

was excellent with a mean of 96.1% (range 95.2%-100%) of pills consumed over the 12-week treatment.

The results of this study add to the existing new evidence of the efficacy of DAAs in patients with inherited blood disorders including thalassemia. Table 1 summarizes the studies treating HCV in thalassaemia patients using DAAs.^{1,4-8} Importantly, the efficacy of these DAA regimens is not impacted by iron overload and is further supported by the high safety profile and lack of evidence of any significant drug-drug interaction. Early treatment of chronic HCV in patients with blood disorders should be considered in attempt to reduce liver-related morbidity and mortality.

ORCID

Ala I. Sharara  <https://orcid.org/0000-0003-0248-9527>

Ali Taher  <https://orcid.org/0000-0001-8515-2238>

Ala I. Sharara¹ 

Luma Basma O. Rustom¹

Majd Marrache¹

Hussein H. Rimmani¹

Halim Bou Daher¹

Suzanne Koussa²

Ali Taher³ 

¹Division of Gastroenterology, Department of Internal Medicine, American University of Beirut Medical Center, Beirut, Lebanon

²The Chronic Care Center, Hazmieh, Lebanon

³Division of Hematology/Oncology, Department of Internal Medicine, American University of Beirut Medical Center, Beirut, Lebanon

Correspondence

Ala I. Sharara, M.D., FACC, AGAF, FRCP, Professor, Division of Gastroenterology, American University of Beirut Medical Center, P.O. Box 11-0236/16-B, Beirut, Lebanon.

Email: ala.sharara@aub.edu.lb

REFERENCES

1. Mehta R, Kabrawala M, Nandwani S, et al. Safety and efficacy of Sofosbuvir and Daclatasvir for hepatitis C virus infection in patients with β -thalassaemia major. *J Clin Exp Hepatol*. 2018;8(1):3-6.
2. Inati A, Taher A, Ghorra S, et al. Efficacy and tolerability of peginterferon alpha-2a with or without ribavirin in thalassaemia major patients with chronic hepatitis C virus infection. *Br J Haematol*. 2005;130(4):644-646.
3. Di Marco V, Capra M, Gagliardotto F, et al. Liver disease in chelated transfusion-dependent thalassaemics: the role of iron overload and chronic hepatitis C. *Haematologica*. 2008;93(8):1243-1246.
4. Mangia A, Sarli R, Gamberini R, et al. Randomised clinical trial: sofosbuvir and ledipasvir in patients with transfusion-dependent thalassaemia and HCV genotype 1 or 4 infection. *Aliment Pharmacol Ther*. 2017;46(4):424-431.
5. Hézode C, Colombo M, Bourlière M, et al. Elbasvir/Grazoprevir for patients with hepatitis C virus infection and inherited blood disorders: a phase III study. *Hepatology*. 2017;66(3):736-745.
6. Sinakos E, Kountouras D, Koskinas J, et al. Treatment of chronic hepatitis C with direct-acting antivirals in patients with β -thalassaemia major and advanced liver disease. *Br J Haematol*. 2017;178(1):130-136.
7. Zamani F, Ajdarkosh H, Safarnezhad-Tameshkel F, et al. The effectiveness of sofosbuvir and daclatasvir in the treatment of hepatitis C in

thalassaemia major patients and their effect on haematological factors. *Indian J Med Microbiol*. 2018;36(2):224-229.

8. Origa R, Ponti ML, Filosa A, et al. Treatment of hepatitis C virus infection with direct-acting antiviral drugs is safe and effective in patients with hemoglobinopathies. *Am J Hematol*. 2017;92(12):1349-1355.

Received: 6 November 2018 | Accepted: 6 November 2018

DOI: 10.1002/ajh.25346

Active smoking and macrocytosis in the general population: Two population-based cohort studies

To the Editor:

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 70s, 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 2 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 3-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 ($n = 6894$) and excluded missing data on smoking behavior ($n = 86$), resulting in 6808 participants eligible for analyses. Smoking status was categorized as never, former, and current (<6, 6-20, or >20 cigarettes/d). To exclude possible misclassification or

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.
© 2018 The Authors. *American Journal of Hematology* published by Wiley Periodicals, Inc.

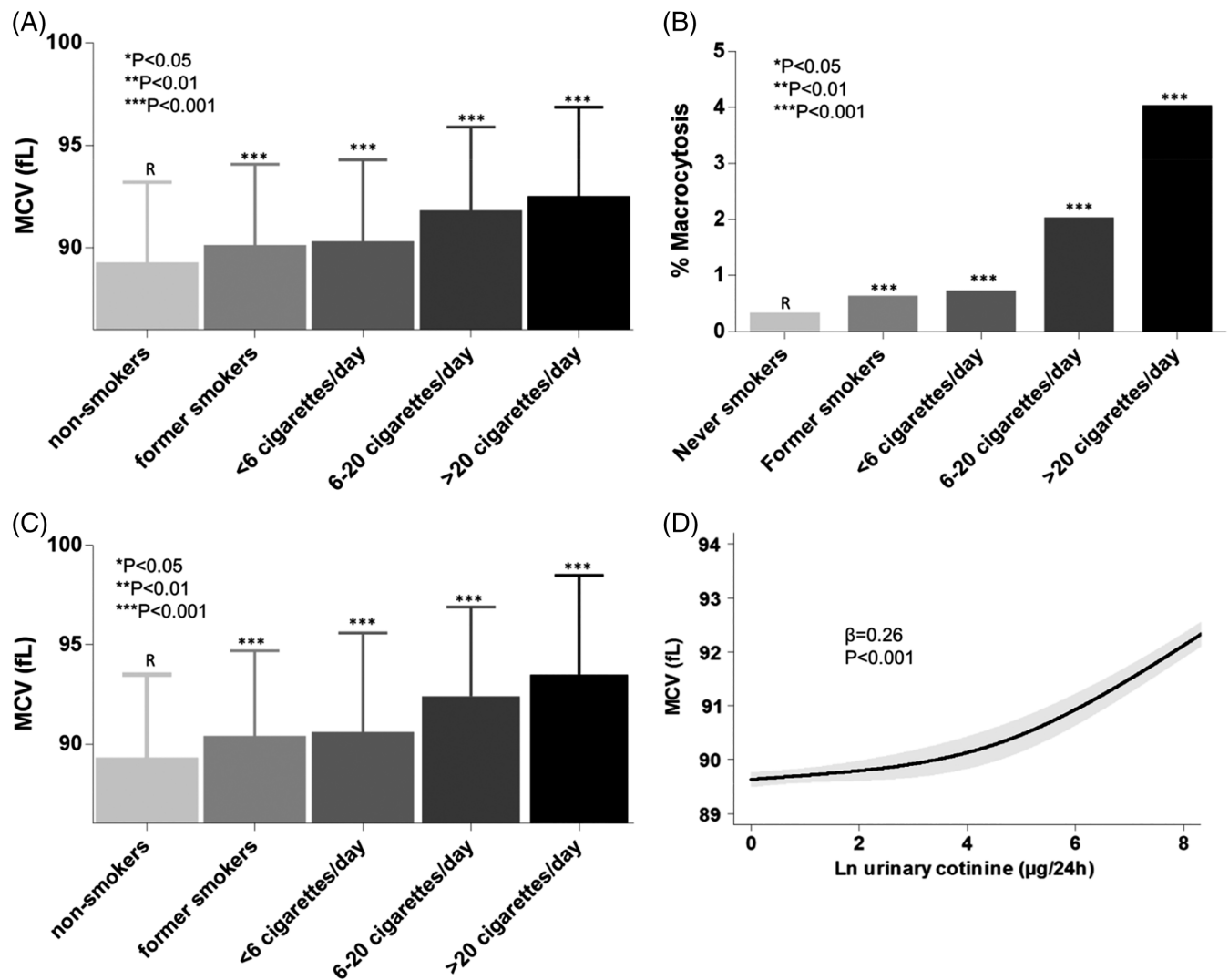


FIGURE 1 Association of smoking and 24-hour urinary cotinine excretion levels with mean corpuscular volume and macrocytosis. A, The association between smoking status and MCV in the lifelines cohort. Reported P-values are shown in respect to reference category of nonsmokers. B, The prevalence of macrocytosis for each smoking status in the lifelines cohort. Reported P-values are shown in respect to reference category of nonsmokers. C, The association between smoking status and MCV in the PREVEND study. Reported P-values are shown in respect to reference category of nonsmokers. D, 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% CIs 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. * $P < .05$ ** $P < .01$ *** $P < .001$

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, 1 U of alcohol per month to 1 U/wk, >1 U/wk to 7 U of alcohol per week, >1 U/d to 3 U of alcohol per day, or > 3 U 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 Supporting Information. Similarly, baseline demographics and clinical characteristics of the included 131 886 community-dwelling participants and 6808 PREVEND participants are shown in Supporting Information Tables S1 and S2.

Of the 131 886 Lifelines participants (age 45 ± 13 years, 40% males), 47% were nonsmokers, 33% were former smokers and 20% were current smokers. Of the current smokers, 28% smoked <6 cigarettes per day, 55% smoked 6-20 cigarettes per day and 18%

smoked > 20 cigarettes per day. Hemoglobin levels were higher in current smokers (14.3 ± 1.2 g/dL) compared with nonsmokers (14.0 ± 1.3 g/dL, $P < .001$). Similarly, MCV levels were higher in current smokers (91.4 ± 4.3 fL) compared with nonsmokers (89.2 ± 4.0 fL, $P < .001$, Figure 1A). Macrocytosis was present in 494 (1.9%) of current smokers compared with 166 (0.3%) of nonsmokers ($P < .001$, Figure 1B).

In univariable linear regression analysis, current smoking, compared with nonsmoking, was positively associated with MCV ($\beta = 0.24$, $P < .001$). In multivariable regression analysis, performed in the whole cohort, current smoking compared with nonsmoking, remained positively associated with MCV ($\beta = 0.23$, $P < .001$), independent of adjustment for age, sex, estimated glomerular filtration rate (eGFR), body mass index (BMI), and alcohol use. Multivariable regression analysis was also performed in a subgroup of participants from whom also gamma-

glutamyltransferase (GGT), alanine aminotransferase (ALAT), free thyroxine (FT4), and high-sensitivity C-reactive protein (hs-CRP) were available ($n = 36\ 109$) with the same result ($\beta = 0.23$, $P < .001$).

Similarly, in logistic regression, smoking was a strong determinant of macrocytosis (OR 6.25, 95% CI 5.2-7.51; $P < .001$ in the total cohort, OR 6.00, 95% CI 4.12-8.73; $P < .001$ 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 (<6 cigarettes [$\beta = 0.07$, $P < .01$], 6-20 cigarettes [$\beta = 0.22$, $P < .001$], and >20 cigarettes [$\beta = 0.19$, $P < .001$]) were associated with MCV, independent of adjustment for potential confounders. The association remained the same after adjustment for GGT, ALAT, FT4, and hs-CRP (<6 cigarettes [$\beta = 0.06$, $P < .001$], 6-20 cigarettes [$\beta = 0.22$, $P < .001$], and >20 cigarettes [$\beta = 0.21$, $P < .001$]).

Of the 6808 subjects (age 53 ± 12 years, 50% males) in the PRE-VEND study, 29% were nonsmokers, 43% were former smokers, and 28% were current smokers. Of the latter, 16% smoked <6 cigarettes per day, 70% smoked 6-20 cigarettes per day, and 14% smoked >20 cigarettes per day. Hemoglobin levels were higher in current smokers (13.9 ± 1.2 g/dL) compared with nonsmokers (13.6 ± 1.3 g/dL, $P < .001$). Similarly, MCV levels were higher in current smokers (92.3 ± 4.7 fL) compared with nonsmokers (89.2 ± 4.3 fL, $P < .001$, Figure 1C). Macrocytosis was present in 73 (4%) of current smokers compared with 8 (0.4%) of nonsmokers ($P < .001$).

In univariable linear regression analysis, current smoking, compared with nonsmoking, was positively associated with MCV ($\beta = 0.30$, $P < .001$). In multivariable analysis, current smoking, compared with nonsmoking, remained positively associated with MCV ($\beta = 0.24$, $P < .001$), 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; $P < .001$), independent of adjustment for potential confounders.

Hereafter, we divided current smoking into categories of cigarettes smoked per day. In multivariate analysis, smoking <6 cigarettes ($\beta = 0.03$, $P = .06$), was not associated with MCV, whereas smoking 6-20 cigarettes ($\beta = 0.24$, $P < .001$), and smoking >20 cigarettes per day ($\beta = 0.13$, $P < .001$) remained, compared with nonsmoking, associated with MCV, independent of adjustment for potential confounders.

As sensitivity analysis, we repeated in the PREVENT 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 ($\beta = 0.82$, $P < .001$). Similar to the primary analysis, we identified a strong positive association between 24-hour urinary cotinine excretion and MCV ($\beta = 0.26$, $P < .001$, Figure 1D). The association remained independent of adjustment for potential confounders ($\beta = 0.23$, $P < .001$).

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.⁵ and O'Reilly et al.⁶ 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. 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 this study, we underline the importance of this association, and we are the first to utilize an objective measurement that is, 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 this 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.

ACKNOWLEDGMENTS

The cotinine measurement for this research was supported by a grant from the EU Joint Programme Initiative A Healthy Diet for a Healthy Life (JPI HDHL), the Food Biomarker Alliance (FOODBALL). Lifelines has been funded by a number of public sources, notably the Dutch Government, The Netherlands Organization of Scientific Research NOW [grant 175.010.2007.006], the European fund for regional development, Dutch Ministry of Economic Affairs, Pieken in de Delta, Provinces of Groningen and Drenthe, the Target project, BBMRI-NL, the University of Groningen, and the University Medical Center Groningen, The Netherlands. This work was supported by the National Consortium for Healthy Ageing, and funds from the European Union's Seventh Framework program (FP7/2007-2013) through the BioSHaRE-EU (Biobank Standardisation and Harmonisation for Research Excellence in the European Union) project, grant agreement 261.433. LifeLines (BRIF4568) is engaged in a Bioresource research impact factor (BRIF) policy pilot study, details of which can be found at: <http://bioshare.eu/content/bioresource-impact-factor>. Finally, the Lifelines Biobank initiative has also been made possible by funds from FES (Fonds Economische Structuurversterking), SNN (Samenwerkingsverband Noord Nederland) and REP (Ruimtelijk Economisch Programma).

CONFLICT OF INTEREST

Nothing to report.


AUTHOR CONTRIBUTIONS

All authors read and approved the final version of the manuscript. M.F.E., H.J.C.M.W., G.H. and S.J.L.B. contributed to the study design.

M.F.E. and H.J.C.M.W. performed the statistical analysis. M.F.E., H.J.C.M.W., L.M.K., M.M.vd.K., P.vd.M., B.H.R.W., C.A.J.M.G., J.E.K.-R., D.J.T., G.H. and S.J.L.B. contributed to the interpretation of the data and analysis. M.F.E. and H.J.C.M.W. wrote the first draft and all authors edited the paper.

ORCID

Michele F. Eisenga  <https://orcid.org/0000-0002-2484-6233>

Michele F. Eisenga^{1*} 

Hanneke J. C. M. Wouters^{2,3*}

Lyanne M. Kieneker¹

Melanie M. van der Klauw²

Peter van der Meer⁴

Bruce H. R. Wolffenbuttel²

Carlo A. J. M. Gaillard⁵

Jenny E. Kootstra-Ros⁶

Daan J. Touw⁷

Gerwin Huls³

Stephan J. L. Bakker¹

¹Department of Nephrology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

²Department of Endocrinology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

³Department of Hematology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

⁴Department of Cardiology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

⁵Division of Internal Medicine and Dermatology, University Medical Center Utrecht, University of Utrecht, Utrecht, The Netherlands

⁶Department of Laboratory Medicine, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

⁷Department of Clinical Pharmacy and Pharmacology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

Correspondence

Michele F. Eisenga, Department of Internal Medicine, Division of Nephrology, University Medical Center Groningen, P.O. Box 30.001, Groningen 9700 RB, The Netherlands.
Email: m.f.eisenga@umcg.nl

[†]Michele F. Eisenga and Hanneke J. C. M. Wouters contributed equally to this study.

REFERENCES

- Martinsson A, Andersson C, Andell P, Koul S, Engström G, Smith JG. Anemia in the general population: prevalence, clinical correlates and prognostic impact. *Eur J Epidemiol.* 2014;29:489-498.
- Galloway M, Hamilton M. Macrocytosis: pitfalls in testing and summary of guidance. *BMJ.* 2007;335:884-886.
- Chalmers DM, Levi AJ, Chanarin I, North WRS, Meade TW. Mean cell volume in a working population: the effects of age, smoking, alcohol and oral contraception. *Br J Haematol.* 1979;43:631-636.
- Helman N, Rubenstein LS. The effects of age, sex, and smoking on erythrocytes and leukocytes. *Am J Clin Pathol.* 1975;63:35-44.

- McNamee T, Hyland T, Harrington J, et al. Haematinic deficiency and macrocytosis in middle-aged and older adults. *PLoS One.* 2013;8:e77743.
- O'Reilly MA, Millar SR, Buckley CM, et al. Smoking as an independent risk factor for macrocytosis in middle-aged adults: a population-based observational study. *Am J Hematol.* 2015;90:E196-E197.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Received: 22 October 2018

Revised: 7 November 2018

Accepted: 12 November 2018

DOI: 10.1002/ajh.25352

Venetoclax plus decitabine induced complete remission with molecular response in acute myeloid leukemia relapsed after hematopoietic stem cell transplantation

To the Editor:

Recurrent mutations in the isocitrate dehydrogenase gene (*IDH*) have been identified in acute myeloid leukemia (AML), both in the cytosolic *IDH1* enzyme and in its mitochondrial homolog, *IDH2*.¹ Approximately 15%-20% of AML patients have mutations in *IDH1* or *IDH2*.² Normal counterparts of these enzymes play their role in the citric acid cycle, catalyzing the oxidative decarboxilation of isocitrate and producing alpha-ketoglutarate; mutant *IDH1* and *IDH2* gain the activity to convert the alpha-ketoglutarate into 2-hydroxyglutarate, an onco-metabolite that leads to epigenetic changes that promote cellular transformation through deregulation of mitochondrial function increasing BCL-2 dependence (B-cell leukemia/lymphoma-2, an anti-apoptotic protein) in AML cells.¹ A subtle approach to face a malignancy with this molecular profile is to exploit the so called synthetic lethality, a strategy based on the concept of nononcogene addiction, wherein cells expressing an oncogenic mutation (ie, *IDH1/2*) exhibit dependence on subsets of nononcogenes (ie, *BCL-2*) for survival.¹ Venetoclax is a small and orally available molecule that target specifically the BH3 domain of BCL-2 (hence the name BH3 mimetic) approved for the treatment of Chronic Lymphocytic Leukemia. Several studies on the use of venetoclax as single agent in relapsed/refractory AML (r/r AML) demonstrated clinical activity with tolerable and safe profile³; furthermore the addition of venetoclax to decitabine or azacitidine seems to sensitize AML cells to these hypomethylating agents (HMAs), in particular for patients with *IDH* mutations.⁴⁻⁶

Relapsed AML after allogeneic hematopoietic stem cell transplantation (HSCT) have poor prognosis despite numerous therapies