Roxana Mehran, MD
***Gregg W. Stone, MD**
*Mount Sinai Medical Center
Cardiovascular Research Foundation
1700 Broadway, 9th Floor
New York, New York 10019
E-mail: gregg.stone@mountsinai.org
Twitter: @GreggWStone

https://doi.org/10.1016/j.jacc.2020.01.037

Please note: The EXCEL Trial is supported by Abbott. Dr. Serruys has served as a consultant for Abbott, Biosensors, Medtronic, Micell Technologies, SINOMED, Philips/Volcano, Xeltis, and HeartFlow. Dr. Sabik III has served as a consultant for Medtronic, Edwards, and Sorin; and has served on the Advisory Board of Medtronic Cardiac Surgery. Dr. Kappetein is an employee of Medtronic. Dr. Morice is the CEO of the Cardiovascular European Research Center. Dr. Kandzari has received consulting honoraria and research/grant support from Medtronic, Biotronik, and Boston Scientific. Dr. Mehran has received institutional grant support (funding to the institution) from AstraZeneca, Bayer, Beth Israel Deaconess, Bristol-Myers Squibb, CSL Behring, DSI, BSC, Novartis Pharmaceuticals, and OrbusNeich; has received consulting fees from Medscape, Regeneron Pharmaceuticals (no fee), Sanofi, and Abbott Laboratories (personal fees for speaking engagements); has received spousal consultant fees (paid to the institution) from Abiomed and The Medicines Company; has served on the Scientific Advisory Board of PLx Opco Inc./PLx Pharma Inc.; has served on the Advisory Board of Bristol-Myers Squibb (funding to the institution); has equity <1% in Claret Medical and Elixir Medical; has served on the Executive Committee (paid to the institution) of Janssen Pharmaceuticals; and has served as a member of the Data Safety Monitoring Board of Watermark Research Partners. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose. (EXCEL Clinical Trial [EXCEL]; NCT01205776)

REFERENCES

1. Lee PC, Kini AS, Ahsan C, Fisher E, Sharma SK. Anemia is an independent predictor of mortality after percutaneous coronary intervention. J Am Coll Cardiol 2004;44:541–6.

2. van Straten AH, Hamad MA, van Zundert AJ, Martens EJ, Schonberger JP, de Wolf AM. Preoperative hemoglobin level as a predictor of survival after coronary artery bypass grafting: a comparison with the matched general population. Circulation 2009;120:118–25.

3. Pilgrim T, Vetterli F, Kalesan B, et al. The impact of anemia on long-term clinical outcome in patients undergoing revascularization with the unrestricted use of drug-eluting stents. Circ Cardiovasc Interv 2012;5:202–10.

4. Stone GW, Sabik JF, Serruys PW, et al. Everolimus-eluting stents or bypass surgery for left main coronary artery disease. N Engl J Med 2016;375:2223–35.

High-Density Lipoproteins Are the Main Carriers of PCSK9 in the Circulation

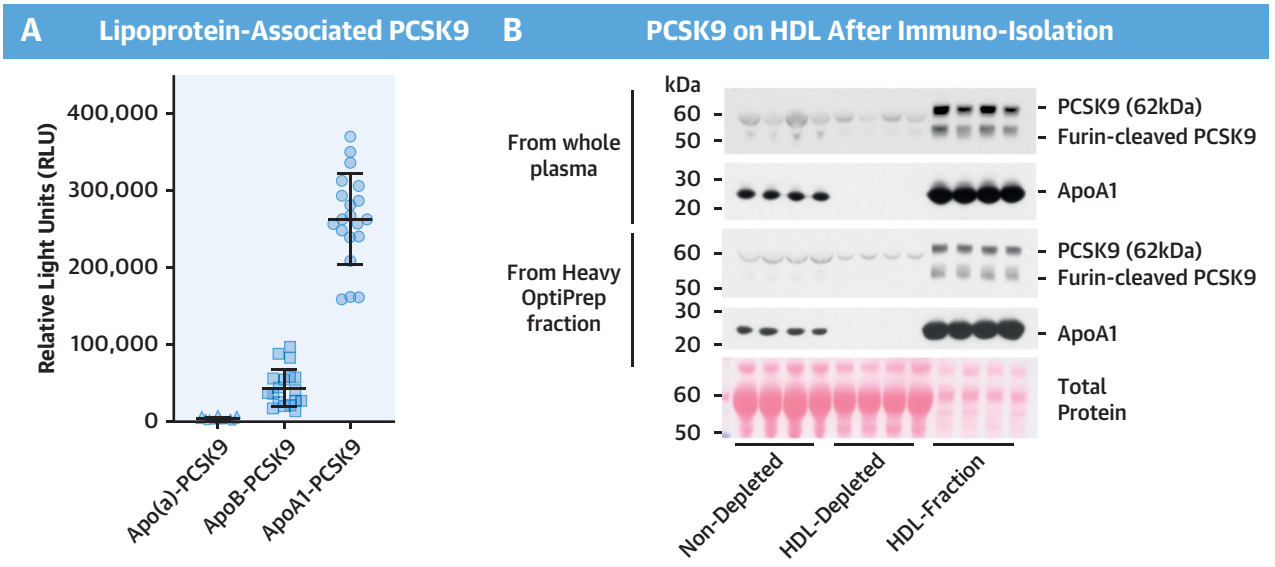
Proprotein convertase subtilisin/kexin type 9 (PCSK9), a secreted protein that regulates circulating low-density lipoprotein (LDL) through the hepatic LDL receptor degradation pathway, is the latest therapeutic target to further lower cholesterol in patients on maximal statin therapy (1). Circulating PCSK9 has been shown to bind to LDL and lipoprotein(a) (Lp[a]). The latter is an LDL particle carrying apolipoprotein(a) as an additional protein component (2,3). Furthermore, the multimeric state of PCSK9 is thought to be influenced by lipoproteins, including high-density lipoprotein (HDL), regulating the LDL receptor–degrading capabilities of PCSK9 (4). However, the presence of PCSK9 on other lipoprotein particles is less well established, in particular in humans.

The potential associations of PCSK9 with different human lipoproteins was first determined by immunocapture, as previously described (5). Alirocumab, a human monoclonal antibody to PCSK9, was coated as a capture antibody to measure plasma PCSK9 in 20 healthy volunteers. Antibodies specific for apolipoprotein(a), apolipoprotein B (ApoB), and apolipoprotein A1 were then used to interrogate PCSK9-lipoprotein associations with Lp(a), ApoB-containing lipoproteins, and HDL, respectively. Measurement of lipoprotein-associated PCSK9 suggested a predominant association of PCSK9 with HDL (**Figure 1A**). For validation, plasma was subject to an anti-ApoB immunoprecipitation (Sun Diagnostics, New Gloucester, Maine). Successful removal of ApoB-carrying lipoproteins, including Lp(a), as confirmed by targeted mass spectrometry, led to only a <20% removal of PCSK9 as measured by enzyme-linked immunosorbent assay (DY3888, R&D Systems, Minneapolis, Minnesota; data not shown). In contrast, HDL removal using an HDL immunodepletion column (Genway Biotech, San Diego, California) resulted in a >90% removal of PCSK9 from fasting plasma (data not shown).

Next, nondepleted plasma, HDL-depleted plasma, and the HDL fraction were compared for apolipoprotein A1 (ab52945, Abcam, Cambridge, United Kingdom) by immunoblotting (**Figure 1B**). Notably, efficient HDL depletion resulted in a profound enrichment of both full-length and furin-cleaved PCSK9 (ab181142, Abcam) in the HDL fraction. Targeted mass spectrometry, however, also revealed a nonspecific removal of up to 50% of ApoB upon HDL depletion. OptiPrep density gradient centrifugation (Sigma Aldrich, St. Louis, Missouri) has been used previously in the determination of PCSK9 association with LDL (2). When pooled fasting plasma was separated using OptiPrep gradients, apolipoprotein A1 was observed in the “heavy” and “medium” fractions. Thus, the LDL-containing “medium” fraction is not devoid of HDL. Finally, to address the ApoB contamination upon HDL depletion, the HDL immune isolation was repeated from the ApoB-depleted, “heavy” OptiPrep fraction. This experiment

JACC VOL. 75, NO. 12, 2020
MARCH 31, 2020:1493–502

FIGURE 1 PCSK9 Is Primarily Associated With HDL in Human Plasma



(A) Proprotein convertase subtilisin/kexin type 9 (PCSK9) levels. Bound PCSK9 on different lipoproteins. **(B)** Immuno-isolated high-density lipoprotein (HDL). Non-depleted plasma, HDL-depleted plasma, and the HDL fraction were subjected to immunoblotting to assess for enrichment of PCSK9 and apolipoprotein A1 (ApoA1). The findings were replicated using fasting plasma separated by OptiPrep density gradient centrifugation, with HDL being immuno-isolated from the apolipoprotein B (ApoB)-depleted, "heavy" fraction. Apo(a) = apolipoprotein(a).

replicated the finding of PCSK9 enrichment in HDL as observed in whole plasma (Figure 1B).

Previous work has explored the associations of PCSK9 with LDL and Lp(a). Our experiments highlight for the first time that PCSK9 is predominantly associated with HDL in human plasma. We provide 5 lines of evidence to support our conclusion: immunocapture of PCSK9-associated lipoproteins, plasma PCSK9 concentrations after ApoB and after HDL depletion, and enrichment of PCSK9 on HDL after immune isolation from plasma and from an ApoB-depleted OptiPrep fraction. Our first experiment (Figure 1A) relies on immunocapture, raising the possibility that the relatively low signal for ApoB and apolipoprotein(a)-associated PCSK9 could be due to epitope masking, alongside the possibility that the different sizes of lipoprotein particles influence their efficiency to be detected. The subsequent experiments, however, provide unambiguous evidence that HDL removal is sufficient to capture the majority of PCSK9 from human plasma (Figure 1B). Unlike other methods for lipoprotein isolation (i.e., ultracentrifugation), immune isolation may reduce the loss of HDL-associated PCSK9. Further studies are now required to determine whether HDL is capable of altering PCSK9 functionality. For example, PCSK9 binding to LDL was shown to inhibit PCSK9-mediated degradation of the LDL receptor (2). The ability of PCSK9 to bind multiple lipoprotein classes raises the possibility that changes in PCSK9-lipoprotein compartmentalization could be an underappreciated regulator of its biological activity.

Sean A. Burnap, MSc
Abhishek Joshi, BA(Hons), BMBCh
Sotirios Tsimikas, MD
Carlos Fernández-Hernando, PhD
Stefan Kiechl, MD
Sarah E. Berry, PhD
Wendy Hall, PhD
Bodo Levkau, MD
***Manuel Mayr, MD, PhD**

*King's British Heart Foundation Centre
King's College London
125 Coldharbour Lane
London, SE5 9NU
United Kingdom
E-mail: manuel.mayr@kcl.ac.uk
Twitter: @Vascular_Prot

https://doi.org/10.1016/j.jacc.2020.01.033

Please note: Prof. Mayr is a British Heart Foundation Chair Holder (CH/16/3/32406) with British Heart Foundation program grant support (RG/16/14/32397). The research was also supported by National Institute of Health Research Biomedical Research Centre based at Guy's and St. Thomas' National Health Service Foundation Trust and King's College London in partnership with King's College Hospital and by VASCage-Research Centre on Vascular Ageing and Stroke. As a COMET centre VASCage is funded within the COMET program-Competence Centers for Excellent Technologies by the Austrian Ministry for Climate Action, Environment, Energy, Mobility, Innovation and Technology, the Austrian Ministry for Digital and Economic Affairs and the federal states Tyrol, Salzburg, and Vienna, and the Deutsche Forschungsgemeinschaft, Sonderforschungsbereich 1116, Project B11 to Bodo Levkau, Institute for Molecular Medicine III, University Hospital Düsseldorf, Heinrich Heine University, Düsseldorf, Germany. Mr. Burnap and Prof. Mayr are named inventors on a patent application filed by King's College London for PCSK9 measurements. Dr. Tsimikas is a consultant to Boston Heart Diagnostics; is a co-inventor and receives royalties from patents owned by University of California-San Diego (UCSD) on oxidation-specific antibodies and of biomarkers related to oxidized lipoproteins; has a dual appointment at UCSD and Ionis Pharmaceuticals; and is a co-founder and has an equity interest in Oxitope, Inc. and Kleanthi Diagnostics, LLC. (Although these relationships have been identified for conflict of interest management based on the overall scope of the project and its potential benefit to Oxitope and Kleanthi Diagnostics LLC, the research findings included in this particular publication may not necessarily relate to the interests of Oxitope, Inc and Kleanthi Diagnostics, LLC. The terms of this arrangement have been reviewed and approved by the University of California, San Diego in accordance with its conflict of interest policies.) All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

REFERENCES

1. Zhang DW, Lagace TA, Garuti R, et al. Binding of proprotein convertase subtilisin/kexin type 9 to epidermal growth factor-like repeat A of low density lipoprotein receptor decreases receptor recycling and increases degradation. J Biol Chem 2007;282:18602–12.

2. Kosenko T, Golder M, Leblond G, Weng W, Lagace TA. Low density lipoprotein binds to proprotein convertase subtilisin/kexin type-9 (PCSK9) in human plasma and inhibits PCSK9-mediated low density lipoprotein receptor degradation. J Biol Chem 2013;288:8279–88.

3. Tavori H, Christian D, Minnier J, et al. PCSK9 association with lipoprotein(a). Circ Res 2016;119:29–35.

4. Fan D, Yancey PG, Qiu S, et al. Self-association of human PCSK9 correlates with its LDLR-degrading activity. Biochemistry 2008;47:1631–9.

5. Viney NJ, Yeang C, Yang X, et al. Relationship between "LDL-C", estimated true LDL-C, apolipoprotein B-100, and PCSK9 levels following lipoprotein(a) lowering with an antisense oligonucleotide. J Clin Lipidol 2018;12:702–10.

Myocardial Edema and Remodeling

A Link Between Acute Myocarditis and Septic Cardiomyopathy?

We read with great interest the paper by Aquaro et al. (1), reporting an association between cardiac magnetic resonance remodeling patterns at the 6-month follow-up and prognosis in acute myocarditis (AM). We recently reported the presence of subepicardial edema in patients with septic cardiomyopathy (SCM), which also evolved with persistent late gadolinium enhancement (LGE) and late T2 hypersignal resolution (2). Importantly, SCM is believed to be reversible, but little is known about long-term outcomes in survivors. Therefore, it is tempting to speculate that, although distinct in etiology, similar inflammation-driven remodeling patterns might carry prognostic implications in both entities. This underscores the fact that, despite seemingly preserved cardiac function in survivors, SCM may also have long-term consequences, and this issue should be addressed in future studies.

Myocardial edema results from an imbalance between microvascular filtration and lymphatic fluid removal. Until now, attention has been mainly focused on microvascular endothelial barrier dysfunction, whereas factors governing myocardial lymphatic clearance remain poorly understood. Interestingly, although the observation of dilated lymphatics in myocardial segments with edema and LGE suggests lymphatic overload as cause of edema in chronic heart failure (3), in the context of acute SCM, we have observed collapsed subepicardial lymphatic pre-collectors (2), a previously unappreciated and potentially relevant mechanism of inflammation-induced lymphatic failure with possible implications in AM.

Notwithstanding the apparent protection associated with persistent edema at 6 months when compared with LGE without edema (definite fibrosis), care should be taken to interpret this as a benign pattern. Actually, it may identify myocardial areas at risk with persistent inflammation or dysfunctional lymphatic clearance, with which a milder remodeling process might be associated, but not without the risk of later progression to fibrosis and impact on prognosis (2). In fact, stimulation of lymphatic clearance has been shown to attenuate experimental myocardial remodeling (4), suggesting edema resolution mechanisms as currently unexplored therapeutic targets in AM.

In conclusion, cardiac magnetic resonance imaging suggests a link between AM and SCM pathophysiology, with edema and inflammation as common mechanisms of myocardial injury and remodeling.

Francisco Vasques-Nóvoa, MD
António Angélico-Gonçalves, MD
Nuno Bettencourt, MD, PhD
Adelino F. Leite-Moreira, MD, PhD
***Roberto Roncon-Albuquerque Jr., MD, PhD**

*Cardiovascular R&D Center
Department of Surgery and Physiology
Faculty of Medicine of the University of Porto
Al. Prof. Hernâni Monteiro
4200-319, Porto
Portugal
E-mail: rra_jr@yahoo.com

https://doi.org/10.1016/j.jacc.2019.12.071