A total of 14 433 participants were included in the analysis with a mean follow-up of 15.8±2.4 years. There were 936 total deaths over 228 304 person-years of follow-up. The crude cumulative mortality rate was 4.1 per 1000 person-years. Among total deaths, 466 were due to cancer, 179 due to CVDs and 291 due to other causes.

Baseline characteristics of the participants by tertile of WCC are reported in table 1. Proportions of men and women were similar across tertiles of WCC. Participants were younger, more obese, less educated, less active physical activity and more current smokers in the higher tertiles of WCC, while alcohol consumption was similar across the tertiles of WCC. Analysis of WCC subtypes revealed significantly higher lymphocyte, monocyte, neutrophil and eosinophils levels among all-cause and disease-specific mortality cases compared with all participants.

Association between total WCC, its subtypes and mortality

The association between WCC, its subtypes and all-cause, cancer and CVD mortality is shown in table 2. After multivariate adjustment, a statistically significant association between total WCC (HR_{T3vsT1}: 1.38, 95% CI 1.17 to 1.64, p<0.0001), lymphocytes ($HR_{T3vs\ T1}$: 1.21, 95% CI 1.02 to 1.43, p=0.025), monocytes ($HR_{T3vs\ T1}$: 1.22, 95% CI 1.04 to 1.43, p=0.016), neutrophils ($HR_{T3vs\ T1}$: 1.29, 95% CI 1.09 to 1.52, p=0.002) and eosinophils ($HR_{T3vs\ T1}$: 1.25, 95% CI 1.05 to 1.49, p=0.017) and all-cause mortality risk was observed. A significant association was observed between total WCC and CVD mortality ($HR_{T3vs\ T1}$: 1.53, 95% CI 1.03 to 2.26, p=0.031), but not cancer mortality ($HR_{T3vs\ T1}$: 1.13, 95% CI 0.89 to 1.44, p=0.282). The associations for subtypes of WCC were not statistically significant for cancer and CVD mortality. No significant association was found for neutrophil-to-Lymphocyte ratio (NLR) and mortality (table 2).

Total WCC, its subtype and mortality by smoking status

Interactions between WCC (or its subtype) and smoking status were consistently significant, while no interaction was found for WCC and BMI, or waist circumference except eosinophils and waist circumference. We further stratified the analyses by smoking status (table 3). The results showed that total WCC and all WCC subtypes were statistically significantly associated with total mortality in current smokers, while the associations were attenuated or disappeared in never and former smokers. Total WCC and lymphocytes were significantly associated with cancer mortality risk in current smokers, whereas only total WCC was significantly associated with CVD mortality in current smokers (data not shown). In former smokers, the NLR was positively associated with CVD mortality, but monocytes count was negatively associated with CVD mortality.

Joint effects of BMI, smoking, WCC and mortality

A high WCC BMI in addition to being a current or former smoker was strongly and significantly associated with all-cause and cancer mortality risk, regardless of BMI status (table 4). Subjects with BMI <25 kg/m^2 who were also current or former smokers with normal WCC showed an increased risk for all-cause and cancer mortality (HR 1.55, 95% CI 1.21 to 1.99; HR 2.00, 95% CI 1.37 to 2.92, respectively). This association was not strengthened when BMI was elevated (HR 1.42, 95% CI 1.11 to 1.80; HR 1.80, 95% CI 1.25 to 2.60, respectively), but apparently enhanced when WCC increases irrespective of level of

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Table 1 Basic characteristics of cohort participants by tertiles of white cell count (WCC)

	WCC							
	All participants		T1 (≤5.7)		T2 (5.8–7.1)		T3 (≥7.2)	
N	14433		5146		4514		4773	
Men (%)	4150	28.75	1502	29.19	1316	29.15	1332	27.91
Women (%)	10283	71.25	3644	70.81	3198	70.85	3441	72.09
Age at recruitment (mean±SD)	47.3±11.8		48.1±12.0		47.1±12.0		46.7±11.3	
BMI, n (%)								
<25 kg/m^2	7022	48.65	2702	52.51	2113	46.81	2207	46.24
≥25 to <30 kg/m^2	5539	38.38	1925	37.41	1784	39.52	1830	38.34
≥30 kg/m^2	1872	12.97	519	10.09	617	13.67	736	15.42
Waist size (cm)	85.1	±11.5	83.6	±10.7	85.3	±11.5	86.3	±12.1
Educational level, n (%)								
Primary school	1794	12.43	546	10.61	571	12.65	677	14.18
Technical/professional	5082	35.21	1760	34.20	1540	34.12	1782	37.34
Secondary school	4078	28.25	1439	27.96	1279	28.33	1360	28.49
Longer education (inclu. university)	3479	24.10	1401	27.23	1124	24.90	954	19.99
Physical activity, n (%)								
Inactive	1015	7.03	249	4.84	306	6.78	460	9.64
Moderately inactive	3510	24.32	1206	23.44	1065	23.59	1239	25.96
Moderately active	3850	26.67	1420	27.59	1209	26.78	1221	25.58
Active	6058	41.97	2271	44.13	1934	42.84	1853	38.82
Smoking habits, n (%)								
Never	5479	37.96	2462	47.84	1774	39.30	1243	26.04
Former	4455	30.87	1889	36.71	1499	33.21	1067	22.35
Current	4499	31.17	795	15.45	1241	27.49	2463	51.60
Alcohol intake, gram/day (median, IQR)	5.9	±16.2	6.1	±15.1	5.9	±16.2	5.8	±18.1

BMI, body mass index.

BMI (in those with BMI <25 hours 3.15, 95% CI 2.07 to 4.81; HR 2.71, 95% CI 1.37 to 5.38 and in those with BMI ≥25 HR 3.92, 95% CI 2.76 to 5.57; HR 3.93, 95% CI 2.30 to 6.72, respectively).

Mediation analysis

Based on the potential causality pathway and previous evidence between smoking and WCC or obesity and WCC, we tested mediation of WCC between smoking (ever smoker vs never smoker) and total mortality, and between overweight (BMI >25 vs BMI ≤25 kg/m^2) and total mortality, when smoking was treated as a primary exposure in the model. Using the counterfactual mediation modelling, we calculated the total effect, direct effect and indirect effect based on Cox regression model and linear regression model. The proportion mediated was 32.7% (95% CI 30.1% to 41.1%) for smoking, and 6.4% (95% CI 5.3% to 5.9%) for BMI. Using the 'change-in-estimate' method, the proportion mediated was 27.9% (95% CI 19.1% to 49.4%) for smoking and 9.6% (95% CI 5.5% to 38.3%) for BMI.

DISCUSSION

In this longitudinal study, we found that higher counts of total WCC and its subtypes are positively associated with a significantly higher risk of all-cause mortality. Highest risk of all-cause mortality was observed in ever smokers with elevated total WCC and higher BMI. WCC may be one of the mediating factors in the association between smoking or BMI and all-cause mortality.

Several previous studies have reported that WCC was associated with mortality even after adjusting for smoking.[1 5 7–12] This is not fully consistent with our analysis in healthy population. In our study, the associations between total WCC and mortality were attenuated when smoking was incorporated into the model. In the analysis stratified by smoking status, all cause, CVD and cancer mortality were especially stronger in current smokers. Current cigarette smoking has been associated with increased WCC in many studies.[15 34] Our data show that current smoking is also strongly related to WCC. The existence of low-grade inflammation in smokers may trigger the increase of WCC that fights against the

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Table 2 HRs and 95% CIs of WCC and its subtype relative to mortality

			All cause of death				Cancer death				CVD death			
		N	Cases	HR (95% CI)			Cases	HR (95% CI)			Cases	HR (95% CI)		
Total (N)		14 433	936				466				179			
WCC	T_1	5146	274	1			144	1			47	1		
	T_2	4514	252	1.01	0.85	1.21	122	0.90	0.70	1.15	53	1.20	0.81	1.79
	T_3	4773	410	1.38	1.17	1.64	200	1.13	0.89	1.44	79	1.53	1.03	2.26
	P trend			<0.0001				0.282				0.031		
Subtype of WCC														
Lymphocytes	T_1	4904	282	1			131	1			50	1		
	T_2	4985	306	1.08	0.92	1.28	160	1.18	0.93	1.49	61	1.19	0.81	1.74
	T_3	4544	348	1.21	1.02	1.43	175	1.22	0.95	1.55	68	1.33	0.90	1.96
	P trend			0.025				0.119				0.154		
Monocytes	T_1	7387	455	1			225	1			76	1		
	T_2	2990	170	1.02	0.85	1.22	90	1.07	0.84	1.38	41	1.40	0.95	2.06
	T_3	4056	311	1.22	1.04	1.43	151	1.15	0.92	1.44	62	1.33	0.92	1.92
	P trend			0.016				0.23				0.105		
Neutrophils	T_1	5008	269	1			138	1			48	1		
	T_2	4927	285	0.94	0.80	1.11	149	0.95	0.75	1.21	58	1.01	0.69	1.49
	T_3	4498	382	1.29	1.09	1.52	179	1.07	0.84	1.36	73	1.35	0.92	1.99
	P trend			0.002				0.572				0.11		
Eosinophils	T_1	7911	484	1			245	1			83	1		
	T_2	3881	262	1.06	0.91	1.23	120	0.93	0.75	1.17	59	1.28	0.91	1.80
	T_3	2641	190	1.25	1.05	1.49	101	1.25	0.98	1.59	37	1.30	0.87	1.95
	P trend			0.017				0.159				0.135		
NLR*	T_1	4831	275	1			146	1			48	1		
	T_2	4796	327	1.13	0.96	1.32	172	1.14	0.91	1.43	59	1.15	0.78	1.68
	T_3	4805	334	1.09	0.93	1.29	148	0.91	0.72	1.15	72	1.34	0.92	1.94
	P trend			0.303				0.431				0.303		

*Model adjusted by age and sex, alcohol consumption, physical activity, educational level, BMI, waist circumference and smoking intensity.
BMI, body mass index; CVD, cardiovascular disease; NLR, neutrophil-to-lymphocyte ratio; WCC, white cell count.

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Table 3 HRs and 95% CIs of white cell count (WCC) and its subtype relative to mortality stratified by smoking status

		Never smoking					Former smoking					Current smoking				
		N	Cases	HR (95% CI)			N	Cases	HR (95% CI)			N	Cases	HR (95% CI)		
WCC	T_1	2462	135	1.00			1889	104	1			795	35	1.00		
	T_2	1774	75	0.77	0.58	1.03	1499	82	0.96	0.71	1.3	1241	95	1.69	1.14	2.50
	T_3	1243	87	1.24	0.94	1.63	1067	67	1.15	0.83	1.58	2463	256	2.03	1.41	2.92
	P trend			0.27					0.45					<0.0001		
Subtype of WCC																
Lymphocytes	T_1	2261	138	1.00			1762	95	1			881	49	1.00		
	T_2	1959	89	0.76	0.58	1.00	1599	90	1.05	0.77	1.42	1427	127	1.70	1.22	2.37
	T_3	1259	70	1.01	0.75	1.36	1094	68	1.09	0.78	1.51	2191	210	1.65	1.20	2.27
	P trend			0.75					0.62					0.01		
Monocytes	T_1	3240	177	1.00			2384	142	1			1763	136	1.00		
	T_2	1079	62	1.29	0.96	1.72	1004	50	0.83	0.59	1.17	907	58	0.91	0.67	1.24
	T_3	1160	58	1.18	0.87	1.60	1067	61	0.87	0.63	1.21	1829	192	1.39	1.10	1.77
	P trend			0.17					0.35					0.01		
Neutrophils	T_1	2268	127	1.00			1794	92	1			946	50	1.00		
	T_2	1942	87	0.70	0.53	0.93	1630	99	1.13	0.84	1.52	1355	99	1.09	0.77	1.54
	T_3	1269	83	1.01	0.76	1.33	1031	62	1.21	0.87	1.69	2198	237	1.62	1.18	2.21
	P trend			0.75					0.25					<0.0001		
Eosinophils	T_1	3266	180	1.00			2588	135	1			2057	169	1.00		
	T_2	1377	79	1.06	0.81	1.39	1129	70	1.07	0.79	1.45	1375	113	1.02	0.80	1.31
	T_3	836	38	0.94	0.66	1.35	738	48	1.27	0.89	1.81	1067	104	1.43	1.10	1.86
	P-trend			0.92					0.21					0.01		
NLR*	T_1	1877	83	1.00			1535	78	1			1419	114	1.00		
	T_2	1827	103	1.15	0.86	1.54	1484	103	1.30	0.96	1.76	1485	121	1.00	0.77	1.30
	T_3	1775	111	1.13	0.84	1.51	1435	72	0.99	0.71	1.38	1595	151	1.08	0.85	1.39
	P trend			0.43					0.99					0.51		

*Model adjusted by age and sex, alcohol consumption, physical activity, educational level, BMI, waist circumference.
BMI, body mass index; NLR, neutrophil-to-lymphocyte ratio.

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Table 4 Joint effect of BMI, smoking status and white cell count (WCC) on mortality

BMI	Smoking	WCC (clinical ranges)	All-cause mortality							Cancer mortality					
			N	Deaths	Model 1			Model 2		N	Deaths	Model 1			Model 2	
<25	Never	<4.0	132	6	0.89	0.36	2.20	0.83	0.34	2.05	132	1	0.47	0.06	3.42	0.45	0.06	3.26
	Never	≥4.0 to ≤10.0	2433	89	Referent			Referent			2433	36	Referent			Referent		
	Never	>10.0	50	4	2.13	0.78	5.85	1.99	0.73	5.45	50	2	2.87	0.68	12.05	2.75	0.66	11.55
	Ever	<4.0	125	9	1.45	0.73	2.88	1.45	0.73	2.89	125	7	2.66	1.18	5.99	2.61	1.15	5.89
	Ever	≥4.0 to ≤10.0	3971	228	1.66	1.30	2.13	1.55	1.21	1.99	3971	126	2.15	1.48	3.12	2.00	1.37	2.92
	Ever	>10.0	311	30	3.63	2.39	5.52	3.15	2.07	4.81	311	11	3.03	1.54	6.00	2.71	1.37	5.38
≥25	Never	<4.0	90	6	1.24	0.54	2.85	1.16	0.51	2.65	90	4	1.96	0.69	5.52	1.81	0.64	5.12
	Never	≥4.0 to ≤10.0	2711	187	1.23	0.96	1.59	1.14	0.88	1.47	2711	84	1.43	0.96	2.11	1.35	0.91	2.00
	Never	>10.0	63	5	1.54	0.63	3.81	1.41	0.57	3.49	63	1	0.82	0.11	5.99	0.78	0.11	5.69
	Ever	<4.0	84	2	0.45	0.11	1.84	0.42	0.10	1.71	84	1	0.55	0.08	4.04	0.52	0.07	3.83
	Ever	≥4.0 to ≤10.0	4111	317	1.57	1.23	1.99	1.42	1.11	1.80	4111	170	1.97	1.37	2.84	1.80	1.25	2.60
	Ever	>10.0	352	53	4.71	3.33	6.65	3.92	2.76	5.57	352	23	4.53	2.66	7.71	3.93	2.30	6.72

Model 1: adjusted by sex and age. Model 2: model 1+alcohol consumption, physical activity and education.
BMI, body mass index.

inflammation and damage caused by smoking.[13] Interestingly, several studies have demonstrated that current cigarette smoking is a reversible cause of elevated WCC and quitting of smoking may lead to recovery of WCC.[34 35] The non-significant results in former smokers in our study may further verify this point. Moreover, obesity has been defined as a state of chronic low-grade inflammation in which leucocytes might be involved.[36] Quite a few studies have observed increased WCC among subjects with overweight or obesity[7 22 37 38] suggesting that leucocytes are involved in the development of obesity-related comorbidities. Stratified analysis by smoking status and joint effect analysis by smoking and BMI status showed apparent evidence for effect modification by smoking and BMI. The further mediation analyses indicated that WCC or its subtype may play in part a mediating role from smoking to mortality, or from obesity to mortality. In fact, the strongest associations between an increased total WCC and all-cause and cancer mortality were observed in ever smoking participants with BMI ≥25 kg/m^2.

Few studies have investigated the risk associated with different WCC subtypes in healthy subjects. Our study showed that subtype of WCC was significantly associated with all-cause mortality after adjustment for smoking (status, intensity and duration), while the associations with cancer and CVD mortality were attenuated and became non-significant. Most previous studies that have evaluated WCC subtype with risk of mortality have been conducted in CVD subjects or patients with cancer, and few in general populations. Among healthy participants at baseline, an Asian cohort study (245 died in 8447 study subjects) showed an inverse association between lymphocyte count and all-cause and cancer mortality, while neutrophil and monocyte counts were positively associated with CVD mortality.[39] The Women's Health and Aging Study (175 died in 624 community-dwelling women age 65–101) demonstrated that high total WCC, high neutrophil counts and low lymphocyte counts were associated with all-cause mortality[3] while among elderly subjects, monocyte counts were associated with an increased risk of cardiovascular and cancer-related mortality.[2] In a recent study from the UK (10 364 died in 478 259 study participants), neutrophil counts were associated with total mortality and CVD mortality, especially consistently associated with fatal and non-fatal CVD in men.

It is important to evaluate WCC subtypes for several reasons. First, they differ in their inflammation and immune functions. In addition, the different associations observed between the WCC subtypes and the outcomes considered (all-cause, cancer and CVD mortality) confirm their differential roles in the organism. Inflammation plays an essential part in initiating tumourigenesis by damaging specific tissues, and neutrophils, monocytes and eosinophils are crucial components of this process.[40] However, in the present study, a stronger association was shown between total WCC and all-cause, cancer and CVD mortality compared with the subtypes. In contrast to our

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findings, several studies have shown high neutrophil and low lymphocyte counts (the increased NLR) associated with mortality in general population. However, the NLR displayed no association with mortality in our study, while a significantly increased association was observed in persons who died of CVD before 70 years (data not showed). More interestingly, when we analysed the association of the NLR with total mortality by 1, 5, 6 and 10 years follow-up, respectively (data not shown), we found the NLR is significantly associated with total mortality in short period of follow-up until 5 years but not in 6 years or 10 years. It has been reported that the NLR is sensitive to the suppressive action of particularly activated myeloid cells. It may reflect the phenotypic action of a disease process and thus be less evident in a long prospective setting. Some studies have shown that the NLR was a good predictor of mortality[41 42] in patients with CVDs or cerebrovascular disease, while our study participants were from the general population in which the role of NLR might be different. The NLR can reflect the balance between the activation of the inflammation pathway and the anti-inflammation immune function, and is a good biomarker for acute inflammation. Therefore, the NLR can be a good predictor for patients with acutely clinical symptoms but not for mortality in general population.

A number of limitations of these analyses must be considered. Only one measurement of WCC was performed. Multiple comparisons were used in the analyses, which might produce smaller p values by chance and exaggerate type I error. Some death cases (n=62) could not be classified because (1) the participant did not consent for linkage with causes of death database (n=18); (2) an error occurred in the linkage procedure (n=42) and (3) for unknown reasons (n=2). Even after adjustment for the known risk factors, residual confounding may occur because of measurement error or unmeasured or unknown risk factors.

The present study has several strengths that should be emphasised as well. EPIC-NL is a large prospective cohort study that included both men and women from the general population, with a broad age range and a long follow-up period. Blood samples were collected at baseline and measured with standardised approaches. Information, including smoking, physical activity, educational level, and alcohol consumption, was collected using a structured questionnaire. Anthropometric variables were measured using standardised procedures. The cause of death of deceased participants was obtained through linkage with the municipal registry and Statistics Netherlands.

In summary, WCC and its subtypes are associated with an increased risk of all-cause mortality, and total WCC is associated with cancer and CVD mortality. WCC may play a partially mediate role on the pathway between smoking and mortality risk, and between obesity and mortality, when smoking was treated as a primary exposure.

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Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval The study complies with the Declaration of Helsinki and was approved by the Institutional Review Board of the University Medical Center Utrecht (Prospect) and the Medical Ethical Committee of TNO Nutrition and Food Research (MORGEN).

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data sets used for the current study are available from the Dutch European Prospective Investigation into Cancer and Nutrition (EPIC-NL) cohort on request.

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