MAYO CLINIC

BRIEF REPORT

# 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, 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 ($\beta=.12$; $P<.001$) and hemoglobin levels ($\beta=.11$; $P<.001$), but inversely associated with EPO levels ($\beta=-.09$; $P<.001$). 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.

 ■ Mayo Clin Proc. 2018;93(3):337-343

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

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concentration will be suppressed.[10,11] 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.[12,13] 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.[14] 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.[15] 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).[16] Renal function was determined by estimating GFR by using the Chronic Kidney Disease Epidemiology Collaboration equation.[17] *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).[18]

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.[16] Furthermore, we specifically adjusted the association between smoking and mean corpuscular volume (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 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 $m^2$. 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


Mayo Clin Proc. ■ March 2018;93(3):337-343 ■ https://doi.org/10.1016/j.mayocp.2018.01.005
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