Validity of Vascular Calcification as a Screening Tool and as a Surrogate End Point in Clinical Research


Online Data Supplement

On a world scale, in 2013, there have been >17 million cardiovascular deaths, one half of which attributable to ischemic heart disease. More than 50% of cases of ischemic heart disease deaths presenting as sudden cardiac death have no history of symptoms heralding the critical event, and most myocardial infarctions occur in low or moderate risk subjects (ie, in the largest segment of the general population). On a lifetime scale, the risk of myocardial infarction at the age of 40 years is 1 in 2 men and 1 in 3 women. These figures underline the potential relevance of timely screening and treatment of individuals at risk of cardiovascular disease.

The Framingham Risk Score or the recently developed risk calculator, which forms the American College of Cardiology/American Heart Association cholesterol guideline, the HeartScore, or the QRISK calculator, are center pieces of current policies for cardiovascular risk assessment and for lipid-lowering treatment. Because in these calculators age is the most informative risk factor for the prediction of cardiovascular events, these instruments have limited precision for the prediction of coronary atherosclerosis at individual level particularly in young subjects who have a low absolute risk for cardiovascular disease events. Novel biomarkers measurable in plasma or serum have been intensively investigated to refine risk prediction in individuals at low or moderate risk, but the gain in prediction power by these biomarkers is just of modest degree.

Imaging biomarkers provide an estimate of the atherosclerotic burden in critical areas of the arterial system like the coronary circulation and may provide an integrated measure of life time exposure to risk factors. Among imaging markers of atherosclerosis assessment of vascular calcification, in particular of coronary calcification, is considered as the most valuable one because it outperforms other imaging techniques such as carotid intima media thickness. Because coronary calcium reflects well the risk for coronary heart disease events and other major cardiovascular events, the coronary calcium score has gained momentum well beyond screening and risk prediction, and this biomarker has been increasingly adopted as a surrogate of clinical events in trials testing disparate interventions for the prevention of cardiovascular disease in various populations.

Vascular calcification is a hallmark in patients with chronic kidney disease (CKD), a population with a high cardiovascular risk, and for this reason, CKD is the condition where the coronary calcium score and calcification at other sites of the cardiovascular system have been most frequently applied as a surrogate end point to investigate experimental treatments in CKD. The interest on coronary calcification as a surrogate end point is not fading. As on January 22, 2015, >100 clinical
These recommendations were class II B, ie, their usefulness in asymptomatic adults at intermediate risk (10%–20% cardiovascular risk assessment). The American Heart Association and the American College of Cardiology recommended measurement of coronary calcium as a reasonable option for cardiovascular risk assessment by the American College of Cardiology/American Heart Association guidelines calculator and again scored this recommendation class II B.

The evidence supporting the use of coronary calcium score in primary prevention was summarized in 2012 by Whelton et al in a meta-analysis of randomized trials testing the impact of coronary calcium screening on lifestyle and risk factors modification. This meta-analysis identified just 4 trials published between 2003 and 2011 totaling 2490 individuals, 3 of 5 of whom enrolled in the Early Identification of Subclinical Atherosclerosis by Noninvasive Imaging Research (EISNER)22 trial. Three trials reported a nonsignificant 15% increase in the probability of smoking cessation in individuals screened with the calcium score when compared with those not submitted to this test. In the EISNER trial, a higher calcium score associated with an increased prescription of lipiddowering medications and, conversely, the absence of calcium in the coronary arteries associated with fewer prescriptions of these medications. Furthermore, in this trial, small but significant reductions in systolic blood pressure (−2 mm Hg) and in low-density lipoprotein cholesterol (−4.3 mg/dL) were registered. However, in the meta-analysis, the pooled estimates of the drop in blood pressure (−0.23 mm Hg/−0.42 mm Hg) and low-density lipoprotein cholesterol (−0.23 mg/dL) were clinically trivial. Thus, the motivational benefit of coronary calcium screening seems to be modest at best. After this meta-analysis, in 2012, a nonrandomized screening intervention that tested the effect of risk evaluation by the coronary calcium and the HeartScore on the use of preventive treatments in 1075 Danish people was published. At follow-up, 21% of patients with a high calcium score (n=462) and 19% of those with high HeartScore (n=233) received lipid-lowering treatment, whereas 25% and 32%, respectively, received antihypertensive treatment. The presence of a high calcium score was associated with an increased use of lipid-lowering treatment (odds ratio, 2.2; 95% confidence interval, 1.2–4.0), whereas the presence of a high HeartScore was associated with an increased use of lipid-lowering treatment (odds ratio, 2.9; 95% confidence interval, 1.6–5.5) and antihypertensive drugs (odds ratio, 3.4; 95% confidence interval, 1.9–6.0), indicating that the calcium screening was not superior to the HeartScore for these motivational outcomes. Therefore, this study provided further proof against the motivational usefulness of coronary calcium screening in primary prevention. A new meta-analysis on the same issue published in June 2014 included the Danish study and trials considered in Whelton meta-analysis and 12 observational studies. The conclusion of this meta-analysis was more optimistic than that of the previous one. However, the apparent benefit of the calcium score for screening was entirely driven by the 12 observational studies. Observational studies looking at motivational outcomes, like those included in Mamudu meta-analysis,
are particularly prone to bias, and their value is highly questionable. Therefore, the most recent meta-analysis does not represent a solid gain in the evidence-based assessment of the usefulness of coronary calcium screening in primary prevention.

Techniques for estimating calcium content in the arterial wall are continuously being refined. Probably because of the unique propensity to calcification of CKD patients, the Agatston score performed well as a risk predictor in a recent subanalysis of the Multi-Ethnic Study of Atherosclerosis (MESA) focused on patients with CKD. Another recent reanalysis of scans collected in the whole MESA cohort applying a novel density score showed that it further improves risk discrimination when compared with traditional risk factors and the Agatston score. The gain in discrimination by this score was highly significant but modest (area under receiver operating characteristic curve from 0.700 to 0.711), but the same score allowed a clinically meaningful (+13.9%) improvement in risk reclassification in patients at intermediate risk. Also taking into account the relevant net reclassification improvement of the Agatston score in previous analyses in MESA and in the Rotterdam study, it can be hypothesized that a normal (ie, zero) calcium score as measured by the new density score in patients at intermediate risk may indicate an underlying true risk level low enough to avoid prevention of cardiovascular disease by drug treatment and an elevated score an indication to start or maintain long-term treatment. However, a prevention policy based on such an approach still remains to be investigated in a formal clinical trial.

The design of a large-scale trial aimed at testing the value of coronary calcium screening in patients at low-to-intermediate risk who are not already candidates for statins and aspirin, a large segment of the general population, was published in 2012. Mainly because of the high number of low-to-intermediate risk individuals to be enrolled (≈3000) and the high cost, such a trial never advanced further than the design phase. Although the new calcium density score or other scores can be useful for increasing the discrimination of coronary calcium, it is unlikely that in the medium-term coronary calcification will be properly tested (ie, incorporated in a clinical trial). The degree of improvement in risk discrimination registered in Criqui’s reanalysis of MESA, although not trivial, is per se considered insufficient to change guidelines recommendations.

**Coronary Calcium as a Surrogate End Point in Clinical Trials**

A systematic review published in 2009 gathered 10 trials testing the effect of various treatments on coronary calcium progression. Five of these trials enrolled patients with established cardiovascular disease (n=2135) and 5 focused on patients with CKD (n=477). The mean weighted annualized coronary calcium score increased both in patients with cardiovascular disease and in those with CKD but without any consistent or reproducible treatment effect of any therapy on this outcome. Therefore, the main conclusion of this meta-analysis was that the change in coronary calcium does not represent a suitable surrogate end point to be applied in randomized clinical trials in patients with cardiovascular or renal disease.

After this meta-analysis, 1 additional trial that tested the effect of an aged garlic extract and a vitamin B complex in patients at intermediate risk for cardiovascular disease was published. Therefore, the 5 trials in cardiovascular disease mentioned above, the trial testing the garlic extract and an additional trial performed in 2007, which was missed in the same meta-analysis, represents the whole analytic base, whereupon we can estimate the effect of treatment on coronary calcium in patients with cardiovascular disease without major renal involvement (Table S1 in the online-only Data Supplement). Three of these trials measured coronary calcium by adopting the volumetric method, and 1 by both methods. Three trials compared a statin with a placebo, 1 compared a high with a low dose of the same statin, one compared 2 different statins, and 1 compared nifedipine with an amiloride–hydrochlorothiazide combination. In the 3 placebo-controlled statins trials, the average annualized weighted progression of coronary calcification of patients treated with statins (+15.9%) was similar to that registered in the corresponding placebo arms (+14.0%). More important, in the largest of these trials, in the St Francis Heart Study, there was a clear dissociation between the progression of coronary calcification, which was completely unaffected by treatment and the cardiovascular event rate, which reduced in the active arm of the trial (placebo 9.9%, atorvastatin 6.9%, relative risk reduction, ~30%; P=0.08). Similarly, in the 2 studies, which compared 2 statins or a low and a high dose of the same statin (Table S1), the progression of calcification was almost identical and largely not significant (P=0.64 and 0.60, respectively). Overall, the lack of effect of statins on coronary calcium is in sharp contrast with the beneficial effect of statins in primary and in secondary prevention. Failure of coronary calcification to capture the solidly established benefit of statins for cardiovascular prevention goes along with biological knowledge that this class of drugs reduces the noncalcified portion of plaques but does not modify, or may even increase, plaque calcification. Atorvastatin significantly reduced noncalcified plaque burden but did not modify the calcification score in a follow-up study in a series of 46 patients at high risk for coronary heart disease. In brief, coronary calcification fails as a surrogate for the effect of statins because it mainly reflects a pathway (calcification), which does not coincide with the pathway conducive to clinical events (occlusive disease; Figure S2). In the Intervention as a Goal in Hypertension Treatment trial (INSIGHT), nifedipine once daily sensibly slowed the progression rate of coronary calcification (40% versus 78% over a 3-year follow-up) when compared with an amiloride–hydrochlorothiazide combination (coamilozide), but the favorable effect on coronary calcification by nifedipine failed to translate into a superior clinical benefit. Indeed, in this trial, the occurrence of overall cardiovascular or cerebrovascular complications (combined end point rate: 6.3% for nifedipine and 5.8% for coamilozide) did not differ. Nifedipine per se may favorably interfere with many calcium-dependent events in the formation of atherosclerosis, whereas, in contrast, thiazides may favor mineralization. The unreliability of coronary calcification as a surrogate in trials
testing nifedipine and chlortalidone may, therefore, depend on the fact that the differing effect of these drugs on coronary calcification has little bearing for cardiovascular outcomes, which depends on their (shared) favorable interference on the main pathway leading to these outcomes in hypertensive patients (ie, the blood pressure burden on the cardiovascular system; Figure S2). Interestingly, the coronary calcium score was substantially reduced by the aged garlic extract/vitamin B complex/l-arginine in the trial by Budoff et al,40 suggesting a beneficial effect by this therapeutic combination in asymptomatic patients with measurable levels of calcium in the coronary arteries. However, because of the lack of a corresponding trial based on clinical end points, no conclusion can be formulated about the validity of the calcium score as a surrogate for measuring the effect of such a combination on coronary heart disease prevention.

Because of the peculiar propensity to vascular calcification of patients with CKD, trials in this population deserve separate discussion. In this population, 4 additional trials were performed between 2009 and 2014.48–51 These trials, all open labeled, and an additional trial published in 2004,52 which was missed in the previous meta-analysis, added 748 patients to the 477 patients with CKD of the previous meta-analysis,29 bringing the total to 1125 patients with CKD. Nine trials compared different strategies for controlling hyperphosphatemia (sevelamer or lanthanum carbonate [1 arm in a trial testing also sevelamer] versus calcium-based phosphate binders). One of these trials included also a placebo arm (one a low phosphate diet and another a rosuvastatin arm) or a calcium receptor agonist (cinacalcet) associated with low-dose vitamin D versus flexible doses of vitamin D for controlling hyperparathyroidism. The 9 trials comparing sevelamer (a pleiotropic noncalcium phosphate-binder with lipid-lowering activity) versus calcium-based binders showed a clear-cut reduction in coronary calcium (Figure 1). This effect apparently goes along with a new meta-analysis of 7 trials in hemodialysis patients and 1 trial in moderate CKD35,38,39,53–57 (updating a meta-analysis by Jamal et al58) that shows a lower mortality in patients treated with sevelamer (risk reduction 57% by the random-effect approach and 30% by the fixed-effect approach). However, this apparent benefit is mainly driven (heterogeneity I²=89%) by a study in hemodialysis patients showing an astounding 91% risk reduction by sevelamer. Given the high heterogeneity (I²=89%) among studies looking at the effect of this drug on mortality (Figure 1), the validity of the coronary calcium score as a surrogate of clinical events in studies testing sevelamer still requires further scrutiny in future trials. Lanthanum carbonate, another noncalcium binder, did not slow the progression of calcification in 18 patients treated with this drug when compared with 22 patients treated with calcium-based phosphate binders in a

Figure 1. Face-to-face comparison of trials looking at coronary calcification as an end point and trials testing the effect of the same drugs on mortality in patients with chronic kidney disease (CKD). CI indicates confidence interval.
trial in patients with predialysis CKD by Block et al. More important, no concordance emerged between the effect of cinacalcet (another major drug applied to treat the bone mineral disorder in CKD) on vascular calcification and on the risk for mortality and cardiovascular events. The ADVANCE study (a randomized study to evaluate the effects of cinacalcet plus low-dose vitamin D on vascular calcification in subjects with CKD receiving hemodialysis) showed that the effect of cinacalcet just failed to meet the primary end point of change in Agatston score but coherently slowed the progression rate in coronary calcification as expressed in volumetric terms ($P=0.009$) and progression of calcification in heart valves and in the thoracic aorta. On the other hand, no benefit by this drug on mortality and cardiovascular outcomes was registered in the Evaluation of Cinacalcet Therapy to Lower Cardiovascular Events (EVALUATE) trial, one of the largest drug trials (n=4000 patients) and one with the longest follow-up ($\leq 64$ months) ever performed in the hemodialysis population (Figure 2). This discrepancy once again suggests that coronary calcification is an unreliable biomarker of the progression of coronary artery disease also in patients with CKD. Thus, trials in patients with CKD largely fail to provide coherent evidence that coronary calcification is a valid surrogate end point in this population.

Overall, results of studies in the general population, in patients with established coronary heart disease and in patients with CKD do not support the adoption of coronary calcification as a surrogate end point in cardiovascular research. Coronary calcification is probably the biomarker that allows the greater gain in risk reclassification improvement in patients at intermediate risk. However, the usefulness of coronary calcium measurement is restricted to its reclassification power with no substantial gain in risk discrimination or calibration. The hypothesis that the gain in reclassification ability by coronary calcium may be helpful for guiding treatment is negated by available trials. Although better predictive of future cardiovascular events than circulating biomarkers, coronary calcium scanning poses the risk of exposure to ionizing radiation. The effective dose of a calcium score study is $\approx 5\times$ less (2–3 mSv) than a 64-slice coronary computed tomographic study and with modern technology exposure to a single calcium score examination is roughly equal to average 1-year environmental radiation exposure. Such an exposure should not be overlooked because of the effects of ionizing radiation on human health are cumulative. Warnings to reduce radiation exposure for the diagnosis or the monitoring of cardiovascular disease have been issued by major cardiology societies like the European Society of Cardiology.

Although the fundamental mechanism(s) underlying the pathogenesis and progression of coronary calcification and vascular calcification in general have been intensively studied during the past years, the relationship between calcium accumulation and atherosclerosis remains incompletely understood. Vascular biology and imaging studies support the interpretation that calcification is a late, difficult to regress, event in the atherosclerosis process. The effect of treatments that reduce the atherosclerosis burden and the risk for clinical events most often are not captured by the dynamics of vascular calcification. By now, imaging studies aimed at quantifying coronary calcium and vascular calcification should be better restricted to research settings aimed at clarifying the pathobiology of atherosclerosis, rather than be extended to screening policies or applied in studies testing interventions in clinical trials. Whether vascular calcification at sites other than the coronary circulation may represent valid surrogate end points remains an open question to be evaluated in additional studies.

Disclosures

C. Zoccali has received speakers’ honoraria from Abbvie. R. Vanholder has received speakers’ and travel honoraria from Bayer. Z. Massy has received speakers’ honoraria and research grants from Amgen, Genzyme, and Fresenius Medical Care. A. Ortiz has received speakers’ and travel honoraria from Genzyme as Sanofi company, Shire and Abbvie. The other authors reports no conflicts.

References


Validity of Vascular Calcification as a Screening Tool and as a Surrogate End Point in Clinical Research


on behalf of the European Renal and Cardiovascular Medicine (EURECA-m) Working Group of the European Renal Association-European Dialysis Transplantation Association (ERA-EDTA)

Hypertension. 2015;66:3-9; originally published online May 11, 2015;
doi: 10.1161/HYPERTENSIONAHA.115.04801

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2015 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/66/1/3

Data Supplement (unedited) at:
http://hyper.ahajournals.org/content/suppl/2015/05/11/HYPERTENSIONAHA.115.04801.DC1
http://hyper.ahajournals.org/content/suppl/2016/04/11/HYPERTENSIONAHA.115.04801.DC2

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org/subscriptions/
Valid surrogate. The surrogate is in the main pathogenic pathway and there is a strong association between the surrogate and the outcome. The intervention acts on the pathway towards the surrogate.

Invalid surrogate. The surrogate is in a pathway parallel to the main pathogenic pathway and the intervention affects the surrogate but not the main pathway.

Invalid surrogate. The surrogate is in a pathway parallel to the main pathogenic pathway and the intervention only affects the main pathway.

Invalid surrogate. The surrogate is in a pathogenic pathway conductive to outcome but the intervention acts also on a separate, second pathogenic pathway that contributes importantly to the outcome.
SUPPLEMENTARY APPENDIX

Full authorship affiliation list

Carmine Zoccali¹  Davide Bolignano¹, Graziella D’Arrigo¹, Friedo W Dekker², Danilo Fliser³, 
Gunnar H Heine³, Kitty J Jager⁴, Mehmet Kanbay⁵, Francesca Mallamaci¹,⁶, Ziad Massy⁷, Alberto 
Ortiz⁸, Gianfranco Parati⁹, Patrick Rossignol¹⁰, Giovanni Tripepi¹, Raymond Vanholder¹¹, Andrzej 
Wieck¹², Gerard London¹³

On behalf of the EUniverse REnal and Cardiovascular Medicine (EURECA-m) working group of 
the European Renal Association – European Dialysis Transplantation Association (ERA-EDTA)

1. CNR-IFC  Pathophysiology of Renal Diseases and Hypertension Unit, Reggio Calabria, 
   Italy. carmine.zoccali@tin.it
2. Department of Clinical Epidemiology, Leiden University Medical Center, Leiden 
   f.w.dekker@lumc.nl
3. Department of Internal Medicine IV, Saarland University Medical Centre, Homburg/Saar, 
   Germany. prof.dr.danilo.fliser@uniklinikum-saarland.de; Gunnar.Heine@uniklinikum- 
   saarland.de
4. European Renal Association-European Dialysis and Transplant Association (ERA-EDTA) 
   Registry, Department of Medical Informatics, Academic Medical Center, University of 
   Amsterdam, Amsterdam, K.J.Jager@amc.uva.nl
5. Division of Nephrology, Department of Medicine, Koc University School of Medicine, 
   Istanbul, Turkey.
6. Nephrology, Dialysis and Transplantation Unit and CNR-IFC Clinical Epidemiology 
   francesca.mallamaci@libero.it;
7. Paris Ille de France Ouest (UVSQ) University, Paris, France  ziad.massv@apr.aphp.fr\textsuperscript{IIIS}-
8. Fundación Jiménez Diaz, Universidad Autónoma de Madrid, Fundación Renal Iñigo 
   Alvarez de Toledo, Madrid, Spain. AOrtiz@fjd.es
9. Department of Cardiovascular, Neural and Metabolic Sciences, S. Luca Hospital, Istituto 
   Auxologico Italiano &University of Milan-Bicocca, Piazzale Brescia 20, Milan 20149, Italy. 
   Gianfranco.parati@unimib.it
10. Inserm, Centre d'Investigations Cliniques-Plurithématique 1433, Inserm U1116, Nancy, 
    France; CHU Nancy, Département de Cardiologie, Institut Lorrain du Cœur et des 
    Vaisseaux, Vandoeuvre lès Nancy, France; Université de Lorraine, France; INI-CRCT 
    (Cardiovascular and Renal Clinical Trialsist), French-Clinical Research Infrastructure 
    Network (F-CRIN), France. p.rossignol@chu-nancy.fr
11. Ghent University Hospital, Department of Nephrology, Department of Internal Medicine, 
    University Hospital Gent, De Pintelaan 185, B9000 Ghent ; raymond.vanholder@ugent.be
12. Department of Nephrology, Endocrinology and Metabolic Diseases, Medical University of 
    Silesia, Francuska 20-24 Str, PL-40-027, Katowice, Poland. awiecek@spskm.katowice.pl
13. INSERM U970, Hopital Européen Georges Pompidou, Paris, France. glondon@club- 
    internet.fr
Vascular Biology of arterial calcification and calcium quantification in the cardiovascular system

Calcification in the arterial system may occur either in the intima or in the media of the vascular wall or at both sites. Intima calcification may have a pro-occlusive effect while media calcification may contribute to vascular stiffening. Calcifications are related to unbalance between inhibitors and activators of calcification process in conditions of saturated calcium and phosphate extracellular fluid concentrations. In both calcification types this unbalance activates and recapitulates mechanisms of embryonic bone formation and is similar to endochondral and membranous ossification process [1]. Intimal calcification is a hallmark of advanced atherosclerosis and is an inherently multifactorial process. Aging, hypercholesterolemia, diabetes, hyperphosphatemia represent pro-calcifying stimuli [1,2] which may lead to arterial wall ossification via oxidative stress and inflammation [3]. Osteogenesis driven by inflammatory mechanism is already evident in the early phases of atherosclerosis when studied by molecular imaging techniques [4]. Proinflammatory cytokines -including IL6 and TNFα and other cytokines- released by macrophages within the context of the arterial wall stimulate bone morphogenetic proteins (BMP2, BMP4) and Msx2 (muscle segment homeobox gene 2). The activation of Msx2 triggers calcification via paracrine WNT (wingless and integration 1 family) signals and β-catenin, which is a co-regulator of a series of transcription factors (Runx2, osterix, and Sox9) which contribute to transform vascular smooth cells and pericytes into ‘osteoblast-like’ cells, i.e. cells endowed with the full enzymatic and protein array (alkaline phosphatase, osteocalcin, osteopontin) needed for the formation and the local deposition of apatite nanocrystals [5]. On the other hand the pro-inflammatory milieu promotes proteolysis via matrix metalloproteinases and cathepsin-S. Proteolysis disrupts elastic lamellae and releases elastin fragments which in turn amplify vascular muscle cells dedifferentiation and calcium deposition. Overall, experimental studies coherently show that inflammatory mechanism(s) herald vascular calcification both in the intimal layer[6] and in smooth muscle cells in the tunica media [1].

Due to the multiple and complex alterations of the hormonal systems regulating calcium and phosphate metabolism [7], CKD patients have a unique propensity to vascular calcification. Hyperphosphatemia is a relevant pro-calcifying stimulus in advanced CKD. High phosphate stimulates production of reactive oxygen species and activates NF-κB, i.e. a critical pathway mediating the cell response to oxidized LDL, cytokines and other factors thereby favoring the transformation of mesenchymal cells into osteoblasts [8]. On the other hand the role of hyperparathyroidism in vascular calcification remains uncertain for two reasons. First, because PTH—besides having osteoclastogenic properties—has an anabolic action on the bone and, when administered intermittently, actually prevents vascular calcification [9]. Second, because in adynamic bone disease in end stage renal disease PTH suppression rather than hyperparathyroidism associates with vascular calcification [10].

In brief, various vascular biology studies in vivo and in vitro point to inflammation as an important driver of vascular calcification. Imaging studies in patients with atherosclerosis nicely confirm biological observations. A survey based on positron emission tomography (PET) adopting 18-flourodeoxyglucose (18-FDG) uptake as biomarker of inflamed areas [11] demonstrated that inflammation and calcification rarely overlap in the same plaque indicating that the two phenomena represent distinct stages of atherosclerosis. Furthermore, elegant longitudinal studies combining PET18-FDG and computed tomography show that inflammation antedates arterial calcification in human atherosclerosis [12] (Figure S1). Vascular calcification reflects the progression of inflammation and atherosclerosis and for this reason is considered as a surrogate marker of plaque burden and disease extension.

Calcium in the cardiovascular system can be quantified by electron beam computed tomography (EBCT) and by multidetector computed tomography (MDCT). EBCT is faster than MDCT but the second technique has a better spatial resolution and is cheaper. A strong association exists between the presence and severity of calcification in thoracic aorta, in heart valves and in
coronary arteries [13]. Even though quantification of calcium in cardiac valves and in the thoracic aorta has been reported in a number of studies and applied as an outcome measure in some clinical trials [14], the coronary arteries are by far the most investigated cardiovascular territory for calcification in diagnostic, prognostic and therapeutic studies. For this reason, the discussion below will mainly focus on coronary calcium quantification.

The Agatston score is calculated by multiplying the lesion area surface in mm² by a density factor (ranging between 1 and 4)[15]. Because the density factor is a 4 point categorical scale, changes in this score might not accurately capture changes in coronary calcium. The calcium volume score (CVS) is calculated as the product of calcified voxels in the volume data set (voxel is a volume element which corresponds to a pixel for a given slice thickness) multiplied by the volume of one voxel. CVS estimates calcium in a well-defined volume rather than on a surface and therefore reduces variability between scans[16]. An increase in the Agatston score over time might depend on a pure increase in plaque signal attenuation by calcium rather than on an increase in calcified plaque size while an increase in CVS provides also a volumetric estimate of the calcified plaque. Progression in coronary calcification can be calculated as absolute or percentage change in the Agatston score or in the CVS. There is no golden standard for measuring coronary calcification progression and there is no precise increase which may be used to define worsening in coronary calcification over time. Even though recent studies are increasingly based on volumetric scores, which are methodologically preferable, the vast majority of clinical trials performed so far (Table S1) adopted the Agatston score which is not a volumetric index[15].

Validity of biomarkers as screening tools and as surrogates of clinical events
Cardiovascular prevention policies demand reliable risk stratification to guide the application of appropriate prevention therapies. Instruments for risk stratification need to be easy to apply and cheap, should accurately predict risk and should have the potential for guiding preventive interventions. Tests for accuracy include risk discrimination (c statistics, area under the ROC curve), calibration (Hosmer-Lemeshow test) and risk reclassification[17]. The previously discussed risk scores which presently inform preventive therapies in the USA and in Europe are based on classical risk factors. These scores are easy to apply and cheap, have good ability for risk discrimination, i.e. provide good rank ordering for cases and non-cases, and reasonably good ability to predict accurately the absolute level of risk that is subsequently observed (calibration)[17]. Importantly, the predictive value of these scores has been confirmed in different prospective cohorts (external validation). In theory the use of these risk estimators should have been compared to non-use in a randomized trial to see whether they may lead to better outcomes by improving underuse while avoiding overuse of preventive therapies. No such trial has been performed for the new ACC/AHA Pooled Cohort Risk Assessment calculator or for the Framingham risk score or the HeartScore or the QRISK. Nonetheless, these risk calculators are pragmatically considered as valid and cost-effective instruments to inform prevention therapies. In other words they have been adopted as the most convenient standard for risk stratification. It is worth remarking that risk calculators serve only to predict risk and therefore do not need to be based on variables which biologically reflect the disease whose risk they are applied to predict. Age is the most important component of the equations wherupon cardiovascular risk scores are based but age per se does not reflect neither cardiovascular disease nor cardiovascular disease severity. We briefly discussed the limitation of current risk scores for individual risk prediction in subjects at intermediate or low risk and alluded to the potential advantage of imaging biomarkers. In order to be considered valid for application as screening tools in cardiovascular prevention, biomarkers should pass the same tests demanded to risk scores (discrimination, calibration, external validation and cost-effectiveness) with the proviso that when tested for accuracy, they should provide prognostic information above and beyond that given by established risk scores[18]. In other words, they should improve the degree of risk discrimination and risk calibration we can
achieve simply on the basis of cardiovascular risk scores. Furthermore, imaging biomarkers should also sensibly improve the risk classification provided by the same scores (risk reclassification). Of particular importance, imaging biomarkers and biomarkers in general also need to be tested in a clinical trial to see whether a prevention policy guided by the same biomarkers may improve clinical outcomes[18].

Clinical correlates are indicators that reflect disease severity or activity and as such they are considered as potential surrogates of clinical events for application in clinical research. According to the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH)[19] a clinical correlate represents a valid surrogate of a corresponding clinical end-point only when (a) it is biologically related to the end point in question, i.e. in the same pathway leading to disease (b) it predicts in proper epidemiological studies the same clinical end point (c) its changes reliably reflect the treatment effects on the corresponding clinical end point in randomized clinical trials. The simple biological association between the clinical correlate and the clinical outcome is insufficient to promote the same correlate to the rank of valid surrogate end point[20]. The list of clinical correlates is virtually innumerable but we have very few validated surrogate end-points. Perhaps the only surrogate endpoints in cardiovascular medicine which reliably predict changes in clinical endpoints in response to experimental interventions are cholesterol for coronary heart disease events and BP for stroke in most clinical settings.

Failure of most clinical correlates to qualify as valid surrogates depends on the fact that correlates do not necessarily reflect the actual change in the relevant clinical end point in response to the experimental intervention. A new anti-platelet agent may decrease platelet aggregation in vitro (a potential marker of the risk for thrombosis) but this effect does not necessarily imply that the use of this drug results in a longer cardiovascular disease free survival. Clinical correlates may fail as surrogate endpoints for various reasons[21]. They may not be in the main causal pathway of the disease process but simply reflect phenomena in a parallel pathway (Figure S2, II) and the experimental intervention may modify the clinical correlate but not the main pathway of the disease in question. Second, the surrogate may not be in the pathway whereby the intervention modifies the disease and the intervention may not affect the surrogate (Figure S2, III). Fourth the surrogate may be in a pathogenic pathway conducive to outcome but the intervention acts by interfering on a separate, second pathogenic pathway that contributes importantly to the outcome (Figure S2, IV). Combinations of these possibilities can also be envisaged.

The validity of a surrogate depends on the clinical or environmental context, i.e. on the particular population or the particular experimental intervention being tested. A classic example of the relevance of the context is bone density. Changes in this biomarker grossly reflect in an inverse fashion the risk of fracture in trials with diphosphonates[22]. However, in a clinical trial in postmenopausal women sodium fluoride, a compound used for the treatment of osteoporosis, increased bone density but increased also the risk of fractures[23].

Using surrogates in clinical research demands caution. Examples of blatant failures of surrogates abound and the case of ventricular premature beats as a surrogate of sudden death in a trial testing flecainide and other Class 1c anti-arrhythmics after myocardial Infarction is often quoted as a classical example of how deceiving surrogates can be. Indeed, when tested in a clinical trial[24] these drugs substantially reduced premature beats but also produced a relevant increase, rather than a decrease, in the risk of sudden death. Such a phenomenon is now attributed to the fact that flecainide interacts with an unexpected translation of the gene coding for the protein-myosin regulatory light chain, a protein which is a crucial component of the contraction of myocardiocytes[25] (Figure S2, IV). Another example of blatant failure is exercise tolerance by the treadmill test as a surrogate for the risk of death in patients with congestive heart failure[26]. Although increasingly applied, vascular calcification is a surrogate whose validity has yet to be proven. Meta-analytic evidence exists that the coronary calcium score may not be a valid
surrogate for risk assessment in primary prevention[27] or for testing the efficacy of treatments [28].
Supplementary reference list


<table>
<thead>
<tr>
<th>Author, year (ref)</th>
<th>Population</th>
<th>Intervention/Comparison (s)</th>
<th>Pts. with evaluable data</th>
<th>CAC-Method of assessment</th>
<th>Baseline CAC score</th>
<th>Follo w-up (mo)</th>
<th>Follow-up CAC score</th>
<th>Annualized rate of %CAC progression</th>
<th>P</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motro, 2001 (34)</td>
<td>Hypertensive patients with 1 or more CVD risk factors, Age ~67 yrs Male gender 62%</td>
<td>Nifedipine 30 mg/ HCTZ 25 mg + amiloride 2.5 mg</td>
<td>101/100</td>
<td>Agatston</td>
<td>108/118</td>
<td>36</td>
<td>151/208</td>
<td>13.3/25.4</td>
<td>NA</td>
<td>Randomized, double-blind study</td>
</tr>
<tr>
<td>Arad, 2005 (30)</td>
<td>Asymptomatic with positive screening for CAC Age ~59 yrs Male gender 74%</td>
<td>Atorvastatin 20 mg + vit-C 1 g + alpha tocopherol 1000U/placebo</td>
<td>490/515</td>
<td>Agatston</td>
<td>528/563</td>
<td>48</td>
<td>859/886</td>
<td>15.7/14.3</td>
<td>0.76</td>
<td>Randomized, double-blind study</td>
</tr>
<tr>
<td>Raggi, 2005 (31)</td>
<td>Postmenopausal women Age ~64 yrs</td>
<td>Atorvastatin 80 mg/ Pravastatin 40 mg</td>
<td>218/257</td>
<td>Volumetric</td>
<td>205/268</td>
<td>12</td>
<td>233/298</td>
<td>13.7/11.1</td>
<td>0.64</td>
<td>Randomized, double-blind study</td>
</tr>
<tr>
<td>Schmermund, 2006 (32)</td>
<td>Asymptomatic with positive screening for CAC with 2 or more CVD risk factors Age ~62 yrs Male gender 74%</td>
<td>Atorvastatin 80 mg/Atorvastatin 10 mg</td>
<td>175/191</td>
<td>Volumetric</td>
<td>348/371</td>
<td>12</td>
<td>396/434</td>
<td>27/25</td>
<td>0.60</td>
<td>Randomized, double-blind study</td>
</tr>
<tr>
<td>Houslay, 2006 (33)</td>
<td>Aortic stenosis Age ~70 yrs Male gender 76%</td>
<td>Atorvastatin 80 mg/ Placebo</td>
<td>39/49</td>
<td>Agatston</td>
<td>195/235</td>
<td>24</td>
<td>246/277</td>
<td>26/18</td>
<td>NA</td>
<td>Randomized, triple-blind study</td>
</tr>
<tr>
<td>Terry, 2007 (41)</td>
<td>Hypertensive patients with 1 or more CVD risk factors,</td>
<td>Atorvastatin 80 mg/ Placebo Simvastatin 80 mg/Placebo</td>
<td>40/40</td>
<td>Agatston</td>
<td>593/659</td>
<td>12</td>
<td>645/691</td>
<td>8.8/4.9</td>
<td>0.12</td>
<td>Randomized, triple-blind study</td>
</tr>
<tr>
<td>Study</td>
<td>Description</td>
<td>Age</td>
<td>Gender</td>
<td>Intervention</td>
<td>Agatston</td>
<td>Follow-up</td>
<td>Delta</td>
<td>P-value</td>
<td>Study Design</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>------------------------------------------------------------------------------</td>
<td>-----</td>
<td>--------</td>
<td>--------------------------------------------------------------------------------</td>
<td>----------</td>
<td>-----------</td>
<td>-------</td>
<td>-----------</td>
<td>--------------------------------</td>
<td></td>
</tr>
<tr>
<td>Budoff, 2009 (40)</td>
<td>Asymptomatic patients with intermediate to high risk for CAC</td>
<td>~66 yrs</td>
<td>91%</td>
<td>Aged garlic extract + B vitamins, L-arginine and folate/Placebo</td>
<td>33/32</td>
<td>12</td>
<td>6.9/26.5</td>
<td>0.03</td>
<td>Randomized, double-blind study</td>
<td></td>
</tr>
<tr>
<td>Russo, 2007 (37)</td>
<td>Pre-dialysis CKD stage 3-5</td>
<td>~54 yrs</td>
<td>85%</td>
<td>Sevelamer HC 1.6 g/Calcium carbonate 2 g/low-phosphate diet</td>
<td>27/28/2 9</td>
<td>24</td>
<td>NA</td>
<td>NA</td>
<td>Randomized, open-label study.</td>
<td></td>
</tr>
<tr>
<td>Chertow, 2002 (35)</td>
<td>Stage 5 CKD in hemodialysis</td>
<td>~52 yrs</td>
<td>85%</td>
<td>Sevelamer HC 6.5 g/Calcium based-phosphate binder 3.9-4.6 g</td>
<td>62/70</td>
<td>12</td>
<td>-3.0/13.4</td>
<td>NA</td>
<td>Randomized, open-label study.</td>
<td></td>
</tr>
<tr>
<td>Braun, 2004 (52)</td>
<td>Stage 5 CKD in hemodialysis</td>
<td>~57 yrs</td>
<td>62%</td>
<td>Sevelamer HC 800 mg/day/Calcium carbonate 500 mg</td>
<td>52/56</td>
<td>12</td>
<td>-7.3/13.6</td>
<td>0.02</td>
<td>Randomized, open-label study.</td>
<td></td>
</tr>
<tr>
<td>Block, 2005 (36)</td>
<td>Incident hemodialysis patients</td>
<td>~58 yrs</td>
<td>63%</td>
<td>Sevelamer HC 8 g/Calcium based-phosphate</td>
<td>45/47</td>
<td>6-18</td>
<td>13.4/25.3</td>
<td>NA</td>
<td>Randomized, open-label study. In the control group 3 patients</td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Stage CKD in hemodialysis</td>
<td>Age (yrs)</td>
<td>Male gender</td>
<td>Treatment Details</td>
<td>Baseline</td>
<td>Atorvastatin</td>
<td>Calcium Acetate</td>
<td>Calcium Carbonate</td>
<td>Agatston Score</td>
<td>p Value</td>
</tr>
<tr>
<td>------------</td>
<td>---------------------------</td>
<td>-----------</td>
<td>-------------</td>
<td>----------------------------------------------------------------------------------</td>
<td>----------</td>
<td>--------------</td>
<td>-----------------</td>
<td>-------------------</td>
<td>----------------</td>
<td>---------</td>
</tr>
<tr>
<td>Qunibi, 2008 (38)</td>
<td>Stage 5 CKD in hemodialysis</td>
<td>~59</td>
<td>51%</td>
<td>Sevelamer HC 7.3 g + atorvastatin 28 mg/Calcium carbonate 5.5 g + atorvastatin 33 mg</td>
<td>68/58</td>
<td>Agatston</td>
<td>889/1069</td>
<td>12</td>
<td>1116/1297</td>
<td>29/30</td>
</tr>
<tr>
<td>Barreto, 2008 (39)</td>
<td>Stage 5 CKD in hemodialysis</td>
<td>~47</td>
<td>68%</td>
<td>Sevelamer HC ≤12 g/Calcium carbonate ≤2.03 g</td>
<td>27/16</td>
<td>Agatston</td>
<td>767/1263</td>
<td>12</td>
<td>976/1602</td>
<td>27.1/26.8</td>
</tr>
<tr>
<td>Raggi, 2011 (48)</td>
<td>Stage 5 CKD hemodialysis</td>
<td>~61</td>
<td>58%</td>
<td>Cinacalcet 30–180 mg/day + Paricalcitol ≤2 μg/Vitamin D (flexible doses)</td>
<td>115/12</td>
<td>Agatston</td>
<td>862/958</td>
<td>12</td>
<td>1069/1255</td>
<td>24/31</td>
</tr>
<tr>
<td>Kakuta, 2011 (49)</td>
<td>Stage 5 CKD in hemodialysis</td>
<td>~58</td>
<td>54%</td>
<td>Sevelamer HC / Calcium carbonate 5.5 g</td>
<td>91/92</td>
<td>Agatston</td>
<td>879/872</td>
<td>12</td>
<td>961/1066</td>
<td>9.3/22.2</td>
</tr>
<tr>
<td>Block, 2012 (50)</td>
<td>Moderate to advanced CKD</td>
<td>~67</td>
<td></td>
<td>Lanthanum carbonate up to</td>
<td>28/30/3</td>
<td>Agatston</td>
<td>547/444/2</td>
<td>9</td>
<td>602/534/31</td>
<td>13.4/27/47.2</td>
</tr>
</tbody>
</table>

*Randomized, open-label study.*
<table>
<thead>
<tr>
<th>Lemos, 2013 (51)</th>
<th>Male gender 50%</th>
<th>1.5g/Sevelamer HC up to 3.2 g/Calcium Acetate up to 2.7 g/Placebo</th>
<th>Male gender 61%</th>
<th>22/26/2</th>
<th>Agatston 170/266/3</th>
<th>24</th>
<th>269/413/46</th>
<th>29.1/27.6/12</th>
<th>0.59</th>
<th>Randomized, open-label study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Moderate CKD</td>
<td>Rosuvastatin 10 mg/day/Sevelamer HC 800 mg/day/Standard therapy</td>
<td>Age ~58 yrs</td>
<td>9</td>
<td>71</td>
<td>24</td>
<td>.3</td>
<td>Standard therapy</td>
<td>27.6</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>assessment blinded</td>
<td>Male gender 61%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Supplementary Figures

Figure S1. Reproduced from supplementary ref.12, with permission by Wolkers-Kluwer (Circulation Cardiovascular Imaging). Baseline positron-emission tomography (PET) and sequential computed tomography (CT) of aortic calcification. A, Axial and coronal PET/CT images show focal 18F-flour deoxy glucose (FDG) uptake in the aorta (yellow arrow). B and C, Baseline and subsequent CT images co-registered to the same locations depicted in the PET/CT images. In the baseline CT images (B) no calcium is seen in the location corresponding to the high FDG uptake (dashed white arrow), on the follow up CT images (C) newly deposited calcium is seen at that same location in the aorta (solid white arrow).
Figure S2 Relationship between surrogates and clinical outcomes. I Valid surrogate. The surrogate is in the main pathogenic pathway and there is a strong association between the surrogate and the outcome. The intervention acts on the pathway towards the surrogate. II The surrogate is in a pathway parallel to the main pathogenic pathway and the intervention affects the surrogate but not the main pathway. III The surrogate is in a pathway parallel to the main pathogenic pathway and the intervention only affects the main pathway. IV The surrogate is in a pathogenic pathway conducive to outcome but the intervention acts also on a separate, second pathogenic pathway that contributes importantly to the outcome (redrawn from supplementary reference 21)
血管钙化作为筛查工具和临床研究替代终点的有效性

Validity of Vascular Calcification as a Screening Tool and as a Surrogate End Point in Clinical Research


全球范围来看, 2013年有超过1700万人死于心血管疾病, 其中半数死于缺血性心脏病[1]。超过半数的缺血性心脏病死亡病例以猝死起病, 既而没有提示缺血性心脏病的症状[2], 大多数梗死发生在微危或中危个体 (即普通人群中占比最大的人群)。从一生来看, 40岁时男性发生心梗的风险为二分之一, 女性为三分之一[3]。这些数字强调了及时筛查和治疗对有心血管疾病风险个体的意义。


影像学标志物能够估计动脉系统关键部位（例如冠脉循环）的动脉粥样硬化负担, 可能提供一个终生危险因素暴露的总体指标。在评估动脉粥样硬化的影像学标志物中, 血管钙化（尤其是冠脉钙化）被看作是具有最有价值的指标。因为血管钙化比颈动脉内中膜厚度等影像学技术更
好[10]。由于冠脉钙化能够很好地反映冠心病事件和其他主要心血管事件的风险[11,12]，因此冠脉钙化评分不仅在筛查和风险预测方面发展势头更好，而且这一生物标志物作为试验中临床事件的替代指标，越来越多地用于检验各种心血管疾病预防手段在不同人群中效果。

血管钙化是慢性肾脏病 (chronic kidney disease, CKD) 的特征性表现，该人群心血管风险增加[13]，基于这一原因，CKD成为冠脉钙化评分和心血管系统其他部位钙化最多被用作替代终点来观察CKD试验性治疗的疾病。当前对冠脉钙化作为替代终点的兴趣并未减退。截止至2015年1月22日，在clinicaltrials.gov登记的临床试验中有超过100项采用冠脉钙化或心血管系统其他部位钙化（主动脉或心脏瓣膜）作为主要终点或次要终点。正如前文所提到的，评价冠脉钙沉积的指标可能总体上反映了当时的危险因素暴露情况，因此基于重复测定冠脉钙化的研究可能建立了疾病活动度及其相关风险的一个替代指标。不过，实验性模型提示，冠脉钙化的进展可能不足以反映动脉粥样硬化潜在严重度的演变[14]，现在怀疑的观点[15]冲淡了对冠脉钙化评估这一方法用于心血管研究潜力的热情。

在本综述中，我们会再次评价冠脉钙化作为心血管疾病预防筛查工具和临床试验替代终点的有效性。为了达到上述目标，我们会直接比较生物标志物用于心血管疾病预防和作为临床终点替代指标时对方法学上的要求，评价冠脉钙化评分用于筛查研究和临床试验的实际表现。本综述参考并整合了荟萃分析, 这些荟萃分析综合了旨在评价冠脉钙化筛查促进治疗干预的临床试验或观察了药物治疗对冠脉钙化进展的影响。在探讨这些临床试验和荟萃分析之前，本文将会概括介绍血管钙化的生理学特点和对心血管系统钙化的定量分析，此外对生物标志物为筛查工具或临床试验的替代终点进行简单的方法学描述（参见仅能在线上获得的补充数据的附录）。

冠脉钙化作为筛查试验和临床研究中的替代终点

冠脉钙化在一级预防中作为筛查试验、生活方式和危险因素调整以及后续检测

有人指出，将冠脉钙化作为筛查试验有助于一级预防，这是由于冠脉钙化可能对生活方式干预、危险因素和后续检测具有有益作用。冠脉钙化水平可测的患者相比未发现冠脉钙化者的患者也许更有可能开始阿司匹林或调脂药物治疗[16]，或调整自己的饮食习惯或体育锻炼习惯[17]。不过，正式推荐某一检测试验用于临床实践还需要证实该试验能够改善临床转归的证据[18,19]。多数针对这一问题的研究在性质上属于观察性研究，因此对这一问题还相当有争议。美国心脏协会和美国心脏病学会2010年指南推荐，测定冠脉钙化作为无症状的中危个体（10年风险10%~20%）评估心血管风险的合理选择，同时冠脉钙化可能中低危风险（10年风险6%~10%）个体的合理选择，但是不推荐冠脉钙化检测用于低危个体（<6%）[19]。上述推荐的级别为II B级，即认为对其作用的评估不足，建议基于来自少数人群的证据（数据来自单中心随机试验或非随机研究）。2013年指南的更新版[20]对上述推荐作出了限制，提出仅在经美国心脏病学会/美国心脏协会指南计算器进行危险度评估后治疗决策仍不明确的情况下进行冠脉钙化检测，推荐级别仍然为II B级。

2012年，Whelton等[21]在一项针对随机试验的荟萃分析中概括了支持在一级预防中使用冠脉钙化评分的证据，这些随机试验观察了冠脉钙化筛查对生活方式和危险因素调整的影响。这一荟萃分析仅纳入2003-2011年发表的4项试验，共纳入2490例患者，其中5分之3的患者被纳入了无创性影像学检查早期发现血管性动脉粥样硬化 (Early Identification of Subclinical Atherosclerosis by Noninvasive Imaging Research, EISNER)试验[22]。接受了冠脉钙化评筛查的患者相比未进行该检测的患者戒烟的可能性增加15%，但未达到统计学显著性。在EISNER试验中，更高的钙化评分与处方的调脂药物更多相关，相反地，无冠脉钙化与处方的调脂药物更少相关。此外，在EISNER试验中观察到了收缩压(-2 mmHg)和低密度脂蛋白胆固醇(-4.3 mg/dL)小幅度的下降，但差异未达到统计学意义。不过，在荟萃分析中，汇总估算的血压下降(-0.23 mmHg/-0.42 mmHg)和低密度脂蛋白降低(-0.23 mg/dL)的临床意义非常小。因此，实施冠脉钙化筛查看起来最多只是轻度获益。在上述荟萃分析之后，2012年发表了一项非随机筛查干预研究，在1075例丹麦受试者中观察了采用冠脉钙化和HearScore评分进行风险评估对预防性用药的影响[23]。在随访过程中，21%的高钙化评分患者(n=462)和19%的高HearScore评分患者(n=233)接受了调脂治疗，同时分别有25%和32%的患者接受了降压治疗。高钙化评分与调脂药物的使用增加存在相关性（比值比，2.2; 95%可信区间; 1.2~4.0），而高HearScore评分与调脂药物（比值比，2.9; 95%可信区间；1.6~5.5）和降压药物（比值比，3.4; 95%可信区间，1.9~6.0）的使用增加相关，提示冠脉钙化评分在促进上述转归上并不优于HearScore评分。因此，这一研究进一步提供了不支持冠脉钙化筛查促进一级预防的证据。在2014年6月，针对这一问题的一项新的荟萃分析发表[24]，纳入了丹麦研究[23]和Whelton等的荟萃分析中涉及的研究[21]和
12项观察性研究。这一荟萃分析的结论比以前的荟萃分析更为乐观[21]。不过，钙化评分用于筛查的明显益处完全来自于12项观察性研究。观察钙化评分促进作用的观察性研究，例如被纳入Mamudu的荟萃分析中的研究[24]尤其容易出现偏倚，对其价值争议很大。因此，最新发表的这项荟萃分析并不意味着对冠脉钙化筛查作为基于证据的评估用于一级预防的作用获得了明确进展。

估计动脉壁内钙含量的技术不断得到改进。在最近对针对CKD患者的多种族动脉粥样硬化研究（Multi-Ethnic Study of Atherosclerosis, MESA）的亚组分析中，发现Agatston评分是一个很好的风险预测因子，这可能是由于该评分具有评价CKD患者钙化的独特特点[25]。最近在对从整个MESA队列收集到的扫描资料进行再次分析时采用了一个全新的密度评分，发现该密度评分相比传统危险因素和Agatston评分能够进一步改善风险识别能力[26]。该密度评分能够轻度改善风险识别，但是具有显著统计学意义（受试者工作特征曲线下面积0.700~0.711），但是该评分用于中危患者使得风险重新分类出现了具有临床意义的改善（+13.9%）。此外，考虑到之前MESA试验亚组分析和Rotterdam研究[11,12]中Agatston评分相应的风险重新分类的净改善，因此可以得出下述假说，即中危患者通过新的密度评分测得的钙评分正常（即0分）提示潜在的真正风险水平较低，以至于不需要通过药物来预防心血管疾病，钙评分升高则提示应当开始或维持长期他汀和阿司匹林治疗。不过，仍然需要一项正式的临床试验来评价基于上述方法的预防策略[18]。在2012年，一项旨在观察冠脉钙化筛查用于不适合他汀和阿司匹林治疗的中危患者（占总体人群的很大一部分）的大型试验的设计得以发表[27]。主要是由于要入选大量的中危受试者（大约30,000人）和高昂的花费，这项试验未能在设计阶段之后继续开展下去。尽管新的钙化密度评分[26]或其他评分有助于增加对冠脉钙化的识别，但是从中期来看，还不足以改变指南推荐[28]。

冠脉钙化作为临床试验中的替代终点


在这一荟萃分析之后，发表了一项另一项试验，观察了陈年大蒜提取物和维生素B复合剂对心血管疾病中危患者的影响[40]。因此，前文提到的5项心血管疾病试验[30-34]、观察大葱提取物的试验和在2007年开展的另一项试验[41]（后者未被纳入同一项荟萃分析）构成了分析的基础，由此我们可以估计治疗对无明显肾脏受累的心血管疾病患者冠脉钙化的影响（参见只能在线获取的补充数据中的表S1）。上述试验中有3项试验采用体积法测定了冠脉钙化，一项试验采用了两种方法[31]。3项试验比较了他汀和安慰剂[30,33,41]，一项试验比较了大剂量和小剂量的同一种他汀[32]，一项试验比较了不同的他汀[33]，一项试验比较了硝苯地平和阿米洛利-氢氯噻嗪联合治疗[34]。在3项安慰剂对照他汀试验中，他汀治疗患者的平均每年加权冠脉钙化进展（+15.9%）与相对应的安慰剂组观察到的结果相似（+14.0%）。更为重要的是，在其中最大的圣弗朗西斯心脏研究中[30]，冠脉钙化进展（完全不受治疗影响）和心血管事件发生率之间明显分离，活性药物治疗组的心血管事件发生率降低（安慰剂组9.9%，阿托伐他汀组6.9%，相对风险降低30%；P =0.08）。同样地，在比较了两种他汀或小剂量和大剂量同一他汀的两项研究中（表S1），发现钙化进展几乎相同，总体上没有显著意义（P =0.64和P =0.60）。总体上来看，他汀对冠脉钙化无影响与他汀在一级和二级预防中的有益效应形成了鲜明的对比。已经被明确证实的他汀的心血管保护益处未能体现在冠脉钙化上，这与生物学知识相符合，即他汀这一类药物能够缩小斑块中未钙化的部分，但是不影响或加重斑块钙化[14,43]。在一项针对46例冠心病高危患者的随访研究中，阿托伐他汀显著降低非钙化斑块负担，但是未能影响钙化评分[44]。一项针对46例冠心病高危患者的研究发现，阿托伐他汀显著降低冠脉钙化进展（40% vs 78%，3年随访），但是硝苯地平对冠脉钙化的有益效应未能转化为更多的临床获益。实际上，在这项试验中，总体心血管或脑血管事件的发生率差异（复合终点发生率：硝苯地平组为6.3%，复发阿米洛利组为5.8%）[45]。硝苯地平本身可能对动脉粥样硬化形成过程中的很多钙依赖性事件具有有益影响[46]，相比之下，噻嗪类利尿剂可能促进矿物质沉积[47]。冠脉钙化作为观察硝苯地平和氢氯噻嗪临床试验的替代终点并不可靠，这可能是由于两者对冠脉钙化的作用不同对于心血管
CKD患者临床试验中司维拉姆对冠脉钙化评分的影响

Chertow 2002[35]
Braun 2004[52]
Block 2005[36]
Russo 2007[37]
Barreto 2008[39]
Quinibi 2008[38]
Sadek 2003[53]
Barreto 2008[39]
Quinibi 2008[38]
Chertow 2002[35]
Block 2005[36]
Suki 2008[55]
Di Ilrio 2012[56]
Di Ilrio 2013[57]

图1. 直接比较将冠脉钙化作为终点的试验和观察了同一种药物对慢性肾脏病患者死亡率影响的试验。CI代表可信区间。

CKD患者临床试验中司维拉姆对死亡率的作用

图2. 西那卡塞和安慰剂在一项观察冠脉钙化的研究中[23]西那卡塞联合低剂量维生素D对血透治疗CKD患者血管钙化作用的研究（ADVANCE）[46]和另一项评价西那卡塞对复合终点作用的研究（死亡、心梗、因不稳定型心绞痛住院、心衰或外周血管事件；评价西那卡塞降低心血管事件比EVOLVE）[59]，下图，另请参见文字描述。
血管钙化的作用与其对死亡和心血管事件的作用一致。ADVANCE研究（一项评价西那卡塞联合小剂量维生素D对血透透析CKD患者血管钙化作用的随机试验）发现，西那卡塞的作用未能达到Agatston评分变化的主要终点（P=0.07），但是能够一致地延缓以体积指标表示的冠脉钙化速度（P=0.009）和心脏瓣膜和动脉钙化的进展。另一方面，评价西那卡塞降低心血管事件（Evaluation of Cinacalcet Therapy to Lower Cardiovascular Events，EVTOLVE）试验中[59]，未观察到西那卡塞对死亡和心血管转归有益处。EVTOLVE试验是最大规模的试验之一（大约4000例患者），也是迄今为止在透人群开展的随访时间最长的研究（<64个月）（图2）。上述不一致的研究结果再次提示，冠脉钙化在CKD患者也不是一个冠脉疾病进展的可靠生物标志物。因此，试验大体上未能就CKD患者冠脉钙化作为人群中可靠的替代终点提供一致的证据。

总的来看，在普通人群、明确冠心病病人和CKD人群开展的研究其结果均不支持采用冠脉钙化作为心血管研究中的替代终点。冠脉钙化可能是有助于对中低危患者更好地进行风险再分类的生物标志物。但是，冠脉钙化测定的作用仅限于其对风险重新分层的能力，风险识别或衡量上并没有明显的益处[9]。冠脉钙化增加风险再分类的能力可能有助于指导治疗这一假说并未被现有临床试验所证实[21,23]。尽管冠脉钙化扫描相比循环生物标志物能够更好地预测未来的心血管事件，但是冠脉钙化扫描带来了暴露于离子射线的风险。钙化评分研究中的有效剂量[2~3 辐射剂量值（mSv）] 大约比64排冠脉计算机断层扫描研究[60]低5倍。在使用现代科技时，单次钙化评分中检测的暴露量大约相当于1年中在环境中中的放射暴露量。这种暴露不应被忽视，因为离子射线对人体健康的严重影响是累积性的。欧洲心脏病学会（European Society of Cardiology）等主要心血管学会已经公布了减少心血管疾病诊断或监测过程中放射暴露的警告[61]。

尽管在过去几年中对冠脉钙化和血管钙化潜在发病机制和进展的基本机制进行了广泛研究[62,63]，但是钙沉积和动脉粥样硬化之间的关系还未完全理解。血管生理学和影像学研究支持下述解释，即钙化是在动脉粥样硬化过程中晚期出现的、难以逆转的事件。通常并有没有发现治疗减少动脉粥样硬化负荷和降低临床事件风险的效应伴随着血管钙化的动态变化。迄今为止，旨在对冠脉钙化和血管钙化进行定量的影像学研究局限于阐明动脉粥样硬化病理学的研究更好[63,64]，而不是将其扩展为筛查策略或用于观察干预的临床试验。冠脉循环以外部位的血管钙化是不是有效的替代指标仍然是一个悬而未决的问题，有待在其他研究中进行评价。

利益声明
C.Zoccali接受了艾伯维公司的讲者酬劳。R.Vanholder接受了拜耳公司的讲者酬劳和差旅费用。Z.Massy接受了安进、健赞和费森尤斯医疗管理公司的讲者酬劳和研究基金。A.Ortiz接受了赛诺菲公司旗下健赞公司、Shire和艾伯维公司的讲者酬劳和差旅费用。其他作者没有利益声明。

参考文献


