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

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ABSTRACT

Cigarette smoking continues to be one of the major risk factors for increased morbidity and mortality worldwide. Among many adverse health effects, it has long been established that smoking can induce an 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 (PREVEND) study, a prospective, population-based cohort. Smoking behavior was quantified as number of cigarettes smoked per day and as 24-hour urinary cotinine excretion, an objective and quantitative measure of nicotine exposure. In 6808 community-dwelling subjects, prevalence of non-smokers, former smokers, and current smokers were 29%, 43%, and 28%, respectively. Hematocrit levels were higher in current smokers (41.4±3.6%) compared to non-smokers (40.3±3.6%; *P*<.001). In contrast, median EPO levels were lower in current smokers (7.5 [Interquartile range (IQR) 5.7-9.6] IU/L) compared to non-smokers (7.9 [6.0-10.7] IU/L; *P*<.001). In multivariable linear regression, current smoking, compared to non-smoking, was independently positively associated with hematocrit (β=0.12, *P*<.001) and hemoglobin (β=0.11, *P*<.001), but inversely associated with EPO (β=−0.09, *P*<.001).

In sensitivity analyses, we observed dose-dependent inverse association of smoking exposure reflected by 24-hour urinary cotinine excretion 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.
INTRODUCTION

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. 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. 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. Previously, multiple studies have shown that smoking leads to higher hematocrit and hemoglobin levels. Currently, it is common belief and even mentioned in textbooks that the erythrocytosis associated with smoking is due to increased circulating erythropoietin (EPO) concentrations. These would arise as a result of tissue hypoxia under 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 workup of erythrocytosis, it is recommended to measure serum EPO concentrations, because they may differentiate between secondary erythrocytosis (e.g. due to carbon monoxide exposure), in which the EPO concentration will be high, while in primary erythrocytosis (i.e. polycythemia vera), 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 90s 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 current study, we aimed to investigate the effect of smoking on hematocrit and EPO concentrations in a large population-based cohort.

METHODS

We analyzed data from the Prevention of Renal and Vascular End-Stage Disease (PREVEND) study, a prospective, population-based cohort of Dutch men and women aged 28-75 years. In total, 8,592 participants constitute the PREVEND study at baseline. For current analysis, we used data from the second survey (n = 6,894) and excluded missing
data on smoking behavior (n=86), resulting in 6,808 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 demographics, 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/day). 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 h in the morning after an overnight fast and 15 min of rest. Twenty-four hour urinary cotinine levels were measured with Enzyme Multiplied Immunoassay Technique on the Abbott Architect c8000 system (Abbott Laboratories, Abbott Park, IL). Serum EPO levels were measured using an immunoassay based on chemiluminescence (Immulite EPO assay, Los Angeles, CA). Renal function was determined by estimating GFR by applying the Chronic Kidney Disease Epidemiology Collaboration equation. Erythrocytosis was defined as hemoglobin level higher than 16.0 g/dL for women, and higher than 16.5 g/dL for men.

Data were analyzed using IBM SPSS software, version 23.0 (SPSS Inc., Chicago, IL) and R version 3.2.3 (Vienna, Austria). We evaluated between-group differences using one way ANOVA, Kruskal-Wallis test, or Chi-square test, as appropriate. Hereafter, we performed linear regression analysis between smoking and outcomes with adjustment for in literature known potential confounders including age, sex, body mass index (BMI), estimated glomerular filtration rate (eGFR), and high-sensitivity C-reactive protein (hs-CRP) levels. Further, we specifically adjusted the association between smoking and MCV for alcohol use, as 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 numbers 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 multivariable, was performed to assess whether current smoking was a major determinant of erythrocytosis. As sensitivity analyses, we excluded all patients with history of cardiovascular disease and renal insufficiency. Cardiovascular disease constituted occurrence of cardiovascular heart disease or cerebrovascular accident and renal insufficiency was defined as an eGFR <60 ml/min/1.73m². Finally, because questionnaire data may be biased, and that the inverse association of EPO with smoking determined by 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 misclassifica-
tion or under- or overestimation of number of cigarettes smoked per day as determined by questionnaire, we repeated as sensitivity analyses the analyses with 24-hour urinary cotinine levels.

RESULTS

Demographics and clinical characteristics of included 6808 subjects, according to non-smokers, former smokers, and current smokers, with the latter subdivided in three groups of number of cigarettes smoked per day are shown in Table 1. Of the 6808 participants, 1969 (29%) were non-smokers, 2922 (43%) were former smokers, and 1917 (28%) were current smokers. Of the latter, 307 (16%) smoked less than 6 cigarettes per day, 1346 (70%) smoked 6-20 cigarettes per day, and 264 (14%) smoked more than 20 cigarettes per day. Hematocrit was higher in current smokers (41.4±3.6%) compared to non-smokers (40.3±3.6%; P<.001). Erythrocytosis was present in 69 (4%) of current smokers compared to 28 (1%) of non-smokers. Median EPO levels were lower in current smokers (7.5 [Interquartile range (IQR) 5.7-9.6] IU/L) compared to non-smokers (7.9 [6.0-10.7] IU/L; P<.001). The EPO index, which constitutes the ratio of EPO versus hemoglobin, was also significantly lower in current smokers (0.85 [0.63-1.13]) compared to non-smokers (0.93 [0.70-1.28]; P<.001).

In univariate linear regression, current smoking, compared to non-smoking, was positively associated with hematocrit (β=0.15 (95% CI 0.12; 0.17); P<.001), hemoglobin (β=0.13 (0.11; 0.16), P<.001), and MCV (β=0.30 (0.27; 0.33), P<.001), and inversely associated with EPO (β=-0.07 (-0.10; -0.05); P<.001). In multivariable linear regression, current smoking, compared to non-smoking, remained positively associated with hematocrit (β=0.12 (0.09; 0.14) P<.001), hemoglobin (β=0.11 (0.08; 0.13), P<.001), and MCV (β=0.29 (0.25; 0.32), P<.001), and inversely associated with EPO (β=-0.09 (-0.12; -0.05), 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=0.25 (0.22; 0.28), P<.001), after further adjustment for categories of alcohol use.

Hereafter, we divided current smoking in number of cigarettes smoked per day. Smoking less than 6 cigarettes per day, compared to non-smoking, was in multivariable linear regression not associated with hematocrit, hemoglobin, or EPO, but was associated with MCV, as shown in Table 2. Both smoking 6 to 20 cigarettes and smoking more than 20 cigarettes per day were positively associated with hematocrit, hemoglobin, MCV, and inversely with EPO, however we did not observe a dose-effect relationship (P=0.50).

In participants with erythrocytosis, median EPO levels (7.2 (5.1-9.8) IU/L) were lower as compared to participants without erythrocytosis (7.8 (5.1-9.8) IU/L). In participants
**Table 1.** Baseline characteristics according to non-smokers, former smokers, and smokers, with the latter subdivided in amount of cigarettes per day

<table>
<thead>
<tr>
<th>Variables</th>
<th>Non-smokers (n=1969)</th>
<th>Former smokers (n=2922)</th>
<th>Current smokers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>&lt;6 cigarettes/day (n=307)</td>
<td>6-20 cigarettes/day (n=1346)</td>
</tr>
<tr>
<td><strong>EPO (IU/L)</strong></td>
<td>7.9 (6.0-10.7)</td>
<td>7.7 (5.7-9.8)</td>
<td>7.3 (5.5-9.4)</td>
</tr>
<tr>
<td><strong>General characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>52±12</td>
<td>57±12</td>
<td>52±12</td>
</tr>
<tr>
<td>Male sex (n, %)</td>
<td>827 (43)</td>
<td>1604 (55)</td>
<td>132 (44)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.6±4.4</td>
<td>27.4±4.4</td>
<td>26.1±4.7</td>
</tr>
<tr>
<td>eGFRb (ml/min/1.73m²)</td>
<td>88.0±16.4</td>
<td>83.4±17.0</td>
<td>86.4±18.3</td>
</tr>
<tr>
<td>No alcohol use (n, %)</td>
<td>628 (32)</td>
<td>647 (22)</td>
<td>64 (21)</td>
</tr>
<tr>
<td>1-4 units/month (n, %)</td>
<td>429 (22)</td>
<td>460 (16)</td>
<td>56 (18)</td>
</tr>
<tr>
<td>1-7 units/week (n, %)</td>
<td>622 (22)</td>
<td>920 (32)</td>
<td>107 (35)</td>
</tr>
<tr>
<td>≥1-3 units/day (n, %)</td>
<td>261 (13)</td>
<td>787 (27)</td>
<td>70 (23)</td>
</tr>
<tr>
<td>&gt;3 units/day (n, %)</td>
<td>29 (2)</td>
<td>108 (4)</td>
<td>10 (3)</td>
</tr>
<tr>
<td><strong>Laboratory parameters</strong></td>
<td></td>
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<tr>
<td>Hemoglobin (g/dL)</td>
<td>13.6±1.3</td>
<td>13.7±1.2</td>
<td>13.5±1.3</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>40.3±3.6</td>
<td>40.7±3.6</td>
<td>40.4±3.8</td>
</tr>
<tr>
<td>Erythrocytosisc (n, %)</td>
<td>28 (1)</td>
<td>52 (2)</td>
<td>6 (2)</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>89±4</td>
<td>90±4</td>
<td>91±5</td>
</tr>
<tr>
<td>Ferritin (µg/L)</td>
<td>87 (41-161)</td>
<td>105 (83-189)</td>
<td>83 (37-156)</td>
</tr>
<tr>
<td>hs-CRP (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>
</tr>
</tbody>
</table>

Data are presented as mean±standard deviation or as median with interquartile range. Abbreviations: BMI, body mass index; eGFR, estimated glomerular filtration rate; EPO, erythropoietin; hs-CRP, high-sensitivity C-reactive protein; MCV, mean corpuscular volume.

a P-values represent the significance across the different smoking categories. P values were determined with one way ANOVA for normally distributed data, Kruskal-Wallis test for skewed distributed data, and Chi-square test for categorical data.

b eGFR was calculated with the CKD-EPI equation

c 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 numbers of cigarettes smoked per day with hematocrit, hemoglobin, MCV, and EPO

<table>
<thead>
<tr>
<th></th>
<th>Ht</th>
<th>Hb</th>
<th>MCV</th>
<th>EPO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current smoking‡</td>
<td>0.12 (0.09; 0.14)***</td>
<td>0.11 (0.08; 0.13)***</td>
<td>0.29 (0.25; 0.32)***</td>
<td>-0.09 (-0.12; -0.05)***</td>
</tr>
<tr>
<td>&lt;6 cigarettes / day‡</td>
<td>-0.003 (-0.03; 0.02)</td>
<td>-0.01 (-0.03; 0.01)</td>
<td>0.04 (0.01; 0.07)***</td>
<td>-0.02 (-0.05; 0.01)</td>
</tr>
<tr>
<td>6-20 cigarettes / day‡</td>
<td>0.11 (0.08; 0.13)***</td>
<td>0.10 (0.07; 0.12)***</td>
<td>0.26 (0.22; 0.28)***</td>
<td>-0.07 (-0.10; 0.04)***</td>
</tr>
<tr>
<td>&gt;20 cigarettes/day‡</td>
<td>0.10 (0.07; 0.12)***</td>
<td>0.10 (0.07; 0.12)***</td>
<td>0.20 (0.17; 0.23)***</td>
<td>-0.04 (-0.07; -0.01)†</td>
</tr>
</tbody>
</table>

‡As compared to the non-smokers group. Standardized betas with 95% confidence interval are shown after adjustment for age, sex, eGFR, BMI, and hs-CRP levels. *P<.05, **P<.01, ***P<.001. Abbreviations: EPO, erythropoietin; Ht, hematocrit; Hb, hemoglobin; MCV, mean corpuscular volume
with erythrocytosis, EPO levels were lower in current smokers (6.7 [4.8-8.9] IU/L) than in non-smokers (7.6 [4.6-10.6] IU/L; P<.001). In univariate logistic regression analysis, current smoking was a major determinant of erythrocytosis (OR, 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 (OR, 2.48; 95%CI 1.61-3.84; P<.001).

As sensitivity analyses, after exclusion of patients with cardiovascular history or renal insufficiency (n=728), current smoking, compared to non-smoking, remained independently associated with hematocrit (β=0.13 (0.10; 0.16) P<.001), hemoglobin (β=0.12 (0.09; 0.15), P<.001), MCV (β=0.29 (0.25; 0.31), P<.001), and inversely associated with EPO (β=-0.07 (-0.10; -0.04), P<.001). Furthermore, as sensitivity analyses, we identified that current smoking status was highly associated with 24-h urinary cotinine

Figure 1. Effect of 24-hour urinary cotinine levels on hematocrit, hemoglobin, mean corpuscular volume, and erythropoietin.
Panel A, B, C, and D show the association between 24-hour urinary cotinine excretion levels and EPO, Hb, Ht, and MCV, respectively. Figures are restricted cubic splines with three knots specified at the 10th, 50th, and 90th Ln 24-hour urinary cotinine percentiles. The 95% confidence intervals are indicated by the shaded areas. Panel E shows the coefficients of 24-hour urinary cotinine levels in linear regression adjusted for age, sex, BMI, eGFR, and hs-CRP levels. Twenty-four urinary cotinine levels and EPO levels have been natural log transformed. Abbreviations: EPO = erythropoietin; Ht = hematocrit; MCV = mean corpuscular volume. *P<.05 **P<.01 ***P<.001;
excretion (β=0.82; 95%CI, 0.81-0.83; P<.001). Similar to primary analyses, we identified positive relationships between 24-h urinary cotinine excretion and hematocrit (β=0.13 (0.10; 0.15), Figure 1A), hemoglobin (β=0.12 (0.09; 0.15), Figure 1B), and MCV (β=0.26 (0.23; 0.28), Figure 1C). Furthermore, we observed an inverse association between 24-h urinary cotinine levels and EPO (β= -0.07 (-0.10; -0.04), Figure 1D). In multivariate linear regression analysis, 24-h urinary cotinine levels remained a main determinant of hematocrit (β=0.15 (0.12; 0.17), P<.001), hemoglobin (β=0.14 (0.12; 0.16), P<.001), MCV (β=0.26 (0.22; 0.28), P<.001), and EPO (β= -0.07 (-0.10; -0.04), P<.001), independent of adjustment for potential confounders (Figure 1E).

**DISCUSSION**

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

Previous studies have extensively shown 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 an hitherto unidentified mechanism, independent of alcohol use.\(^{21,22}\)

To date, it is allegedly assumed that secondary erythrocytosis associated with smoking, occurs due 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 current 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. which reported substantial lower EPO levels in smokers than nonsmokers assessed through questionnaire.\(^{14}\) 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. have indeed described a circadian rhythm of serum EPO in hospitalized patients, with the lowest levels measured in the morning.\(^{24}\) It is not known whether this circadian rhythm is more pronounced in smoking subjects.

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

Limitations of this study are the observational design and 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 impact on the association of smoking status with circulating EPO concentrations. The major strength of the current study is the large patient population and that as one of the first large studies smoking behavior was measured in sensitivity analyses by reliable 24-hour urinary cotinine excretion levels next to smoking behavior gathered by 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 current study might draw more attention to the mechanism by which smoking causes erythrocytosis despite lower EPO levels. Future studies might want to consider to measure serum EPO levels at various time moments during the day to see whether in smokers an variation in EPO levels exists.

**Acknowledgments**

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**Disclosures**

The authors have no disclosures to report.
REFERENCES


**Supplemental Table 1.** Association of smoking, and numbers of cigarettes smoked per day with hematocrit, hemoglobin, MCV, and EPO with unstandardized betas

<table>
<thead>
<tr>
<th></th>
<th>Ht (%)</th>
<th>Hb (g/dL)</th>
<th>MCV (fl)</th>
<th>EPO (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current smoking‡</td>
<td>0.9 (0.7; 1.1)***</td>
<td>0.29 (0.22; 0.36)***</td>
<td>2.93 (2.61; 3.30)***</td>
<td>-0.09 (-0.13; -0.06)***</td>
</tr>
<tr>
<td>&lt;6 cigarettes / day‡</td>
<td>0.0 (-0.4; 0.4)</td>
<td>-0.04 (-0.17; 0.09)</td>
<td>1.25 (0.66; 1.83)†</td>
<td>-0.06 (-0.13; 0.001)</td>
</tr>
<tr>
<td>6-20 cigarettes / day‡</td>
<td>1.1 (0.8; 1.3)***</td>
<td>0.33 (0.25; 0.40)***</td>
<td>3.08 (2.74; 3.43)***</td>
<td>-0.09 (-0.13; -0.06)***</td>
</tr>
<tr>
<td>&gt;20 cigarettes/day‡</td>
<td>1.6 (1.2; 2.1)***</td>
<td>0.56 (0.42; 0.70)***</td>
<td>4.48 (3.83; 5.14)***</td>
<td>-0.09 (-0.16; -0.02)†</td>
</tr>
</tbody>
</table>

‡As compared to the non-smokers group. Unstandardized betas with 95% confidence interval are shown after adjustment for age, sex, eGFR, BMI, and hs-CRP levels. Units in which the dependent variable is expressed are indicated in the heading. †P<.05, ‡P<.01, ***P<.001. Abbreviations: EPO, erythropoietin; Ht, hematocrit; Hb, hemoglobin; MCV, mean corpuscular volume.