Associations between Tooth Loss and Prognostic Biomarkers and the Risk for Cardiovascular Events in Patients with Stable Coronary Heart Disease

Short title: Vedin et al.: Tooth Loss and Biomarkers in CHD

Ola Vedin, MD, PhD[1,2], Emil Hagström, MD, PhD[1,2], Ollie Östlund, PhD[2], Alvaro Avezum, MD, PhD[3], Andrzej Budaj, MD, PhD[4], Marcus D. Flather, MBBS[5], Robert A. Harrington, MD[6], Wolfgang Koenig, MD[7-9], Joseph Soffer, MD[10], Agneta Siegbahn, MD, PhD[2,11], Philippe Gabriel Steg, MD[12-15], Ralph A. H. Stewart, MD[16], Lars Wallentin, MD, PhD[1,2], Harvey D. White, MB, ChB, DSc[16], Claes Held, MD, PhD[1,2] on behalf of the STABILITY Investigators

[1]Department of Medical Sciences, Cardiology, Uppsala University, Uppsala, Sweden; [2]Uppsala Clinical Research Center (UCR), Uppsala University, Uppsala, Sweden; [3]Dante Pazzanese Institute of Cardiology, São Paulo, Brazil; [4]Postgraduate Medical School, Grochowski Hospital, Warsaw, Poland; [5]Norwich Medical School, University of East Anglia and Norfolk and Norwich University Hospital, UK; [6]Department of Medicine, Stanford University, Stanford, CA, USA; [7]Department of Internal Medicine II-Cardiology, University of Ulm Medical Center, Ulm, Germany; [8]Deutsches Herzzentrum München, Technische Universität München, Munich, Germany; [9]DZHK (German Centre for Cardiovascular Research), partner site Munich Heart Alliance, Munich, Germany; [10]Metabolic Pathways and Cardiovascular Therapeutic Area, GlaxoSmithKline, King of Prussia, PA, USA; [11]Department of Medical Sciences, Clinical Chemistry, Uppsala University, Uppsala, Sweden; [12]INSERM-Unité 1148, Paris, France; [13]Assistance Publique-Hôpitaux de Paris, Département Hospitalo-Universitaire FIRE, Hôpital Bichat, Paris, France; [14]Université Paris-Diderot, Sorbonne-Paris Cité, Paris, France; [15]NHLI Imperial College, ICMS, Royal

Brompton Hospital, London, UK; [16]Green Lane Cardiovascular Service, Auckland City Hospital and University of Auckland, Auckland, New Zealand

All authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

Address for correspondence: Dr. Ola Vedin; Uppsala Clinical Research Centre/MTC, Dag Hammarskjölds väg 14B, 752 37, Uppsala, SWEDEN; Tel: + 46 18 6119500;
Fax: +46 18 515570; Email: ola.vedin@ucr.uu.se

ACKNOWLEDGEMENT OF GRANT SUPPORT

The STABILITY study was funded by GlaxoSmithKline. Roche Diagnostics, Rotkreuz, Switzerland supported the research by providing GDF-15 assay free of charge.

CONFLICTS OF INTEREST

OV: institutional research grant from GlaxoSmithKline; expert committee member and lecture fees from Boehringer Ingelheim, Fresenius Medicare, Novartis. **EH**: expert committee member, lecture fees and institutional research grant from Sanofi; institutional research grant and lecture fees from Amgen; institutional research grants from AstraZeneca, GlaxoSmitKline; expert committee member for Ariad and Merck Sharp & Dohme. **OÖ**: institutional research grants from GlaxoSmithKline. **AA**: personal fees from Boehringer Ingelheim, Bristol-Myers Squibb, Pfizer, Bayer, AstraZeneca. **AB**: research grants/investigator's fees and honoraria for lectures from GlaxoSmithKline, AstraZeneca, Bristol-Myers Squibb/Pfizer; research grants/investigator's fees from Sanofi-Aventis, Boehringer Ingelheim, Novartis, Eisai, Duke Research Institute. **MDF**: speaker's bureau and

consultancy fees from AstraZeneca; grants from GlaxoSmithKline, Novartis, Menarini; honoraria from Eli Lilly. **RAH**: research grants and consultant/advisory fees from Merck, The Medicines Company; research grants from AstraZeneca, CSL Behrig, GlaxoSmithKline, Portola, Regado, Sanofi; consultant/advisory fees from Amgen, Gilead, MyoKardia, WebMD; stock ownership in Element Science, Scanadu, Signal Path. **WK**: lecture and consultancy fees from Novartis, Amgen, AstraZeneca, Servier; lecture fees from Actavis; consultancy fees from BioInvent, diaDexus, Cerenis, GlaxoSmithKline, The Medicines Company, Genzyme, Pfizer, Merck Sharpe & Dohme; research grants from Roche Diagnostics, Abbott, Singulex, Beckmann. **JS**: employee of and having stock ownership in GlaxoSmithKline. **AS**: institutional research grants from AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb/Pfizer, GlaxoSmithKline. **PGS**: institutional research grants, honoraria and non-financial support from Sanofi, Servier; honoraria and non-financial support from AstraZeneca; honoraria from Amarin, Bayer, Boehringer Ingelheim, Bristol-Myers Squibb, Daiichi-Sankyo, Eli Lilly, Merck Sharp & Dohme, Novartis, Pfizer, Medtronic, Janssen, The Medicines Company, CSL-Behring, GlaxoSmithKline; stockholder in Aterovax. **RAHS**: grants and non-financial support from GlaxoSmithKline. **LW**: institutional research grant, consultancy and lecture fees, travel support and honoraria from GlaxoSmithKline; institutional research grants, consultancy and lecture fees and travel support from AstraZeneca, Bristol-Myers Squibb/Pfizer, Boehringer Ingelheim; institutional research grant from Merck, Roche; consultancy fees from Abbott; holds two patents involving GDF-15. **HDW:** research grants and advisory board for AstraZenecea; advisory board for The Medicines Company; research grants from Sanofi Aventis, Eli Lilly and Company, National Institute of Health, Merck Sharpe & Dohm, GlaxoSmithKline, Omthera Pharmaceuticals, Pfizer New Zealand, Intarcia Therapeutics Inc, Elsai Inc., DalGen Products and Services, Daiichi Sankyo Pharma Development. **CH**: institutional research grant and speaker's bureau

from AstraZeneca; institutional research grants from Bristol-Myers Squibb Merck & Co, GlaxoSmithKline, Roche.

KEYWORDS

Tooth loss, periodontal disease, stable coronary heart disease, biomarkers, risk factors.

ABSTRACT

BACKGROUND: Underlying mechanisms behind the hypothesized relationship between periodontal disease (PD) and coronary heart disease (CHD) have been insufficiently explored. We evaluated associations between self-reported tooth loss, a marker of PD, and prognostic biomarkers in 15,456 (97%) patients with stable CHD in the global STABILITY trial.

METHODS AND RESULTS: Baseline blood samples were obtained and patients reported their number of teeth according to the following tooth loss levels: "26–32 (All)" [lowest level], "20–25", "15–19", "1–14", and "No Teeth" [highest level]. Linear and Cox regression models assessed associations between tooth loss levels, biomarker levels and the relationship between tooth loss levels and outcomes, respectively.

After multivariable adjustment, the relative biomarker increase between the highest and the lowest tooth loss level was: high-sensitivity C-reactive protein 1.21 (95% confidence interval, 1.14-1.29), interleukin 6 1.14 (1.10-1.18), lipoprotein-associated phospholipase A_2 activity 1.05 (1.03-1.06), growth differentiation factor 15 1.11 (1.08-1.14), and N-terminal pro−B-type natriuretic peptide (NT-proBNP) 1.18 (1.11-1.25). No association was detected for high-sensitivity troponin T 1.02 (0.98-1.05). Some attenuation of the relationship between tooth loss and outcomes resulted from the addition of biomarkers to the multivariable analysis, of which NT-proBNP had the biggest impact.

CONCLUSIONS: A graded and independent association between tooth loss and several prognostic biomarkers was observed, suggesting that tooth loss and its underlying mechanisms may be involved in multiple pathophysiological pathways also implicated in the development and prognosis of CHD. The association between tooth loss and cardiovascular

death and stroke persisted despite comprehensive adjustment including prognostic biomarkers.

Clinical trial registration: www.clinicaltrials.gov; NCT00799903

1. INTRODUCTION

There is a growing body of evidence favouring an independent association between oral health and coronary heart disease (CHD) [1–3]. We have recently reported an association between tooth loss and cardiovascular (CV) outcomes, but not myocardial infarction (MI), in a chronic CHD population [4]. In the literature, the hypothesized relationship between dental disease and CV disease is often attributed to deleterious effects of periodontal disease (PD), a highly prevalent chronic inflammatory condition ranging from early gingivitis to end-stage tooth loss, on the atherosclerotic process but the specific mechanistic nature of such a relationship remains elusive and debated [5].

Pathophysiological information on multiple aspects of etiology and progression of CV disease is reflected by several biomarkers, many of which also have robust capabilities of predicting prognosis [6–11]. Thus, associations between markers of PD, biomarkers and CV outcomes could provide further insights about possible mechanisms connecting PD and CV disease. However, such existing observations mainly stem from smaller populations and are limited to selected inflammatory markers such as C-reactive protein and interleukin-6 [12,13], whereas reported associations with other important biomarkers are either scarce or non-existent.

We evaluated associations between self-reported tooth loss, a marker of oral disease and PD, and a wide range of prognostic biomarkers, including high-sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), lipoprotein-associated phospholipase A_2 activity (Lp-PLA_2), growth differentiation factor 15 (GDF-15), high-sensitivity troponin T (hs-Troponin T) and N-terminal pro-B-type natriuretic peptide (NT-proBNP) in a large global CHD population. Further, we assessed the influence of these biomarkers on the ability of self-reported tooth loss to predict CV outcomes.

2. METHODS

2.1. Study population

The STabilization of Atherosclerotic plaque By Initiation of darapLadIb TherapY (STABILITY) study evaluated the efficacy of darapladib, an oral inhibitor of lipoprotein-associated phospholipase A_2 activity (Lp-PLA_2) compared to placebo in addition to optimal medical treatment in 15,828 participants from 39 countries with stable CHD, defined as prior myocardial infarction (MI), prior coronary revascularization (percutaneous coronary intervention or coronary artery bypass grafting), or multivessel CHD without revascularization. At least one additional enrichment criterion was required: age ≥60 years; diabetes mellitus requiring pharmacotherapy; high-density lipoprotein cholesterol <1.03mmol/L; current or previous smoker defined as ≥5 cigarettes per day on average; moderate renal dysfunction (estimated glomerular filtration rate ≥30 and <60mL/min/1.73 m^2 or urine albumin:creatinine ratio ≥30mg albumin/g creatinine); or polyvascular disease (co-existing disease in at least two arterial territories). Patients with an estimated glomerular filtration rate <30mL/min/1.73 m^2 were excluded [14]. After a median follow-up of 3.7 years, no difference in major adverse cardiovascular events (MACE, i.e. first occurrence of CV death, myocardial infarction or stroke) was observed for patients randomized to darapladib compared to placebo [15]. The ethics committees of each participating country approved the study and all patients provided written informed consent prior to inclusion. The STABILITY trial was performed in accordance with the Declaration of Helsinki.

2.2. Data collection

At baseline, 15,456 (97%) patients reported their number of teeth according to the following categories: “26–32 (All teeth)”, “20–25”, “15–19”, “1–14”, and “No Teeth”. For the purposes

of this report, these categories are termed tooth loss levels with 26-32 teeth corresponding to the lowest level and no teeth, the highest level.

Blood samples for routine laboratory tests and storage for later analyses were obtained in all 15,456 patients at baseline and prognostic biomarker analyses were performed in the majority of patients. Plasma aliquots were stored at -70°C until biochemical analysis. All routine biochemical and hs-CRP analyses were performed at a central laboratory with standardized methods (Quest Diagnostics Clinical Laboratories, Inc., Valencia, California, USA). Plasma concentrations of hs-CRP were analyzed using a particle-enhanced immunonephelometry assay, *CardioPhase*© hsCRP, Siemens Healthcare. Plasma concentrations of high-sensitivity IL-6 were analyzed using an ELISA technique, R&D Systems Inc., Minneapolis, MN, U.S.A. Lp-PLA_2 activity was measured in an automated enzyme assay system (PLAC® Test for Lp-PLA_2 Activity, diaDexus, San Francisco, CA, USA). The other biomarker assays were performed at the UCR Laboratory at Uppsala University, Uppsala, Sweden, GDF-15 was measured with the GDF-15 precommercial assay (Roche Diagnostics, Penzberg, Germany), composed of a monoclonal mouse antibody for capture and a monoclonal mouse antibody fragment, [F(ab´)2], for detection in a sandwich assay format. Detection was based on an electrochemiluminiscence immunoassay using a ruthenium (II) complex label. Levels of hs-Troponin T and NT-proBNP were also determined by electrochemiluminescence (Roche Diagnostics, Penzberg, Germany). The Cobas Analytics e601 was used for the Roche immunoassays.

2.3. Statistical analysis

Baseline variables are presented as mean, standard deviation and percentages. Baseline biomarker levels are presented as median and interquartile range. Baseline biomarker levels by tooth loss level were compared using the Kruskal-Wallis tests.

To determine associations between tooth loss levels and biomarker levels, each biomarker was modelled as a function of tooth loss level (five levels). All biomarkers were analyzed on a log-transformed scale using linear models. Geometric mean ratios are presented with 95% confidence intervals (CI) with the lowest tooth loss level (26-32 teeth) as reference, and according to three adjustment models. Model 1 adjusted for randomized treatment. Model 2 adjusted for Model 1 and prior MI, prior coronary revascularization, multi-vessel CHD, age, sex, geographic region, diabetes mellitus, hypertension, renal dysfunction, body mass index, smoking (current, former or never), systolic blood pressure and polyvascular disease. Model 3 adjusted for Model 2 and estimated glomerular filtration rate (according to The Chronic Kidney Disease Epidemiology Collaboration equation, replacing significant renal dysfunction) [16], hemoglobin, white blood cells, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol and triglycerides. Cox proportional hazards models were used to calculate hazard ratios for MACE, CV death and stroke in relation to tooth loss levels, adjusting for biomarkers in addition to a previously reported multivariable model [4], co-variables of which are also listed in Table 3. In these models, all biomarkers except Lp-PLA_2 activity were added after log-transformation. The association between tooth loss and MI has previously been found to be absent in this cohort and was not re-analysed in the present analysis.

A p-value <0.05 was considered statistically significant in all analyses. Analyses were performed at the Uppsala Clinical Research Center, Uppsala, Sweden using SAS version 9.3 (SAS Institute, Inc., Cary, NC, USA).

3. RESULTS

3.1. Baseline characteristics and associations with biomarkers

Patients with higher tooth loss levels were older, were more likely to be female and had a greater CV risk factor burden, particularly a higher prevalence of smoking, diabetes mellitus, hypertension and impaired renal function compared to those with lower tooth loss levels (Table 1). Patients with more tooth loss had progressively higher baseline levels of hs-CRP, IL-6, Lp-PLA_2 activity, GDF-15, hs-Troponin T and NT-proBNP (Table 2). As demonstrated in Figure 1, higher tooth loss levels were associated with progressively greater relative increases for all prognostic biomarkers in relation to the lowest tooth less level in Model 1. After multivariable adjustment the association remained statistically significant for all biomarkers, except for hs-Troponin T.

3.2. Tooth loss and outcomes

Table 3 demonstrates the association between a one-level increase in tooth loss and the risk of MACE, CV death and stroke according to the three adjustment models after a median follow-up of 3.7 years. The data up until Model 2 have been previously presented [4] showing a relative risk increase of 1.06, 1.17 and 1.14 for MACE, CV death and for stroke, respectively, for every one-level increase in tooth loss. The subsequent addition of biomarkers, individually and in groups, only attenuated the results slightly. Nevertheless, the addition of IL-6 and NT-proBNP rendered the association with MACE non-significant. Despite adjustment for all biomarkers and other co-variables, the association between tooth loss and CV death and stroke persisted.

4. DISCUSSION

We found that tooth loss, a marker of PD, was associated with higher levels of several CV biomarkers indicating a potential relationship between tooth loss and several pathophysiological mechanisms relevant to CV morbidity and mortality, including inflammation, cellular integrity and myocardial dysfunction [6,7,9,17]. Adjustment for the biomarkers somewhat attenuated the risk of adverse outcome, signalling a potential relevance of the pathways they represent in the relationship between tooth loss and increased CV risk. However, much of the risk increase remained, suggesting that other and presently unknown mechanisms could be of importance, although confounding cannot be excluded.

The host-mediated inflammatory response to PD constitutes the most commonly proposed mechanism linking oral disease with CHD and CV disease [18], mainly based on evidence from smaller studies of moderate associations between various measures of PD and elevation of inflammatory markers [12,13,19] and studies showing reductions of CRP and IL-6 levels after PD treatment [20]. This is the first study to corroborate these findings on a large scale in a global CHD population, including both upstream and downstream inflammatory markers, with comprehensive adjustment and with tooth loss as the exposure measure. Moreover, the association with Lp-PLA_2 activity, previously only assessed in small patient populations [21], could represent an additional alternative and independent inflammatory link [22]. However, our findings could represent a challenge to the inflammation hypothesis as the highest levels of inflammatory markers were observed among the edentulous, who are most likely rid of oral substrates for inflammation. This could indicate that intrinsic factors and not PD explain the inflammatory activity, for instance pro-inflammatory genetic traits and epigenetic modifications common to both advanced PD and CHD [23,24]. However, it is also conceivable that many years' exposure to chronic oral inflammation could instigate a secondary response in other locations, e.g.

atherosclerosis in blood vessels, which in turn could promote or maintain an elevated systemic inflammatory response.

Associations with biomarkers that reflect other than inflammatory processes could suggest a relevance of alternative pathophysiological pathways in the tooth loss-CV disease relationship. The association with GDF-15 for instance, has not been previously described and could partly reflect a relationship between tooth loss and inflammation but also other harmful mechanisms [17] of relevance to several cardiac afflictions, including MI and heart failure, to which GDF-15 has been previously associated [25,26]. An independent association with cardiac biomarkers could support a more specific link between tooth loss, its antecedents, and cardiac pathology. This was previously investigated in a small study where 44 patients with clinical evidence of PD had higher levels of troponin and NT-proBNP compared to controls, however these associations were unadjusted [27]. The association with NT-proBNP in our study suggests a relationship between tooth loss and baseline cardiac dysfunction that persisted after adjustment for traditional important heart failure determinants, including prior MI, hypertension and diabetes. Cross-reactivity between periodontal and myocardial antigens and chronic toll-like receptor activation have been suggested as potential mechanisms linking chronic infection, e.g. PD, to heart failure [28]. Furthermore, a recent study demonstrated a greater burden of PD among patients with advanced HF awaiting heart transplantation compared to controls without HF. However, the observed differences were attenuated to non-significance after adjustment for markers of oral hygiene, race and 25- hydroxyvitamin D levels [29]. Overall, data on the role of PD in the pathophysiology of heart failure is largely lacking but the results from our present study and other studies are interesting from a hypothesis generating perspective and should be explored further.

Conversely to the association with NT-proBNP, tooth loss did not appear to be related to

mechanisms generating elevated levels of troponin [30], which is an interesting finding concurrent with the absent association between tooth loss and MI in this population [4].

In contrast to the absent MI association we recently reported a significant association between tooth loss and MACE, CV death and stroke. Although the demonstrated risk increases were relatively modest between adjacent tooth loss levels, they were more substantial and likely to be clinically relevant when comparing patients with severe or complete tooth loss to those with little or no tooth loss, particularly for CV death and stroke [4]. In the present analysis, we added biomarkers to the previous outcomes model to assess their influence on the demonstrated relationship between tooth loss and prognosis. Of the inflammatory markers, IL-6, an upstream marker, attenuated the results the most and eliminated the MACE association, supporting the hypothesis of inflammation as a mediator in the PD-CV outcomes relationship further. However, for most of the biomarkers, resulting attenuations were relatively small. The addition of NT-proBNP had the most pronounced attenuating effect on the risk of adverse outcome, indicating a prognostic relevance of the observed relationship between tooth loss and baseline myocardial dysfunction. The fact that a significant portion of the increased risk for CV death and stroke persisted despite full adjustment implies that alternate and presently unidentified pathways could be involved. The observation of stronger associations for CV death and stroke compared to MI further suggests that such pathways may be other than atherosclerotic and inflammatory, which is also consistent with the associations to biomarkers reflecting other than primarily inflammatory mechanisms, including GDF-15 and NT-proBNP. While the additional adjustment for biomarkers could lead to underestimation of the true influence of tooth loss on prognosis by removing effects of potential intermediate pathways, it also likely lessens the impact of residual confounding, which is undoubtedly a considerable obstacle when assessing causality. Nevertheless, as our findings are purely observational, causality cannot be inferred and they

may in fact be a product of long-term influence by causal factors common to both tooth loss and CV disease.

From a prediction perspective and irrespective of causality, the observations show promise for the potential use of self-reported tooth loss as a prognostication tool in chronic CHD. Given its ability to predict several important outcomes combined with other qualities, including its straightforward obtainability and affordability, the clinical predictive utility of self-reported tooth loss should be explored further.

4.1. Study limitations

Self-reported tooth loss was the sole marker of PD in this analysis and the use of additional exposure measures, e.g. clinical, could have provided better information regarding the underlying etiology of tooth loss in the individual subjects. However, the literature generally exhibits a lack of consistency in the use of PD definitions [5] and PD has been shown to be the most common cause of tooth loss in older populations [31], although other causes, most importantly caries, must be acknowledged. Moreover, self-reported tooth loss has been validated against number of teeth on clinical examination with concurring results [32]. The cross-sectional multivariable analysis of the association between tooth loss and biomarker levels did not include markers of socioeconomic status, which could be important confounders. Finally, the analysis does not specifically disclose the nature of the observed associations, i.e. whether tooth loss and its underlying mechanisms increases CV risk or vice versa, or if the two conditions are driven by a common etiology.

4.2. Conclusion

Self-reported tooth loss was associated with progressively higher levels of several prognostic CV biomarkers suggesting possible involvement of tooth loss-generating mechanisms,

including PD, in multiple pathophysiological pathways relevant to both CHD development and prognosis. Adjustment for a wide range of CV risk factors, socioeconomic status and biomarkers eliminated the association with MACE but the association with CV death and stroke persisted.

ACKNOWLEDGMENTS

We thank all participants, study personnel and investigators in the STABILITY study. We also thank Ebba Bergman, Susanna Thörnqvist, and Vendela Roos, Uppsala Clinical Research Centre, for editorial support.

FUNDING

The STABILITY study was funded by GlaxoSmithKline. Roche Diagnostics, Rotkreuz, Switzerland supported the research by providing GDF-15 assay free of charge.

REFERENCES

[1] L.L. Humphrey, R. Fu, D.I. Buckley, M. Freeman, M. Helfand, Periodontal disease and coronary heart disease incidence: a systematic review and meta-analysis, J. Gen. Intern. Med. 23 (2008) 2079–86.

[2] A.A. Bahekar, S. Singh, S. Saha, J. Molnar, R. Arora, The prevalence and incidence of coronary heart disease is significantly increased in periodontitis: a meta-analysis, Am. Heart J. 154 (2007) 830–7.

[3] L. Rydén, K. Buhlin, E. Ekstrand, U. de Faire, A. Gustafsson, J. Holmer, B. Kjellström, B. Lindahl, A. Norhammar, Å. Nygren, P. Näsman, N. Rathnayake, E. Svenungsson, B. Klinge, Periodontitis Increases the Risk of a First Myocardial Infarction: A Report From the PAROKRANK Study, Circulation. (2016).

[4] O. Vedin, E. Hagström, A. Budaj, S. Denchev, R.A. Harrington, W. Koenig, J. Soffer, P. Sritara, A. Stebbins, R.H. Stewart, H.P. Swart, M. Viigimaa, D. Vinereanu, L. Wallentin, H.D. White, C. Held, Tooth loss is independently associated with poor outcomes in stable coronary heart disease, Eur. J. Prev. Cardiol. (2015) 2047487315621978-.

[5] P.B. Lockhart, A.F. Bolger, P.N. Papapanou, O. Osinbowale, M. Trevisan, M.E. Levison, K. a Taubert, J.W. Newburger, H.L. Gornik, M.H. Gewitz, W.R. Wilson, S.C. Smith, L.M. Baddour, Periodontal disease and atherosclerotic vascular disease: does the evidence support an independent association?: a scientific statement from the American Heart Association., Circulation. 125 (2012) 2520–44.

[6] S. Kaptoge, E. Di Angelantonio, G. Lowe, M.B. Pepys, S.G. Thompson, R. Collins, J. Danesh, C-reactive protein concentration and risk of coronary heart disease, stroke,

and mortality: an individual participant meta-analysis., Lancet. 375 (2010) 132–40. [7] J. Danesh, S. Kaptoge, A.G. Mann, N. Sarwar, A. Wood, S.B. Angleman, F. Wensley, J.P.T. Higgins, L. Lennon, G. Eiriksdottir, A. Rumley, P.H. Whincup, G.D.O. Lowe, V. Gudnason, Long-term interleukin-6 levels and subsequent risk of coronary heart disease: Two new prospective studies and a systematic review, PLoS Med. 5 (2008) 0600–0610.

[8] A. Thompson, P. Gao, L. Orfei, S. Watson, E. Di Angelantonio, S. Kaptoge, C. Ballantyne, C.P. Cannon, M. Criqui, M. Cushman, A. Hofman, C. Packard, S.G. Thompson, R. Collins, J. Danesh, Lipoprotein-associated phospholipase A(2) and risk of coronary disease, stroke, and mortality: collaborative analysis of 32 prospective studies, Lancet. 375 (2010) 1536–1544.

[9] S. Blankenberg, M.J. McQueen, M. Smieja, J. Pogue, C. Balion, E. Lonn, H.J. Rupprecht, C. Bickel, L. Tiret, F. Cambien, H. Gerstein, T. Münzel, S. Yusuf, Comparative impact of multiple biomarkers and N-terminal pro-brain natriuretic peptide in the context of conventional risk factors for the prediction of recurrent cardiovascular events in the Heart Outcomes Prevention Evaluation (HOPE) study, Circulation. 114 (2006) 201–208.

[10] T. Omland, J.A. de Lemos, M.S. Sabatine, C.A. Christophori, M. Rice, K.A. Jablonski, S. Tjora, M.J. Domanski, B.J. Gersh, J.L. Rouleau, M.A. Pfeffer, E. Braunwald, A Sensitive Cardiac Troponin T Assay in Stable Coronary Artery Disease, N Engl J Med. 361 (2009) 2538–2547.

[11] D.W. Schopfer, I.A. Ku, M. Regan, M.A. Whooley, Growth differentiation factor 15 and cardiovascular events in patients with stable ischemic heart disease (The Heart and Soul Study), Am. Heart J. 167 (2014) 186–192.e1.

[12] S. Paraskevas, J.D. Huizinga, B.G. Loos, A systematic review and meta-analyses on C-reactive protein in relation to periodontitis, J. Clin. Periodontol. 35 (2008) 277–90.

[13] B.G. Loos, J. Craandijk, F.J. Hoek, P.M. Wertheim-van Dillen, U. van der Velden, Elevation of systemic markers related to cardiovascular diseases in the peripheral blood of periodontitis patients, J. Periodontol. 71 (2000) 1528–34.

[14] H. White, C. Held, R. Stewart, D. Watson, R. Harrington, A. Budaj, P.G. Steg, C.P. Cannon, S. Krug-Gourley, J. Wittes, T. Trivedi, E. Tarka, L. Wallentin, Study design and rationale for the clinical outcomes of the STABILITY Trial (STabilization of Atherosclerotic plaque By Initiation of darapLadIb TherapY) comparing darapladib versus placebo in patients with coronary heart disease, Am. Heart J. 160 (2010) 655–661.

[15] H.D. White, C. Held, R. Stewart, E. Tarka, R. Brown, R.Y. Davies, A. Budaj, R.A. Harrington, P.G. Steg, D. Ardissino, P.W. Armstrong, A. Avezum, P.E. Aylward, A. Bryce, H. Chen, M.-F. Chen, R. Corbalan, A.J. Dalby, N. Danchin, R.J. De Winter, S. Denchev, R. Diaz, M. Elisaf, M.D. Flather, A.R. Goudev, C.B. Granger, L. Grinfeld, J.S. Hochman, S. Husted, H.-S. Kim, W. Koenig, A. Linhart, E. Lonn, J. López-Sendón, A.J. Manolis, E.R. Mohler, J.C. Nicolau, P. Pais, A. Parkhomenko, T.R. Pedersen, D. Pella, M.A. Ramos-Corrales, M. Ruda, M. Sereg, S. Siddique, P. Sinnaeve, P. Smith, P. Sritara, H.P. Swart, R.G. Sy, T. Teramoto, H.-F. Tse, D. Watson, W.D. Weaver, R. Weiss, M. Viigimaa, D. Vinereanu, J. Zhu, C.P. Cannon, L. Wallentin, Darapladib for Preventing Ischemic Events in Stable Coronary Heart Disease, N. Engl. J. Med. 370 (2014).

[16] A.S. Levey, L.A. Stevens, C.H. Schmid, Y.L. Zhang, A.F. Castro, H.I. Feldman, J.W. Kusek, P. Eggers, F. Van Lente, T. Greene, J. Coresh, A new equation to estimate

glomerular filtration rate, Ann. Intern. Med. 150 (2009) 604–12.

[17] T. Ago, J. Sadoshima, GDF15, a cardioprotective TGF-beta superfamily protein, Circ. Res. 98 (2006) 294–7.

[18] M. Kebschull, R.T. Demmer, P.N. Papapanou, "Gum bug, leave my heart alone!"--epidemiologic and mechanistic evidence linking periodontal infections and atherosclerosis, J. Dent. Res. 89 (2010) 879–902.

[19] T. Nakajima, T. Honda, H. Domon, T. Okui, K. Kajita, H. Ito, N. Takahashi, T. Maekawa, K. Tabeta, K. Yamazaki, Periodontitis-associated up-regulation of systemic inflammatory mediator level may increase the risk of coronary heart disease, J. Periodontal Res. 45 (2010) 116–22.

[20] M.S. Tonetti, F. D'Aiuto, L. Nibali, A. Donald, C. Storry, M. Parkar, J. Suvan, A.D. Hingorani, P. Vallance, J. Deanfield, Treatment of periodontitis and endothelial function, N. Engl. J. Med. 356 (2007) 911–20.

[21] W. Lösche, G.J. Marshal, D.A. Apatzidou, S. Krause, T. Kocher, D.F. Kinane, Lipoprotein-associated phospholipase A2 and plasma lipids in patients with destructive periodontal disease, J. Clin. Periodontol. 32 (2005) 640–4.

[22] F.D. Kolodgie, A.P. Burke, K.S. Skorija, E. Ladich, R. Kutys, A.T. Makuria, R. Virmani, Lipoprotein-associated phospholipase A2 protein expression in the natural progression of human coronary atherosclerosis, Arterioscler. Thromb. Vasc. Biol. 26 (2006) 2523–2529.

[23] A.S. Schaefer, G.M. Richter, B. Groessner-Schreiber, B. Noack, M. Nothnagel, N.-E. El Mokhtari, B.G. Loos, S. Jepsen, S. Schreiber, Identification of a shared genetic susceptibility locus for coronary heart disease and periodontitis, PLoS Genet. 5 (2009) e1000378.

[24] S. Schulz, U.D. Immel, L. Just, H.-G. Schaller, C. Gläser, S. Reichert, Epigenetic characteristics in inflammatory candidate genes in aggressive periodontitis, Hum. Immunol. 77 (2016) 71–75.

[25] K.C. Wollert, T. Kempf, T. Peter, S. Olofsson, S. James, N. Johnston, B. Lindahl, R. Horn-Wichmann, G. Brabant, M.L. Simoons, P.W. Armstrong, R.M. Califf, H. Drexler, L. Wallentin, Prognostic value of growth-differentiation factor-15 in patients with non-ST-elevation acute coronary syndrome, Circulation. 115 (2007) 962–971.

[26] T. Kempf, S. von Haehling, T. Peter, T. Allhoff, M. Cicoira, W. Doehner, P. Ponikowski, G.S. Filippatos, P. Rozentryt, H. Drexler, S.D. Anker, K.C. Wollert, Prognostic Utility of Growth Differentiation Factor-15 in Patients With Chronic Heart Failure, J. Am. Coll. Cardiol. 50 (2007) 1054–1060.

[27] W.Y. Loo, Y. Yue, C. Fan, L. Bai, Y. Dou, M. Wang, H. Liang, M.N.B. Cheung, L.W.C. Chow, J. Li, Y. Tian, L. Qing, Comparing serum levels of cardiac biomarkers in cancer patients receiving chemotherapy and subjects with chronic periodontitis, J. Transl. Med. 10 Suppl 1 (2012) S5.

[28] Z. Kaya, C. Leib, H. a. Katus, Autoantibodies in heart failure and cardiac dysfunction, Circ. Res. 110 (2012) 145–158.

[29] U. Schulze-Späte, I. Mizani, K.R. Salaverry, J. Chang, C. Wu, M. Jones, P.J. Kennel, D.L. Brunjes, T.-H. Choo, T.S. Kato, D. Mancini, J. Grbic, P.C. Schulze, Periodontitis and bone metabolism in patients with advanced heart failure and after heart transplantation, ESC Hear. Fail. 4 (2017) 169–177.

[30] H.D. White, Pathobiology of troponin elevations: do elevations occur with myocardial ischemia as well as necrosis?, J. Am. Coll. Cardiol. 57 (2011) 2406–8.

[31] P.S. Hull, H. V Worthington, V. Clerehugh, R. Tsirba, R.M. Davies, J.E. Clarkson,

The reasons for tooth extractions in adults and their validation, J. Dent. 25 (1997) 233–7.

[32] W. Pitiphat, R.I. Garcia, C.W. Douglass, K.J. Joshipura, Validation of self-reported oral health measures, J. Public Health Dent. 62 (2002) 122–8.

FIGURE LEGENDS

Figure 1 legend. Geometric mean ratio of biomarker levels according to tooth loss level with the lowest tooth loss level (26-32 teeth) as reference. Included biomarkers are high-sensitivity C-reactive protein (hs-CRP), interleukin 6 (IL-6), lipoprotein-associated phospholipase A2 activity (Lp-PLA2), growth differentiation factor 15 (GDF-15), high-sensitivity troponin T (hs-Troponin T), and N-terminal pro−B-type natriuretic peptide (NT-proBNP).

Adjusted for darapladib treatment, prior MI, prior revascularization, multi-vessel coronary heart disease, age, sex, geographic region, diabetes mellitus, hypertension, body mass index, smoking (current, former or never), systolic blood pressure, polyvascular disease, estimated glomerular filtration rate, Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI), hemoglobin, white blood cells, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides.

TABLES

Table 1. Demographic and baseline characteristics by tooth loss level

		Tooth Loss Level					
		Lowest				Highest	
Characteristics	**All Patients (n=15456)**	**26-32 Teeth (n=3310)**	**20-25 Teeth (n=3680)**	**15-19 Teeth (n=2167)**	**1-14 Teeth (n=3785)**	**No Teeth (n=2514)**	**p-value**
Age, years	64.4 +/- 9.3	61.1 +/- 10.0	63.2 +/- 9.2	64.5 +/- 9.0	65.8 +/- 8.5	68.2 +/- 8.0	<0.0001
Sex							<0.0001
Female	18.6	13.7	15.1	19.9	21.6	24.9	
Smoking status							<0.0001
Never smoked	30.8	38.9	32.9	30.0	27.2	22.9	
Former smoker	51.2	46.2	50.5	50.4	53.2	56.6	
Current smoker	18.0	14.9	16.5	19.6	19.6	20.5	
Diabetes	38.8	35.4	37.4	36.6	41.3	43.0	<0.0001
Chronic kidney disease	30.2	23.9	27.3	31.4	32.7	38.0	<0.0001
Prior MI	58.9	55.3	58.1	59.9	62.4	58.7	<0.0001
Prior revascularization	74.9	76.9	75.6	73.5	72.8	75.7	0.0078

1420
1421

Education							<0.0001
None	3.6	3.5	2.6	3.1	3.8	5.2	
1-8 years	19.2	14.7	15.5	16.8	23.1	27.1	
9-12 years	30.7	26.1	29.0	32.5	32.8	34.4	
Trade school	18.2	14.5	17.8	19.7	20.7	18.6	
College/university	28.3	41.2	35.0	27.9	19.7	14.7	
Alcohol consumption/week							<0.0001
None	54.8	53.6	50.1	51.8	57.1	62.1	
1-4 drinks	18.9	19.1	20.3	20.2	18.5	16.4	
5-14 drinks	15.8	16.3	18.0	16.4	14.9	12.9	
≥15 drinks	10.5	11.0	11.6	11.7	9.5	8.7	
Leisure time physical activity							0.0002
Sedentary	32.8	33.3	30.0	30.4	35.1	35.0	
Mild exercise	44.6	44.4	45.1	45.5	43.0	45.4	
Moderate exercise	22.6	22.3	25.0	24.1	21.8	19.6	
LDL cholesterol, mmol/L	2.2 ± 0.9	2.1 ± 0.8	2.2 ± 0.8	2.2 ± 0.8	2.3 ± 0.9	2.2 ± 0.8	<0.0001
HDL cholesterol, mmol/L	1.2 ± 0.3	1.2 ± 0.3	1.2 ± 0.3	1.2 ± 0.3	1.2 ± 0.3	1.2 ± 0.3	<0.0001
eGFR, mL/min/1.73m^2	75.8 ± 18.1	78.7 ± 17.8	77.2 ± 17.8	75.3 ± 18.1	74.5 ± 18.2	72.3 ± 18.0	<0.0001

Systolic BP, mmHg	131.5 ± 16.6	128.8 ± 16.0	131.3 ± 16.1	131.6 ± 17.0	132.6 ± 16.4	133.4 ± 17.3	<0.0001
Diastolic BP, mmHg	78.7 ± 10.4	78.4 ± 10.2	78.7 ± 10.3	78.8 ± 10.4	79.2 ± 10.3	78.2 ± 10.6	<0.0001
BMI, kg/m^2	28.9 ± 5.0	28.5 ± 5.0	29.1 ± 5.1	28.9 ± 5.0	29.0 ± 4.8	29.3 ± 5.2	<0.0001

Data are percentages and mean ± SD

MI, myocardial infarction; LDL, low-density lipoprotein; HDL, high-density lipoprotein; eGFR, estimated glomerular filtration rate; BP, blood pressure; BMI, body mass index

Table 2. Baseline biomarker levels by tooth loss level

		Tooth Loss Level				
		Lowest			Highest	
Biomarker	**All Patients (n=15456)**	**26-32 Teeth (n=3310)**	**20-25 Teeth (n=3680)**	**15-19 Teeth (n=2167)**	**1-14 Teeth (n=3785)**	**No Teeth (n=2514)**	**p-value**
hs-CRP, mg/L							
n	14076	2977	3389	1972	3467	2271	
Median (Q1; Q3)	1.3 (0.6; 3.1)	1.1 (0.5; 2.6)	1.3 (0.6; 2.8)	1.4 (0.7; 3.0)	1.5 (0.7; 3.3)	1.7 (0.8; 4.0)	< 0.0001
IL-6, pg/mL							
n	14263	2974	3400	2007	3545	2337	
Median (Q1; Q3)	2.1 (1.4; 3.2)	1.8 (1.3; 2.8)	2.0 (1.4; 2.9)	2.1 (1.4; 3.3)	2.2 (1.5; 3.4)	2.4 (1.7; 3.8)	< 0.0001
Lp-PLA$_2$, µmol/min/L							
n	14155	2960	3366	1984			
Median (Q1; Q3)	173 (143; 204)	171 (140; 203)	172 (143; 202)	172 (143; 204)			
GDF-15, pg/mL							

	3520	2325
	173 (144; 206)	176 (148; 207)
		< 0.0001

n	14237	2974	3385	2011	3528	2339	
Median (Q1; Q3)	1254 (915; 1827)	1120 (824; 1615)	1181 (876; 1709)	1225 (907; 1792)	1314 (962; 1888)	1486 (1069; 2172)	< 0.0001
hs-Troponin T, ng/L							
n	14147	2952	3386	1996	3497	2316	
Median (Q1; Q3)	9.3 (6.2; 14.2)	8.5 (5.7; 12.7)	8.7 (6.0; 13.3)	9.4 (6.2; 14.3)	9.8 (6.4; 14.8)	10.4 (6.9; 16.2)	< 0.0001
NT-proBNP, ng/L							
n	14181	2960	3392	2003	3505	2321	
Median (Q1; Q3)	173 (83; 377)	132 (64; 278)	152 (72; 328)	177 (88; 392)	202 (97; 450)	224 (108; 493)	< 0.0001

hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin 6; Lp-PLA_2, lipoprotein-associated phospholipase A_2 activity; hs-Troponin T, high-sensitivity troponin T; NT-proBNP, N-terminal pro−B-type natriuretic peptide; GDF-15, growth differentiation factor 15

Table 3. Risk of major adverse cardiac events (MACE), cardiovascular (CV) death and stroke for one increase in tooth loss level after adjustment for risk factors and biomarkers

Adjustment	MACE (HR [95% CI])	p-value	CV death (HR [95% CI])	p-value	Stroke (HR [95% CI])	p-value
Model 1	1.16 (1.12-1.20)	<0.0001	1.30 (1.23-1.37)	<0.0001	1.22 (1.23-1.32)	<0.0001
Model 2	1.06 (1.02-1.10)	0.0041	1.17 (1.10-1.24)	<0.0001	1.14 (1.04-1.25)	0.0040
Model 2 + hs-CRP	1.05 (1.00-1.09)	0.0581	1.16 (1.08-1.23)	<0.0001	1.12 (1.02-1.23)	0.0163
Model 2 + IL-6	1.04 (1.00-1.09)	0.0691	1.14 (1.07-1.22)	<0.0001	1.10 (1.01-1.20)	0.0335
Model 2 + Lp-PLA_2	1.06 (1.01-1.10)	0.0091	1.16 (1.09-1.24)	<0.0001	1.14 (1.04-1.26)	0.0057
Model 2 + GDF-15	1.05 (1.01-1.10)	0.0273	1.15 (1.08-1.23)	<0.0001	1.12 (1.02-1.23)	0.0188

<
0
.
0
0
0
1

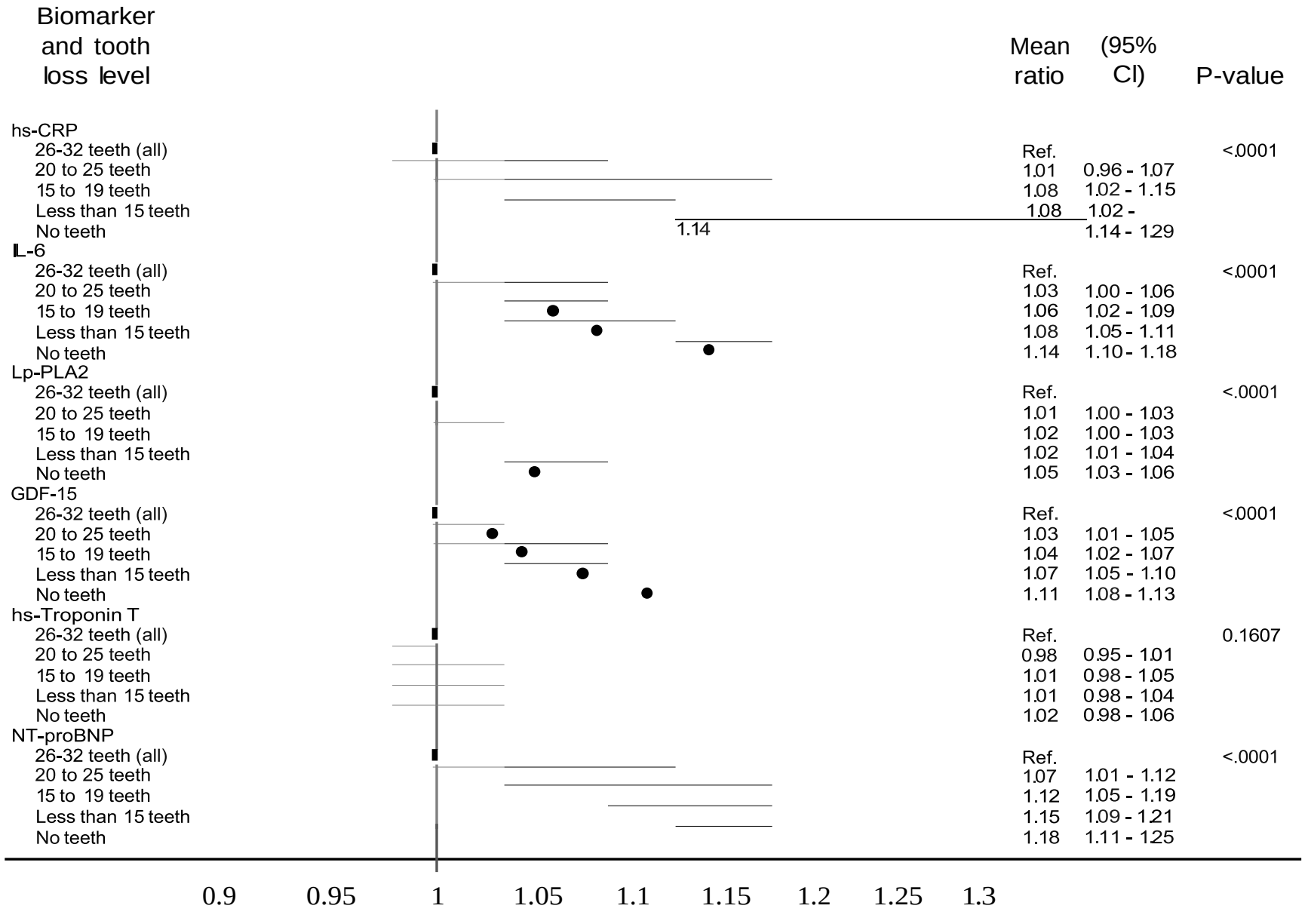
0
.
0
0
6
2

0
.
0
0
6
5

0
.
0
2
2
4

Model 2 + hs-Troponin T, NT-proBNP	1.03 (0.99-1.07)	0.1893	1.12 (1.05-1.19)	0.0006	1.10 (1.00-1.21)	0.0488
Model 2 + hs-CRP, IL-6, Lp-PLA_2, GDF-15	1.04 (0.99-1.08)	0.0973	1.13 (1.05-1.20)	0.0005	1.13 (1.03-1.25)	0.0146
Model 2 + hs-CRP, IL-6, Lp-PLA_2, GDF-15, hs-Troponin T, NT-proBNP	1.02 (0.98-1.07)	0.3335	1.10 (1.03-1.18)	0.0046	1.11 (1.00-1.23)	0.0406

HR, hazard ratio; CI, confidence interval; Model 1 is adjusted for study treatment only. Model 2 is adjusted for model 1 and age, systolic blood pressure, diastolic blood pressure, BMI, LDL cholesterol, HDL cholesterol, history of diabetes, prior MI, sex, smoking status, waist hip ratio, eGFR, family history of coronary heart disease, alcohol consumption, years of education, level of physical activity and country income level. Abbreviations as in Tables 1 and 2.


Biomarker and tooth loss level
Mean ratio
(95% CI)
P-value
hs-CRP
26-32 teeth (all) Ref. <.0001
20 to 25 teeth 1.01 0.96 - 1.07
15 to 19 teeth 1.08 1.02 - 1.15
Less than 15 teeth 1.08 1.02 -
No teeth 1.14 1.14 - 1.29
IL-6
26-32 teeth (all) Ref. <.0001
20 to 25 teeth 1.03 1.00 - 1.06
15 to 19 teeth 1.06 1.02 - 1.09
Less than 15 teeth 1.08 1.05 - 1.11
No teeth 1.14 1.10 - 1.18
Lp-PLA2
26-32 teeth (all) Ref. <.0001
20 to 25 teeth 1.01 1.00 - 1.03
15 to 19 teeth 1.02 1.00 - 1.03
Less than 15 teeth 1.02 1.01 - 1.04
No teeth 1.05 1.03 - 1.06
GDF-15
26-32 teeth (all) Ref. <.0001
20 to 25 teeth 1.03 1.01 - 1.05
15 to 19 teeth 1.04 1.02 - 1.07
Less than 15 teeth 1.07 1.05 - 1.10
No teeth 1.11 1.08 - 1.13
hs-Troponin T
26-32 teeth (all) Ref. 0.1607
20 to 25 teeth 0.98 0.95 - 1.01
15 to 19 teeth 1.01 0.98 - 1.05
Less than 15 teeth 1.01 0.98 - 1.04
No teeth 1.02 0.98 - 1.06
NT-proBNP
26-32 teeth (all) Ref. <.0001
20 to 25 teeth 1.07 1.01 - 1.12
15 to 19 teeth 1.12 1.05 - 1.19
Less than 15 teeth 1.15 1.09 - 1.21
No teeth 1.18 1.11 - 1.25
0.9
0.95
1
1.05
1.1
1.15
1.2
1.25
1.3

Author Agreement Form – International Journal of Cardiology

Manuscript Title: Associations between Tooth Loss and Prognostic Biomarkers and the Risk for

Cardiovascular Events in Patients with Stable Coronary Heart Disease

List of all Authors: Ola Vedin, MD, PhD; Emil Hagström, MD, PhD; Ollie Östlund, PhD; Alvaro Avezum, MD, PhD; Andrzej Budaj, MD, PhD; Marcus D. Flather, MBBS; Robert A. Harrington, MD; Wolfgang Koenig, MD; Joseph Soffer, MD; Agneta Siegbahn, MD, PhD; Philippe Gabriel Steg, MD; Ralph A. H. Stewart, MD; Lars Wallentin, MD, PhD; Harvey D. White, MB, ChB, DSc; Claes Held, MD, PhD.

Corresponding Author: Ola Vedin

This statement is to certify that all authors have seen and approved the manuscript being submitted, have contributed significantly to the work, attest to the validity and legitimacy of the data and its interpretation, and agree to its submission to the *International Journal of Cardiology*.

We attest that the article is the Authors' original work, has not received prior publication and is not under consideration for publication elsewhere. We adhere to the statement of ethical publishing as appears in the International of Cardiology (citable as: Shewan LG, Rosano GMC, Henein MY, Coats AJS. A statement on ethical standards in publishing scientific articles in the International Journal of Cardiology family of journals. Int. J. Cardiol. 170 (2014) 253-254 DOI:10.1016/j.ijcard.2013.11).

On behalf of all Co-Authors, the corresponding Author shall bear full responsibility for the submission. Any changes to the list of authors, including changes in order, additions or removals will require the submission of a new author agreement form approved and signed by all the original and added submitting authors.

All authors are requested to disclose any actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence, or be perceived to influence, their work. If there are no conflicts of interest, the COI should read: “The authors report no relationships that could be construed as a conflict of interest”.