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PCSK9 as a Biomarker for Cardiovascular Risk

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Abstract

Background: Proprotein convertase subtilisin/kexin 9 (PCSK9) is a protein that prevents low-density lipoprotein (LDL) clearance from plasma through degradation of low-density lipoprotein receptors (LDL-R). Mutations that up- or down-regulate PCSK9 have been shown to affect risk of cardiovascular disease independently of plasma LDL level. In light of these discoveries, plasma PCSK9 level may prove useful as a biomarker for cardiovascular risk.

Methods: An exhaustive search of the available medical literature was performed using the MEDLINE (Ovid), CINAHL and Web of Science databases. Keywords included PCSK9, evolocumab, alirocumab, myocardial infarction, cardiovascular disease and coronary artery disease (CAD). Inclusion criteria consisted of studies published in English that were performed on a human population, in the absence of statin therapy with endpoints including occurrence of a first cardiovascular event and severity of coronary artery stenosis.

Results: Two studies met eligibility criteria for this systematic review. One was a cross-sectional study and the other was a nested case-control study. The first study was performed on two separate cohorts, with the first cohort of 771 patients showing no correlation between serum PCSK9 level and coronary artery disease (CAD), and the second cohort of 822 patients showing a positive correlation. Both cohorts showed a positive correlation between elevated serum PCSK9 level and acute MI but not prior MI. The second study of 716 women showed no correlation between serum PCSK9 level and risk for future cardiovascular event. Both studies had very low quality of evidence as judged by the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) guideline.

Conclusion: The two studies failed to reach a consensus on whether PCSK9 can be useful as a biomarker for cardiovascular risk; however, transiently elevated serum PCSK9 levels occurred with acute MI. Future studies could be performed to further evaluate the potential use of PCSK9 as a cardiovascular biomarker, as well as a diagnostic marker for myocardial infarction (MI).

Keywords: *PCSK9, Evolocumab, Alirocumab, Myocardial Infarction, Cardiovascular Disease*

and Coronary Artery Disease

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PCSK9, Cardiovascular Risk, Myocardial Infarction, LDL-R, Evolocumab, Alirocumab, Coronary Artery Disease, CAD, MI

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Biography

Tyler McKinnon is a native of Utah, where he majored in Biochemistry with a minor in Spanish Language at Utah State University. He served a religious mission to Bahia Blanca, Argentina for two years, where he became interested in Hispanic culture and pursued fluency in the Spanish language. Tyler was a recipient of the Irving Condie Frost Award for excellence in Organic Chemistry in 2012. He is currently pursuing a Master of Physician Assistant Studies from Pacific University in order to provide healthcare to underserved Latino communities.

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To my wife, *Catherine*, thank you for your many hours of studying with me on road trips. I'm glad we didn't fall asleep.

To *my parents*: Thank you for your support, encouragement and for teaching me how to read.

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List of Abbreviations

CAD……...Coronary Artery Disease
EmCB……..Emory Cardiology Biobank Study
GRADE……Grading of Recommendations, Assessment, Development and Evaluation
LDL……...Low Density Lipoprotein
LDL-R……Low Density Lipoprotein Receptor
LRP8…….Low Density Lipoprotein Related Protein 8
MI……...Myocardial Infarction
OHGS……Ottawa Heart Genomics Study
PCSK9…….Proprotein Convertase Subtilisin/Kexin 9
WHS…….Women's Health Study

PCSK9 as a Biomarker for Cardiovascular Risk

BACKGROUND

Proprotein convertase subtilisin/kexin 9 (PCSK9) is a protein that opposes the regulation of plasma low-density lipoprotein (LDL) levels through degradation of LDL receptors (LDL-R). Because of this fact, PCSK9 has recently become a promising target for therapy aimed at reducing plasma LDL levels and, hence, cardiovascular risk in patients.[1] Monoclonal antibodies against PCSK9, also called PCSK9 inhibitors, have demonstrated effectiveness in lowering LDL levels when taken either as monotherapy[2] or in addition to traditional statin therapy,[3] but have not yet completed trials to demonstrate effective improvement of cardiovascular outcomes. Plasma LDL levels are an important biomarker for cardiovascular risk in patients, and cardiovascular disease is the leading cause of death in both men and women in the United States, resulting in 610 000 mortalities annually, or 1 in 4 deaths.[4]

Interestingly, mutations of PCSK9 have shown greater influence on cardiovascular risk than would be expected from their accompanied LDL level. Gain-of-function mutations in PCSK9 have been found to cause familial hypercholesterolemia and are associated with premature coronary heart disease.[5] PCSK9 loss-of-function mutations can reduce LDL levels and are associated with protection against coronary heart disease. In fact, multiple studies[6-8] have shown that loss of PCSK9 function reduces risk of cardiovascular disease to a magnitude much greater than the LDL reduction alone would predict.

As an independent biomarker for cardiovascular disease, PCSK9 may be useful. This idea has been investigated before,[9] but due to the confounding effect of statins to raise serum PCSK9 levels[10] in those studies, further investigation in patients not on statin therapy is

necessary. The fact that most patients with cardiovascular risk are on chronic statin therapy makes this type of study difficult. This systematic review examines the current evidence for PCSK9 as a biomarker for cardiovascular risk in patients not on statin therapy.

METHODS

An exhaustive search of the literature was performed using the MEDLINE (Ovid), CINAHL, and Web of Science databases. Keywords included PCSK9, evolocumab, alirocumab, myocardial infarction, cardiovascular disease and coronary artery disease (CAD). Article titles and abstracts were used to determine inclusion/exclusion. Inclusion criteria consisted of studies published in English that were performed on a human population, in the absence of statin therapy with endpoints including subsequent occurrence of a first cardiovascular event and severity of coronary artery stenosis. Articles were assessed for quality using the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) guidelines. [11]

RESULTS

The initial search of MEDLINE using Ovid yielded 371 results, with 369 excluded that did not meet inclusion criteria. An identical search of CINAHL showed 30 results, with 1 relevant duplicate from Ovid. Twenty-nine results did not meet inclusion criteria. A final search of Web of Science yielded 382 results, with 1 duplicate from Ovid. The other 381 results did not meet inclusion criteria. Of the 2 included articles, one is a cross-sectional study design [12] and one is a case control design. [13] See Table I.

Almontashiri et al

This study [12] measured serum PCSK9 levels at time of angiography for 1593 adults in two separate cross-sectional studies: the Ottawa Heart Genomics Study (OHGS) and the Emory Cardiology Biobank (EmCB) study.

In the OHGS, a total of 3918 patients were enrolled. There were 3147 patients who were taking statins, so this review will focus on the 771 patients who were not on statin therapy. These patients were sorted into a case (492 patients) and a control (279 patients) group based on presence of significant coronary artery stenosis of greater than 50% for the cases and insignificant stenosis of less than 30% for the controls. Patients with intermediate stenosis between 30-50% were excluded. Patients with previous diagnosis of diabetes mellitus were also excluded. [12]

OHGS showed no significant difference in serum PCSK9 levels between cases and controls in patients not taking statin therapy. Cases had an average serum PCSK9 level of 309.0 +/- 99.7 ng/mL while serum PCSK9 level, on average, for controls, was 317.1 +/- 131.9 ng/mL ($p = 0.376$). [12]

Elevated serum PCSK9 levels in OHGS were associated with acute myocardial infarction, but not with prior incidence of MI. Of patients not taking statins in OHGS, 94 had an MI, with 45 experiencing an acute MI and 49 having experienced a prior MI. Average serum PCSK9 level was 363.5 +/- 140.0 ng/mL for patients experiencing acute MI compared to 302.0 +/- 91.3 ng/mL for 398 cases without MI ($p = 0.004$). Serum PCSK9 levels were, on average, 315.9 +/- 107.5 ng/mL for patients with prior MI ($p = 0.977$). [12] See Table II.

In the EmCB study, a total of 2357 patients were enrolled, of which 822 plasma samples were taken for this study. Patients were excluded if they were on statin therapy or had a prior diagnosis of diabetes mellitus. These patients were sorted in a similar fashion to the OHGS study, with 465 cases showing greater than 50% stenosis of a coronary artery and 357 controls showing less than 20% stenosis. Patients with stenosis between 20-50% were excluded. [12]

In contrast to OHGS, EmCB showed a significant association between serum PCSK9 levels and coronary artery stenosis. Average serum PCSK9 level for cases was 385.0 +/- 146.9 ng/mL, while the average level for controls was 340.4 +/- 125.2 ng/mL ($p < 0.001$). [12]

Elevated serum PCSK9 levels were associated with acute MI in EmCB, but, like OHGS, there was no association with prior MI. Acute MI occurred in 74 patients, with an average PCSK9 serum level of 445.0 +/- 171.7 ng/mL. This is compared to 273 patients who did not experience acute or prior MI and had an average serum PCSK9 level of 369.9 +/- 139.1 ng/mL ($p = 0.0037$). There were 118 patients who experienced a prior MI, and they had an average PCSK9 level of 382.3 +/- 139.1 ng/mL ($p = 0.0954$). [12] See Table II.

Ridker et al

This nested case control study [13] measured baseline serum PCSK9 levels in 716 women from a total prospective cohort of 28 263 women in the Women's Health Study (WHS). The cohort was initially free of reported cardiovascular disease and patients were subsequently followed for an average of 17 years to evaluate occurrence of cardiovascular events, including MI, stroke or death from coronary heart disease. There were 358 cases, consisting of patients who experienced a cardiovascular event during follow-up, and 358 matched controls who did not

experience a cardiovascular event. Patients were excluded from this study if they were on statin therapy. [13]

Elevated serum PCSK9 levels were not shown to correlate with subsequent occurrence of cardiovascular events in the Ridker et al study. The average serum PCSK9 level for cases was 304.4 ng/mL (252.9-365.9) compared to an average of 299.7 (252.9-358.8) for the controls (p = 0.94). [13] See Table III.

DISCUSSION

The results from the reviewed studies are unclear as to whether serum PCSK9 levels correlate with future cardiovascular risk in patients. The authors of the two studies [12,13] cited in this review support a lack of correlation; however, this picture is not entirely clear. Almontashiri et al [12] found no association between serum PCSK9 level and coronary atherosclerosis in patients not taking statin therapy in OHGS, but did find correlation in patients not taking statin therapy in EmCB. Ridker et al [13] found no correlation between plasma PCSK9 level in women not taking statins and risk of future cardiovascular events. The very low quality of the evidence presented herein necessitates further study to ascertain an accurate picture of the role of PCSK9 as a cardiovascular biomarker.

The discrepancy between analogous findings in the OHGS and EmCB by Almontashiri et al [12] may be explained by the inclusion of controls with aortic valve disease and minimal coronary artery stenosis in OHGS [14], due to the unknown relationship between aortic valve disease and serum PCSK9 levels. The authors put forward an additional possibility that the correlation found between serum PCSK9 level and CAD incidence in EmCB was caused by the differential control criteria between OHGS and EmCB, with controls having less than 30%

coronary stenosis in OHGS and less than 20% stenosis in EmCB. [12] This theory seems paradoxical, however, because it uses a correlation between PCSK9 level and CAD to explain away a correlation between PCSK9 and CAD, and is therefore unlikely.

The finding of plasma PCSK9 level elevation with acute MI is of note in the study by Almontashiri et al. [12] Though plasma PCSK9 level was elevated with acute MI, it was found to have no elevation associated with prior MI, pointing to the probability of transient PCSK9 elevation with MI. Further investigation into the cause of this transient elevation may help explain why PCSK9 mutations appear to affect coronary heart disease risk independently of LDL level.

The possibility that PCSK9 functions as an acute phase reactant in acute MI was suggested by Almontashiri et al [12] as an explanation for its' transient elevation in acute MI. A study [15] is cited as finding that serum PCSK9 levels are not elevated acutely in severe trauma, however, which points away from this hypothesis.

Another interesting theory proposed by the authors was that PCSK9 elevation may transiently occur prior to acute MI. [12] PCSK9 has been shown to bind to LDL-R related protein 8 (LRP8), [16] which may promote platelet aggregation [17] in patients. Serum PCSK9 levels, incidence of acute coronary events and platelet aggregation are all highest in the morning, [18-20] providing support for this idea.

Transient elevation of serum PCSK9 level with MI suggests that further study may be done to evaluate PCSK9 as a clinical diagnostic marker for acute MI, as well as pharmaceutical PCSK9 inhibition for management of acute MI. The completion of further studies evaluating

long-term cardiovascular outcomes with PCSK9 inhibitors will guide future treatment guidelines for this therapy and possibly help clarify the role PCSK9 plays in cardiovascular disease.

The study by Ridker et al [13] was performed on a cohort completely composed of women. This is a drawback, because the majority of cardiovascular deaths in the United States occur in men. [4]

Future research should be directed at cohorts composed of both men and women who are not being treated with statin therapy in order to accurately determine whether PCSK9 can be used as a biomarker for future cardiovascular risk, or to help in the diagnosis of acute MI.

Until further research can fully assess its' value, it appears that the use of PCSK9 as a biomarker for cardiovascular risk is limited.

CONCLUSION

Although two studies performed on patients in the absence of statin therapy have failed to reach a concrete consensus as to whether elevated plasma PCSK9 levels correlate with future cardiovascular risk, serum PCSK9 level showed a correlation with acute MI and not with prior MI, indicating a transient elevation with MI occurrence. Further investigation in cohorts consisting of men and women not currently being treated with statin therapy will be necessary to evaluate whether PCSK9 can be used as a biomarker for cardiovascular risk or as a diagnostic marker for acute MI.

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Table I. Characteristics of Reviewed Studies

Study	Design	Downgrade Criteria					Upgrade Criteria	Quality
		Limitations	Indirectness	Inconsistency	Imprecision	Publication Bias		
Almontashiri et al[1]	Cross-Sectional	Not Serious	Not Serious	Serious[a]	Not Serious	Unlikely	NA	Very Low
Ridker et al[2]	Case-Control	Not Serious	Serious[b]	Not Serious	Not Serious	Unlikely	NA	Very Low

[a] Serum PCSK9 level was not predictive of CAD in OHGS, but was predictive of CAD in EmCB

[b] Serum PCSK9 levels in WHS were only collected on women

Table II. Summary of Findings from Almontashiri et al [12]

Authors	Study Design	Cohort	Group	Number of Patients	Age (Years)	Male Sex, N (%)	PCSK9 (ng/mL)	p-value
Almontashiri et al	Cross-Sectional	OHGS	Cases	492	65 +/- 11	334 (67.9)	309.0 +/- 99.7	0.376
			Controls	279	63 +/- 12	132 (47.3)	317.1 +/- 131.9	
		EmCB	Cases	465	65 +/- 12	352 (75.7)	385.0 +/- 146.9	0.000003
			Controls	357	56 +/- 12	193 (54.1)	340.4 +/- 125.2	

Table III. Summary of Findings from Ridker et al [13]

Authors	Study Design	Cohort	Group	Number of Patients	Age (Years)	PCSK9 (ng/mL)	p-value
Ridker et al	Case-Control	WHS	Cases	358	63 +/- 5	304.4 (252.9-365.9)	0.94
			Control	358	63 +/- 5	299.7 (252.9-358.8)	