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Active smoking and Macrocytosis in the General Population: Two Population-based Cohort Studies

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Macrocytosis, an elevated mean corpuscular volume (MCV) of erythrocytes, is a highly prevalent phenomenon in adult individuals [1]. MCV is the measurement of the average volume of red blood cells, and macrocytosis is defined as a MCV exceeding 100 fL. Currently, in textbooks and guidelines a myriad causes are being mentioned for macrocytosis, with vitamin B12 and folate deficiency, alcohol use, myeloid dysplastic syndromes, and liver disease as the most prominent ones [2]. In the seventies, a number of papers have reported a positive association between smoking and MCV [3, 4]. This has nowadays, however, not resulted in inclusion of cigarette smoking as an important cause of macrocytosis in textbooks and guidelines. Hence, in the current study, we aimed to investigate the association between smoking, assessed by both questionnaire and 24-hour urinary cotinine excretion, as objective measurement of nicotine exposure, with MCV in two large population-based cohorts.

First, we analyzed data from the Lifelines cohort study. Lifelines is a large multi-disciplinary prospective population-based cohort study which examines, in a unique three-generation design, the health and health-related behaviors of persons living in the north of The Netherlands. For the present study, we included 131,886 of the 167,729 subjects (aged 18-93 years) of whom hematology indices, drinking and smoking behavior were available. Second, 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. For current analyses, 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 analyses. Smoking status was categorized as never, former, and current (less than six, six to 20, or more than 20 cigarettes/day). To exclude possible misclassification or under- or overestimation of number of cigarettes smoked per day as determined by questionnaire, 24-hour urinary cotinine levels were measured. Alcohol use was categorized as no alcohol use, one unit of alcohol per month to one unit per week, more than one unit per week to seven units of alcohol per week, more
than one unit per day to three units of alcohol per day, or more than three units of alcohol per
day. Details of the Lifelines cohort and PREVEND study regarding clinical examination,
biochemical measurements, data description and statistical analyses are described in the
Supplemental Methods. Similarly, baseline demographics and clinical characteristics of the
included 131,886 community-dwelling participants and 6,808 PREVEND participants are
shown in Supplemental Table 1 and 2.

Of the 131,886 Lifelines participants (age 45±13 years, 40% males), 47% were non-
smokers, 33% were former smokers and 20% were current smokers. Of the current smokers,
28% smoked less than six cigarettes per day, 55% smoked six to 20 cigarettes per day and
18% smoked more than 20 cigarettes per day. Hemoglobin levels were higher in current
smokers (14.3±1.2 g/dL) compared to non-smokers (14.0±1.3 g/dL, \( P<0.001 \)). Similarly,
MCV levels were higher in current smokers (91.4±4.3 fl) compared to non-smokers
(89.2±4.0 fl, \( P<0.001 \), Figure 1A). Macrocytosis was present in 494 (1.9%) of current
smokers compared to 166 (0.3%) of non-smokers (\( P<0.001 \), Figure 1B).

In univariable linear regression analysis, current smoking, compared to non-smoking,
was positively associated with MCV (\( \beta=0.24, P<0.001 \)). In multivariable regression analysis,
performed in the whole cohort, current smoking compared to non-smoking, remained
positively associated with MCV (\( \beta=0.23, P<0.001 \)), independent of adjustment for age, sex,
eGFR, BMI, and alcohol use. Multivariable regression analysis was also performed in a
subgroup of participants from whom also GGT, ALAT, FT4 and hs-CRP were available
(N=36,109) with the same result (\( \beta=0.23, P<0.001 \)).

Similarly, in logistic regression, smoking was a strong determinant of macrocytosis
(OR 6.25, 95% CI 5.2–7.51; \( P<0.001 \) in the total cohort, OR 6.00, 95% CI 4.12–8.73;
\( P<0.001 \) in the subgroup of N=36,109), independent of adjustment for potential confounders.

Hereafter, we divided current smoking into categories of cigarettes smoked per day. In
multivariate analysis, all smoking categories (less than six cigarettes ($\beta=0.07, P<0.001$), six to 20 cigarettes ($\beta=0.22, P<0.001$) and more than 20 cigarettes ($\beta=0.19, P<0.001$)) were associated with MCV, independent of adjustment for potential confounders. The association remained the same after adjustment for GGT, ALAT, FT4 and hs-CRP (less than six cigarettes ($\beta=0.06, P<0.001$), six to 20 cigarettes ($\beta=0.22, P<0.001$) and more than 20 cigarettes ($\beta=0.21, P<0.001$)).

Of the 6,808 subjects (age 53±12 years, 50% males) in the PREVEND study, 29% were non-smokers, 43% were former smokers, and 28% were current smokers. Of the latter, 16% smoked less than six cigarettes per day, 70% smoked six to 20 cigarettes per day, and 14% smoked more than 20 cigarettes per day. Hemoglobin levels were higher in current smokers (13.9±1.2 g/dL) compared to non-smokers (13.6±1.3 g/dL, $P<0.001$). Similarly, MCV levels were higher in current smokers (92.3±4.7 fL) compared to non-smokers (89.2±4.3 fL, $P<0.001$, Figure 1C). Macrocytosis was present in 73 (4%) of current smokers compared to 8 (0.4%) of non-smokers ($P<0.001$).

In univariable linear regression analysis, current smoking, compared to non-smoking, was positively associated with MCV ($\beta=0.30, P<0.001$). In multivariable analysis, current smoking, compared to non-smoking, remained positively associated with MCV ($\beta=0.24, P<0.001$), independent of adjustment for age, sex, eGFR, BMI, hs-CRP, alcohol use, GGT, ALAT, FT4, vitamin B12, and folic acid. Similarly, in logistic regression, smoking was a strong determinant of macrocytosis (OR, 8.54, 95% CI 2.57–28.37; $P<0.001$), independent of adjustment for potential confounders.

Hereafter, we divided current smoking into categories of cigarettes smoked per day. In multivariate analysis, smoking less than six cigarettes ($\beta=0.03, P=0.06$), was not associated with MCV, whereas smoking six to 20 cigarettes ($\beta=0.24, P<0.001$), and smoking more than
20 cigarettes per day ($\beta=0.13$, $P<0.001$) remained, compared to non-smoking, associated with MCV, independent of adjustment for potential confounders.

As sensitivity analysis, we repeated in the PREVEND study the analysis with 24-hour urinary cotinine excretion levels as objective reflection of smoking. Twenty-four hour urinary cotinine excretion was strongly correlated with current smoking ($\beta=0.82$, $P<0.001$). Similar to the primary analysis, we identified a strong positive association between 24-hour urinary cotinine excretion and MCV ($\beta=0.26$, $P<0.001$, **Figure 1D**). The association remained independent of adjustment for potential confounders ($\beta=0.23$, $P<0.001$).

In this study, we have shown that smoking, assessed both by means of a self-administered questionnaire and by 24-hour urinary cotinine excretion levels, was strongly positively associated with MCV. Importantly, this association was independent of known causes of macrocytosis, including alcohol use. A few years ago, McNamee et al. and O’Reilly et al. reinvestigated the association between smoking as unrecognized cause of macrocytosis and showed that cigarette smoking was a significant risk factor for macrocytosis, independent of other known causes [5, 6]. Unfortunately, at present cigarette smoking is still not mentioned in textbooks and major guidelines, and clinicians are generally unaware of this association. The major drawback of the previously performed studies was that smoking status was assessed by means of a self-administered questionnaire, which might still be regarded as a subjective measurement of smoking status. In the current study, we underline the importance of this association, and we are the first to utilize an objective measurement, i.e. urinary cotinine excretion levels, for the current association. The latter combined with the large patient populations can be regarded also as the major strength of this study. Due to the observational design of the current study, we cannot discern potential mechanisms for the strong association between smoking and MCV. Finally, despite the extensive number of factors for which we adjust, residual confounding can still not be excluded.
In conclusion, smoking is an important determinant of MCV levels and macrocytosis, independent of prominent causes such as alcohol intake, liver disease, vitamin B12, and folic acid deficiency. Smoking should be included in current guidelines regarding known causes of an elevated MCV, and the current study might draw more attention to the mechanism by which smoking causes macrocytosis independent of alcohol intake.

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References


Contribution

Conflict-of-interest disclosures

None
Figure 1. Association of smoking and 24-hour urinary cotinine excretion levels with mean corpuscular volume and macrocytosis
Panel A shows the association between smoking status and MCV in the Lifelines cohort. Reported P-values are shown in respect to reference category of non-smokers. Panel B shows the prevalence of macrocytosis for each smoking status in the Lifelines cohort. Reported P-values are shown in respect to reference category of non-smokers. Panel C shows the association between smoking status and MCV in the PREVEND study. Reported P-values are shown in respect to reference category of non-smokers. Panel D shows the association between 24-hour urinary cotinine excretion levels and MCV by means of restricted cubic splines. Three knots have been specified at the 10th, 50th, and 90th of 24-hour urinary cotinine percentiles. The 95% confidence intervals are indicated by the shaded areas. Twenty-four urinary cotinine levels have been natural log transformed. Abbreviations: MCV, mean corpuscular volume; PREVEND, Prevention of Renal and Vascular End-Stage Disease.

* $P < 0.05$  ** $P < 0.01$  *** $P < 0.001$