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Active Smoking and Hematocrit and Fasting Circulating Erythropoietin Concentrations in the General Population

Michele F. Eisenga, MD; Lyanne M. Kieneker, MSc; Daan J. Touw, PharmD, PhD; Ilja M. Nolte, PhD; Peter van der Meer, MD, PhD; Gerwin Huls, MD, PhD; Carlo A.J.M. Gaillard, MD, PhD; and Stephan J.L. Bakker, MD, PhD

Abstract

Cigarette smoking continues to be one of the major risk factors for increased morbidity and mortality worldwide. Among many adverse health effects, smoking can induce erythrocytosis, which is commonly believed to result from elevated serum erythropoietin (EPO) levels. Currently, however, this notion is only alleged, without data available to substantiate it. Hence, we analyzed data from the Prevention of Renal and Vascular End-Stage Disease study, a prospective population-based cohort study. Smoking behavior was quantified as number of cigarettes smoked per day and as 24-hour urinary cotinine excretion levels, an objective and quantitative measure of nicotine exposure. In 6808 community-dwelling participants, the prevalence of nonsmokers, former smokers, and current smokers were 29%, 43%, and 28%, respectively. Hematocrit levels were higher in current smokers (41.4%±3.6%) than in nonsmokers (40.3%±3.6%) (P<.001). In contrast, median EPO levels were lower in current smokers (7.5 IU/L; interquartile range [IQR], 5.7-9.6 IU/L) than in nonsmokers (7.9 IU/L; IQR, 6.0-10.7 IU/L) (P<.001). In multivariate linear regression analysis, current smoking, compared with nonsmoking, was independently positively associated with hematocrit levels (β=.12; P<.001) and hemoglobin levels (β=.11; P<.001), but inversely associated with EPO levels (β=−.09; P<.001). In sensitivity analyses, we observed a dose-dependent inverse association of smoking exposure reflected by 24-hour urinary cotinine excretion levels with EPO levels. Contrary to common belief, we identified that in the general population, smoking is inversely associated with EPO levels. Future mechanistic insight is needed to unravel the currently identified association, and if reproduced in other studies, guidelines for diagnosis of secondary erythrocytosis may need to be revisited.

Cigarette smoking is one of the major public health concerns worldwide. Although efforts for tobacco control have led to reduced tobacco consumption in developed countries, global tobacco use continues to substantially augment.1 Smokers have an increased risk of malignant neoplasms, atherosclerosis, cardiovascular disease, and a plethora of other diseases including chronic obstructive pulmonary disease and gastrointestinal disorders.2-4 It has been postulated that the detrimental effects of cigarette smoking are caused by increased oxidative stress, free radicals, and by alterations in blood rheology.5,6 Previously, multiple studies have reported that smoking leads to higher hematocrit and hemoglobin levels.7 Currently, it is common belief and even mentioned in textbooks that erythrocytosis associated with smoking is due to increased circulating erythropoietin (EPO) concentrations.8,9 These would arise as a result of tissue hypoxia under the influence of continuous exposure to carbon monoxide in tobacco smoke. The increased circulating EPO concentrations will stimulate erythropoiesis and lead to an increased red cell volume. In fact, for the diagnostic work-up of erythrocytosis, it is recommended to measure serum EPO concentrations because they may differentiate between secondary erythrocytosis (eg, owing to carbon monoxide exposure), in which the EPO concentration will be high, and primary erythrocytosis (ie, polycythemia vera), in which the EPO
concentration will be suppressed. This suppression would be a compensatory response to constitutively increased EPO signaling, resulting from JAK2 V617F exon 14 sequence variations—present in at least 90% of cases—and JAK2 exon 12 sequence variations. Strikingly, there are no data available to support the alleged increase in circulating EPO concentrations in response to smoking. In fact, a study performed in the 1990s describes an inverse association between smoking and circulating EPO concentrations, but this study is not mentioned in guidelines. For diagnostic purposes and to unravel the pathophysiologic mechanisms, it is necessary to determine the role of EPO in smoking-induced erythrocytosis.

In the present study, we aimed to investigate the effect of smoking on hematocrit and EPO concentrations in a large population-based cohort.

PATIENTS AND METHODS
We analyzed data from the Prevention of Renal and Vascular End-Stage Disease study, a prospective population-based cohort study of Dutch men and women aged 28 to 75 years. In total, 8592 participants constitute the Prevention of Renal and Vascular End-Stage Disease study sample at baseline. For the present analysis, we used data from the second survey (n=6894) and excluded missing data on smoking behavior (n=86), resulting in 6808 participants eligible for analysis. The study has been approved by the medical ethics committee of the University Medical Center Groningen, and written informed consent was obtained from all participants. All participants completed a self-administered questionnaire regarding demographic characteristics, cardiovascular and renal disease history, smoking habits, alcohol consumption, and medication use. Smoking status was categorized as never, former, and current (<6, 6-20, or >20 cigarettes/d). Alcohol use was categorized as no alcohol use, 1 unit of alcohol per month to 1 unit per week, >1 unit per week to 7 units of alcohol per week, >1 unit per day to 3 units of alcohol per day, or >3 units of alcohol per day.

Venous blood samples were taken from participants between 08:00 and 10:00 AM after an overnight fast and 15 minutes of rest. Twenty-four-hour urinary cotinine levels were measured using the enzyme multiplied immunoassay technique on the Architect c8000 system (Abbott Laboratories). Serum EPO levels were measured using an immunoassay based on chemiluminescence (IMMULITE EPO assay). Renal function was determined by estimating GFR by using the Chronic Kidney Disease Epidemiology Collaboration equation. Erythrocytosis was defined as hemoglobin levels higher than 16.0 g/dL in women and higher than 16.5 g/dL in men (to convert to mol/L, multiply by 0.6206).

Data were analyzed using SPSS version 23.0 (IBM Corp.) and R version 3.2.3 (R Foundation for Statistical Computing). We evaluated between-group differences using a 1-way analysis of variance, Kruskal-Wallis test, or chi-square test, as appropriate. Hereafter, we performed linear regression analysis between smoking and outcomes with adjustment for the literature known potential confounders including age, sex, body mass index (BMI, calculated as the weight in kilograms divided by the height in meters squared), estimated glomerular filtration rate (eGFR), and high-sensitivity C-reactive protein (hs-CRP) levels. Furthermore, we specifically adjusted the association between smoking and mean corpuscular volume (MCV) for alcohol use, as a categorized variable, to account for potential confounding. We repeated the analyses for categories of number of cigarettes smoked per day and assessed by means of a dummy variable of smoking dose across the 3 categories of number of cigarettes smoked per day while concomitantly adjusting for current smoking, whether a dose-effect relationship exists between smoking and EPO levels. Logistic regression analysis, both univariate and multivariate, was performed to assess whether current smoking was a major determinant of erythrocytosis. In sensitivity analyses, we excluded all patients with a history of cardiovascular disease and renal insufficiency. Cardiovascular disease constituted the occurrence of cardiovascular heart disease or cerebrovascular accident, and renal insufficiency was defined as eGFR less than 60 mL/min per 1.73 m². Finally, because questionnaire data may be biased and the fact that the inverse association of EPO levels with smoking determined by the questionnaire was rather unexpected, we measured in all 24-hour urine samples urinary cotinine concentrations to provide an objective and quantitative measure of nicotine exposure. Therefore, to exclude possible
misclassification or under- or overestimation of number of cigarettes smoked per day as determined by the questionnaire, we repeated sensitivity analyses as the analyses with 24-hour urinary cotinine levels.

RESULTS

Demographic and clinical characteristics of the included 6808 participants are summarized in Table 1, according to nonsmokers, former smokers, and current smokers, with the last group subdivided into 3 subgroups of number of cigarettes smoked per day. Of the 6808 participants, 1969 (29%) were nonsmokers, 2922 (43%) were former smokers, and 1917 (28%) were current smokers. Among the last group, 307 (16%) smoked less than 6 cigarettes/d, 1346 (70%) smoked 6 to 20 cigarettes/d, and 264 (14%) smoked more than 20 cigarettes/d. Hematocrit levels were higher in current smokers (41.4%±3.6%) than in nonsmokers (40.3%±3.6%) (P<.001). Erythrocytosis was present in 69 (4%) of current smokers compared with 28 (1%) of nonsmokers. Median EPO levels were lower in current smokers (7.5 IU/L; IQR, 5.7-9.6 IU/L) than in nonsmokers (7.9 IU/L; IQR, 6.0-10.7 IU/L) (P<.001). The EPO index, which constitutes the ratio of EPO levels to hemoglobin levels, was significantly lower in current smokers (0.85; 95% CI, 0.82-0.88) than in nonsmokers (0.93; 95% CI, 0.91-0.95) (P<.001).

In univariate linear regression analysis, current smoking, compared with nonsmoking, was positively associated with hematocrit levels (β=.13; 95% CI, .12-.17; P<.001), hemoglobin levels (β=.13; 95% CI, .11-.16; P<.001), and MCV (β=.30; 95% CI, .27-.33; P<.001) and inversely associated with EPO.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Nonsmokers (n=1969)</th>
<th>Former smokers (n=2922)</th>
<th>&lt;6 cigarettes/d (n=307)</th>
<th>6-20 cigarettes/d (n=1346)</th>
<th>&gt;20 cigarettes/d (n=264)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPO level (IU/L)</td>
<td>7.9 (6.0-10.7)</td>
<td>7.9 (6.0-10.4)</td>
<td>7.7 (5.7-9.8)</td>
<td>7.3 (5.5-9.4)</td>
<td>7.5 (5.4-9.9)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>General characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>52±12</td>
<td>57±12</td>
<td>52±12</td>
<td>52±11</td>
<td>50±8</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Male sex</td>
<td>927 (47)</td>
<td>1604 (55)</td>
<td>132 (44)</td>
<td>670 (50)</td>
<td>131 (50)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.4±4.1</td>
<td>27.4±4.4</td>
<td>26.1±4.7</td>
<td>25.8±4.0</td>
<td>26.5±4.5</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>eGFR* (mL/min per 1.73 m²)</td>
<td>88.0±16.4</td>
<td>83.4±17.0</td>
<td>86.4±18.3</td>
<td>85.4±15.6</td>
<td>89.7±14.1</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>No alcohol use</td>
<td>628 (32)</td>
<td>647 (22)</td>
<td>64 (21)</td>
<td>337 (25)</td>
<td>65 (25)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>1-4 units/mo</td>
<td>429 (22)</td>
<td>460 (17)</td>
<td>56 (18)</td>
<td>197 (15)</td>
<td>16 (6)</td>
<td></td>
</tr>
<tr>
<td>1-7 units/wk</td>
<td>622 (22)</td>
<td>920 (32)</td>
<td>107 (35)</td>
<td>412 (31)</td>
<td>66 (25)</td>
<td></td>
</tr>
<tr>
<td>&gt;1-3 units/d</td>
<td>261 (13)</td>
<td>787 (27)</td>
<td>70 (23)</td>
<td>313 (23)</td>
<td>63 (24)</td>
<td></td>
</tr>
<tr>
<td>&gt;3 units/d</td>
<td>29 (2)</td>
<td>108 (4)</td>
<td>10 (3)</td>
<td>87 (7)</td>
<td>54 (21)</td>
<td></td>
</tr>
<tr>
<td>Laboratory parameters</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin level (g/dL)</td>
<td>13.6±1.3</td>
<td>13.7±1.2</td>
<td>13.5±1.3</td>
<td>14.0±1.2</td>
<td>14.2±1.2</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Hematocrit level (%)</td>
<td>40.3±3.6</td>
<td>40.7±3.6</td>
<td>40.4±3.8</td>
<td>41.6±3.5</td>
<td>42.1±3.5</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Erythrocytosis†</td>
<td>28 (1)</td>
<td>52 (2)</td>
<td>6 (2)</td>
<td>45 (3)</td>
<td>18 (7)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>89±4</td>
<td>90±4</td>
<td>91±5</td>
<td>92±4</td>
<td>94±5</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Ferritin level (µg/L)</td>
<td>87 (91-161)</td>
<td>105 (83-189)</td>
<td>83 (37-156)</td>
<td>95 (49-164)</td>
<td>101 (54-181)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>hs-CRP level (mg/L)</td>
<td>1.1 (0.5-2.6)</td>
<td>1.4 (0.7-3.0)</td>
<td>1.1 (0.5-3.1)</td>
<td>1.7 (0.8-3.7)</td>
<td>2.5 (1.1-4.6)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

*BMI = body mass index; eGFR = estimated glomerular filtration rate; EPO = erythropoietin; hs-CRP = high-sensitivity C-reactive protein; MCV = mean corpuscular volume.

† Conversion factors: to convert g/dL values to mol/L, multiply by 0.6206; to convert µg/dL values to µmol/L, multiply by 2.247; and to convert mg/dL values to mmol/L, multiply by 0.0259.

*Data are presented as mean ± SD, as median (interquartile range), or as No. (percentage).

P values represent the significance across the different smoking categories. P values were determined using a 1-way analysis of variance for normally distributed data, Kruskal-Wallis test for skewed distributed data, and χ² test for categorical data.

*eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation.

†Erythrocytosis has been defined as hemoglobin levels >16.0 g/dL in women and >16.5 g/dL in men.
Table 2. Association of Smoking and Number of Cigarettes Smoked per Day With Hematocrit Levels, Hemoglobin Levels, MCV, and EPO Levels

<table>
<thead>
<tr>
<th>Variable</th>
<th>Ht levels</th>
<th>Hb levels</th>
<th>MCV</th>
<th>EPO levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current smoking</td>
<td>.12 (.09 to .14)**</td>
<td>.11 (.08 to .13)**</td>
<td>.29 (.25 to .32)**</td>
<td>−.09 (−.12 to −.05)**</td>
</tr>
<tr>
<td>&lt;6 cigarettes/d</td>
<td>−.003 (−.03 to .02)</td>
<td>−.01 (−.03 to .01)</td>
<td>.04 (.01 to .07)**</td>
<td>−.02 (−.05 to .01)</td>
</tr>
<tr>
<td>6-20 cigarettes/d</td>
<td>.11 (.08 to .13)**</td>
<td>.10 (.07 to .12)**</td>
<td>.26 (2.2 to .28)**</td>
<td>−.07 (−.10 to .04)**</td>
</tr>
<tr>
<td>&gt;20 cigarettes/d</td>
<td>.10 (.07 to .12)**</td>
<td>.10 (.07 to .12)**</td>
<td>.20 (.17 to .23)**</td>
<td>−.04 (−.07 to −.01)**</td>
</tr>
</tbody>
</table>

*EPO = erythropoietin; Hb = hemoglobin; Ht = hematocrit; MCV = mean corpuscular volume.

†Data are presented as β (95% CI).

As compared with the nonsmokers group. Standardized β coefficients with 95% CIs are shown after adjustment for age, sex, estimated glomerular filtration rate, body mass index, and high-sensitivity C-reactive protein levels. ***P<.001; **P<.01; *P<.05.

levels (β=−.07; 95% CI, −.10 to −.05; P<.001). In multivariate linear regression analysis, current smoking, compared with nonsmoking, remained positively associated with hematocrit levels (β=12; 95% CI, .09-.14; P<.001), hemoglobin levels (β=.11; 95% CI, .08-.13; P<.001), and MCV (β=.29; 95% CI, .25-.32; P<.001) and inversely associated with EPO levels (β=−.09; 95% CI, −.12 to −.05; P<.001), independent of adjustment for age, sex, BMI, eGFR, and hs-CRP levels. The association of current smoking with MCV remained materially unchanged (β=.25; 95% CI, .22-.28; P<.001) after further adjustment for categories of alcohol use.

Hereafter, we divided current smoking into fewer cigarettes smoked per day. In multivariable linear regression analysis, smoking less than 6 cigarettes/d, compared with nonsmoking, was not associated with hematocrit, hemoglobin, or EPO levels, but was associated with MCV, as shown in Table 2 (also see the Supplemental Table, available online at http://www.mayoclinicproceedings.org). Both smoking 6 to 20 cigarettes/d and smoking more than 20 cigarettes/d were positively associated with hematocrit levels, hemoglobin levels, and MCV and inversely associated with EPO levels; however, we did not observe a dose-effect relationship (P=.50).

In participants with erythrocytosis, median EPO levels (7.2 IU/L; IQR, 5.1-9.8 IU/L) were lower than those in participants without erythrocytosis (7.8 IU/L; IQR, 5.1-9.8 IU/L). In participants with erythrocytosis, EPO levels were lower in current smokers (6.7 IU/L; IQR, 4.8-8.9 IU/L) than in nonsmokers (7.6 IU/L; IQR, 4.6-10.6 IU/L) (P<.001). In univariate logistic regression analysis, current smoking was a major determinant of erythrocytosis (odds ratio, 2.26; 95% CI, 1.63-3.13; P<.001). After adjustment for age, sex, eGFR, BMI, and hs-CRP levels, current smoking remained a major determinant of erythrocytosis (odds ratio, 2.48; 95% CI, 1.61-3.84; P<.001).

In sensitivity analyses, after exclusion of patients with a history of cardiovascular disease or renal insufficiency (n=728), current smoking, compared with nonsmoking, remained independently associated with hematocrit levels (β=.13; 95% CI, .10-.16; P<.001), hemoglobin levels (β=.12; 95% CI, .09-.15; P<.001), and MCV (β=.29; 95% CI, .25-.31; P<.001) and inversely associated with EPO levels (β=−.07; 95% CI, −.10 to −.04; P<.001). Furthermore, in sensitivity analyses, we identified that current smoking status was strongly associated with 24-hour urinary cotinine excretion levels (β=.82; 95% CI, .81-.83; P<.001). Similar to primary analyses, we identified positive relationships between 24-hour urinary cotinine excretion levels and hematocrit levels (β=.13; 95% CI, .10-.15) (Figure A), hemoglobin levels (β=.12; 95% CI, .09-.15) (Figure B), and MCV (β=.26; 95% CI, .23-.28) (Figure C). Furthermore, we observed an inverse association between 24-hour urinary cotinine levels and EPO levels (β=−.07; 95% CI, −.10 to −.04) (Figure D). In multivariate linear regression analysis, 24-hour urinary cotinine levels remained a major determinant of hematocrit levels (β=.15; 95% CI, .12-.17; P<.001), hemoglobin levels (β=.14; 95% CI, .12-.16; P<.001), MCV (β=.26; 95% CI, .22-.28; P<.001), and EPO levels (β=−.07; 95% CI, −.10 to −.04; P<.001), independent of adjustment for potential confounders (Figure E).
DISCUSSION

In the present study, we confirm that smoking, defined as current smoking and by 24-hour urinary cotinine levels, is positively associated with hematocrit levels, hemoglobin levels, and MCV. Strikingly, contrary to common belief, our data indicate that secondary erythrocytosis that ensues from smoking is not associated with up-regulated EPO levels.

Previous studies have extensively reported that cigarette smoking leads to elevated hematocrit and hemoglobin levels.19,20 Similarly, it has previously been established that smoking leads to increased MCV through a hitherto unidentified mechanism, independent of alcohol use.21,22

To date, it is allegedly assumed that secondary erythrocytosis associated with smoking occurs owing to tissue hypoxia, which consequently increases secretion of EPO and augments erythropoiesis in an attempt to increase oxygen delivery. Indeed, it has been documented that circulating EPO concentrations increase in response to phlebotomy.23 In the present study, we identified that smoking is associated with lower rather than higher EPO levels. This is in keeping with a previous report of Tanabe et al,14 which reported substantially lower EPO levels in smokers than in nonsmokers assessed by the questionnaire. As potential mechanism for the currently found results, we hypothesize that smokers will have high EPO levels in the course of the day, leading to erythrocytosis, which, through a negative feedback loop, will inhibit EPO production at night during smoking cessation. With a reported half-life of endogenous circulating EPO in the order of 6 to 8 hours, this could then
result in low EPO levels in the morning when blood samples are drawn. Wide et al.24 have indeed described a circadian rhythm of serum EPO in hospitalized patients, with the lowest levels measured in the morning. It is not known whether this circadian rhythm is more pronounced in participants who smoke.

An alternative hypothesis might be as suggested by Weinberg et al.23 that smokers have a higher incidence of JAK2 V617F sequence variation, implicating that erythropoiesis observed with smoking is due to erythroid cell-intrinsic EPO-independent mechanism attributable to constitutively activated EPO receptor signaling. Finally, there might be a hitherto unidentified direct effect of smoking on erythropoiesis.

Limitations of this study are the observational design and the fact that there may be residual confounding despite the factors for which we adjusted. Although serum EPO concentrations were assessed from blood samples taken in the fasting state in the morning, we have no data on the exact time of blood sampling, precluding us from investigating whether controlling for time of collection would have an effect on the association of smoking status with circulating EPO concentrations. The major strengths of the present study are the large patient population and the fact that as one of the first large studies, it measured smoking behavior by sensitivity analyses using reliable 24-hour urinary cotinine excretion levels next to smoking behavior gathered by the questionnaire.

CONCLUSION
We identified an inverse association between smoking and EPO levels, contrary to common belief that smoking as the most important cause of secondary erythrocytosis presents with elevated EPO levels. The present study might draw more attention to the mechanism by which smoking causes erythrocytosis despite lower EPO levels. Future studies might want to consider measuring serum EPO levels at various times during the day to see whether a variation in EPO levels exists in smokers.

SUPPLEMENTAL ONLINE MATERIAL
Supplemental material can be found online at: http://www.mayoclinicproceedings.org. Supplemental material attached to journal articles has not been edited, and the authors take responsibility for the accuracy of all data.

Abbreviations and Acronyms: BMI = body mass index; eGFR = estimated glomerular filtration rate; EPO = erythropoietin; hs-CRP = high-sensitivity C-reactive protein; IQR = interquartile range; MCV = mean corpuscular volume

Potential Competing Interests: The authors report no competing interests.

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REFERENCES


