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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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 (4,150 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/m², 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.

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, 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. 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. Smokers have higher WCC than non-smokers, and the extent of the increase rises with the number of cigarettes smoked. Obesity, an important risk factor for the development of CVD such as hypertension, diabetes and dyslipidaemia, is also associated with substantial increases in WCC. 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.
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 or lower lymphocytes were associated with increased mortality, but no associations were found in other subtype in the same study. Several studies have shown that monocyte counts have been independently associated with all-cause, CVD or cancer-related mortality, while other studies have reported no association. A U-shaped association of eosinophil count with all-cause mortality was reported in a Danish study. Smoking or obesity has been associated with differential WCC subtype, 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. 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, 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. 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. Body mass index (BMI, kg/m²) was calculated dividing body weight (kg) by the square of the body height (m²), 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 were shown by tertiles (T1=WCC ≤5.7×10⁹/L; T2=WCC >5.7×10⁹/L <7.2×10⁹/L;
T_1=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), WCC categories (≤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 (≥24 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. 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. 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 14433 participants were included in the analysis with a mean follow-up of 15.8±2.4 years. There were 936 total deaths over 228304 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 multi-variate adjustment, a statistically significant association between total WCC (HR_{T3=T1}: 1.38, 95% CI 1.17 to 1.64, p<0.0001), lymphocytes (HR_{T3=T1}: 1.21, 95% CI 1.02 to 1.43, p=0.025), monocytes (HR_{T3=T1}: 1.22, 95% CI 1.04 to 1.43, p=0.016), neutrophils (HR_{T3=T1}: 1.29, 95% CI 1.09 to 1.52, p=0.002) and eosinophils (HR_{T3=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_{T3=T1}: 1.53, 95% CI 1.03 to 2.26, p=0.031), but not cancer mortality (HR_{T3=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
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         | 29.15     | 1332      | 27.91     |
| Women (%) | 10283        | 71.25     | 3644         | 70.81     | 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/m² | 7022      | 48.65        | 2702      | 52.51     | 2113      | 46.81     | 2207      | 46.24     |
| ≥25 to <30 kg/m² | 5539      | 38.38     | 1925         | 37.41     | 1784      | 39.52     | 1830      | 38.34     |
| ≥30 kg/m² | 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 (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/m²) 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. 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. 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
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              |                    |              |            |       |
| T1               | 5146               | 274          | 1          | 144   | 1           | 47    | 1           |
| T2               | 4514               | 252          | 1.01 0.85 1.21 | 122   | 0.90 0.70 1.15 | 53    | 1.20 0.81 1.79 |
| T3               | 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      |                    |              |            |       |
| T1               | 4904               | 282          | 1          | 131   | 1           | 50    | 1           |
| T2               | 4985               | 306          | 1.08 0.92 1.28 | 160   | 1.18 0.93 1.49 | 61    | 1.19 0.81 1.74 |
| T3               | 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        |                    |              |            |       |
| T1               | 7387               | 455          | 1          | 225   | 1           | 76    | 1           |
| T2               | 2990               | 170          | 1.02 0.85 1.22 | 90    | 1.07 0.84 1.38 | 41    | 1.40 0.95 2.06 |
| T3               | 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      |                    |              |            |       |
| T1               | 5008               | 269          | 1          | 138   | 1           | 48    | 1           |
| T2               | 4927               | 285          | 0.94 0.80 1.11 | 149   | 0.95 0.75 1.21 | 58    | 1.01 0.69 1.49 |
| T3               | 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      |                    |              |            |       |
| T1               | 7911               | 484          | 1          | 245   | 1           | 83    | 1           |
| T2               | 3881               | 262          | 1.06 0.91 1.23 | 120   | 0.93 0.75 1.17 | 59    | 1.28 0.91 1.80 |
| T3               | 2641               | 190          | 1.25 1.05 1.49 | 101   | 1.25 0.98 1.59 | 37    | 1.30 0.87 1.95 |
| P trend          | 0.002              |              |            | 0.572 |            | 0.135 |
| NLR*             |                    |              |            |       |
| T1               | 4831               | 275          | 1          | 146   | 1           | 48    | 1           |
| T2               | 4796               | 327          | 1.13 0.96 1.32 | 172   | 1.14 0.91 1.43 | 59    | 1.15 0.78 1.68 |
| T3               | 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.
### 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 |             |
|-------------------|---------------|-------------|----------------|-------------|----------------|-------------|
|                   |               |            |                |                   |               |            |
| WCC               |               |            |                |                   |               |            |
| T<sub>1</sub>     | 2462          | 135        | 1.00           | 1889         | 104           | 1           |
| T<sub>2</sub>     | 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<sub>3</sub>     | 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<sub>1</sub>     | 2261          | 138        | 1.00           | 1762         | 95            | 1           |
| T<sub>2</sub>     | 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<sub>3</sub>     | 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<sub>1</sub>     | 3240          | 177        | 1.00           | 2384         | 142           | 1           |
| T<sub>2</sub>     | 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<sub>3</sub>     | 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<sub>1</sub>     | 2268          | 127        | 1.00           | 1794         | 92            | 1           |
| T<sub>2</sub>     | 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<sub>3</sub>     | 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<sub>1</sub>     | 3266          | 180        | 1.00           | 2588         | 135           | 1           |
| T<sub>2</sub>     | 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<sub>3</sub>     | 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<sub>1</sub>     | 1877          | 83         | 1.00           | 1535         | 78            | 1           |
| T<sub>2</sub>     | 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<sub>3</sub>     | 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.
Table 4  Joint effect of BMI, smoking status and white cell count (WCC) on mortality

| WCC (clinical ranges) | BMI | Smoking | Model 1 | Model 2 |
|-----------------------|-----|---------|---------|---------|
|                       |     |         | All-cause mortality | Cancer mortality |
|                       | N   | Deaths  | Model 1 | Model 2 |
|<25 Never <4.0            | 132 | 6       | 0.89   | 0.36 |
|<25 Never ≥4.0 to ≤10.0   | 2433| 89      | Referent| Referent|
|<25 Never >10.0           | 50  | 4       | 2.13   | 0.78 |
|<25 Ever <4.0             | 125 | 9       | 1.45   | 0.73 |
|<25 Ever ≥4.0 to ≤10.0    | 3971| 228    | 1.30   | 0.65 |
|<25 Ever >10.0            | 311 | 30      | 2.39   | 1.10 |
|≥25 Never <4.0            | 90  | 6       | 1.24   | 0.54 |
|≥25 Never ≥4.0 to ≤10.0   | 2711| 187    | 1.23   | 0.63 |
|≥25 Never >10.0           | 633 | 5       | 1.54   | 0.63 |
|≥25 Ever <4.0             | 125 | 9       | 1.45   | 0.73 |
|≥25 Ever ≥4.0 to ≤10.0    | 4111| 317    | 1.37   | 0.65 |
|≥25 Ever >10.0            | 352 | 53      | 4.71   | 1.33 |

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. 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. 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. Recent studies have observed increased WCC among subjects with overweight or obesity suggesting that leucocytes are involved in the development of obesity-related comorbidities. Stratiﬁcation analysis by smoking status and joint effect analysis by smoking and BMI status showed apparent evidence for effect moderation by smoking and BMI. More 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/m².

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. 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 while among elderly subjects, monocyte counts were associated with an increased risk of cardiovascular and cancer-related mortality. 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 tumourogenesis by damaging speciﬁc tissues, and neutrophils, monocytes and eosinophils are crucial components of this process. 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
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 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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Contributors

YL and IA are the correspondence authors with equal contribution. YL is acting as the submission’s guarantor. YL, IA and BB-d-M are responsible for the integrity of the work as a whole, from inception to publishing article. BB-d-M proposed the conception of the study, designed the study based on the Dutch European Prospective Investigation into Cancer and Nutrition (EPIC-NL) cohort. YL and IA performed the data analysis and drafted the manuscript; Ci participated in the data analysis, results interpretation and manuscript writing; MV, YvdS and BB-d-M are the key persons for the EPIC-NL cohort. They also contributed to conception of the study, data analysis, results interpretation and manuscript writing.

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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).

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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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