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EDITORIAL

A biomarker for vascular calcification: shedding light on an unfinished story?

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This editorial refers to 'A combined microRNA and target protein-based panel for predicting the probability and severity of uremic vascular calcification: a translational study' by C.T. Chao *et al.*, pp. 1958–1973.

Vascular calcification (VC) is highly prevalent in patients with chronic kidney disease (CKD), and particularly those with end-stage renal disease (ESRD) on dialysis.[1] In this patient population, VC, especially coronary artery calcification, is a strong predictor of mortality.[2] Even in young adults, VC progresses, particularly with declining kidney function (eGFR<60–75 mL/min/1.73 m^2),[3] worsening albuminuria,[4] and longer duration of dialysis.[3] The adverse clinical outcomes associated with higher levels of VC include acute myocardial infarction, ischaemic cardiac events, left ventricular hypertrophy, and sudden death.[4]

In addition to traditional risk factors for VC, patients with CKD have disordered mineral metabolism, particularly hyperphosphataemia, resulting from impaired renal clearance, secondary hyperparathyroidism, alterations in vitamin D status, and abnormal bone metabolism.[5] Increased levels of serum phosphate, a known contributor to ectopic calcification, and reduced levels of calcification inhibitors, including fetuin-A, osteocalcin, osteoprotegerin, matrix GLA protein, and pyrophosphate, all create a milieu for trans-differentiation of vascular smooth muscle cells (VSMCs) and unopposed mineralization of the extracellular matrix.[6] Towler has aptly termed this a 'perfect storm' for mineralization.

VC is currently identified using non-invasive imaging modalities, such as chest radiography and computed tomography (CT), or invasive techniques, such as intravascular ultrasound of the coronary arteries. While VC is often incidentally found on such studies, there has been a marked rise in the use of cardiac CT as a screening test to calculate a coronary artery calcium (CAC) score, owing to the strong association of higher CAC scores with worse prognoses. These imaging modalities are widely used, yet factors such as cost, availability, and radiation exposure may limit their accessibility to some patients. Accordingly, the identification of circulating biomarkers in the blood that can identify the presence of VC could offer a more appealing alternative.

In this issue, using meticulous, step-wise miRNA and transcriptomic profiling, Chao *et al.*[7] identified a novel panel of circulating serum biomarkers for diagnosing the presence and severity of uraemic VC in patients with ESRD. The researchers used miRNA and mRNA microarrays of samples from uraemic cell culture and rodent models to search for differentially regulated miRNAs. Their initial analysis identified 122 down-regulated and 119 up-regulated miRNAs with increasing levels of VC. Using a bioinformatics-assisted approach that sorted for matching trends with target genes, they narrowed the miRNA biomarker candidates to nine miRNAs that were down-regulated with VC. These nine candidates were further validated experimentally using the original *in vitro* and *in vivo* models, as well as an additional *ex vivo* VC model, which further narrowed their candidates to four miRNAs (miR-10b-5p, miR-195, miR125b-2-3p, and miR-378a-3p) and one mRNA (*SULF1*). Finally, they measured circulating levels of these candidates in two cohorts of patients: dialysis-dependent patients with ESRD and non-dialysis-dependent patients with CKD. In both cohorts, they found that VC severity correlated with decreased serum levels of miR-125b-2-3p and mir-378a-3p, whereas it correlated with increased levels of *SULF1*, a potential target of miR-378a-3p. In their regression analyses, they conclude that the miRNA/mRNA pair—miR-378a-30/*SULF1*—in combination with traditional clinical features appears to be useful for improved diagnosis and classification of severity of uraemic VC in patients with CKD/ESRD.

The authors of this study employed a fastidious, tiered approach to identify the novel biomarker candidates. Their bioinformatic-assisted method centred not only on identifying differentially expressed miRNAs in the disease process, but also on capturing those with expression trends matching their purported gene targets. More importantly, by validating their final candidates using *in vitro, in vivo*, and *ex vivo* models as well as CKD patient sera, they not only strengthen the utility of potential biomarkers, but also raise interest in their potential as therapeutic targets for uraemic VC. In functional characterization assays of their identified biomarkers, Chao *et al.* found that overexpression of miR-378a-3p in high phosphate-treated aortic VSMCs reduced osteocalcin and alkaline phosphatase levels. Similarly, silencing of its target gene *SULF1* attenuated VSMC calcification.

The search for VC biomarkers is not a new one. Indeed, a number of circulating proteins have previously been found to associate with increased VC, such as fibroblast growth factor-23, fetuin-A, and

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Table 1 Serum biomarkers of VC[1,2]

Biomarker	Description	Correlation with VC	Additional information
Fibroblast growth factor-23 (FGF-23)	Bone-derived hormone that regulates phosphate and vitamin D metabolism	Positive	Mainly in patients with CKD
Matrix Gla protein (MGP)	Vitamin K-dependent gamma-carboxylated protein. Inhibitor of mineralization and of bone morphogenetic protein-2 (BMP-2)	Positive	Correlation is specifically with the dephosphorylated, uncarboxylated MGP (dp-ucMGP) isoform in patients with CKD
Osteopontin (OPN)	Highly phosphorylated glycoprotein found in various tissues. Inhibitor of mineralization	Positive	Association seen in patients with coronary artery disease, but not in patients with CKD
Osteoprotegerin (OPG)	Inhibitor of osteoclastogenesis	Positive	
Phosphate	Anion component of hydroxyapatite. Serum levels are regulated by kidney	Positive	Phosphate binders failed to show benefits
Fetuin-A	Liver-secreted glycoprotein. Inhibitor of mineralization	Negative	Patients with CKD
Pyrophosphate	Inhibitor of mineralization	Negative	Patients with CKD & ESRD on dialysis
Osteocalcin	Vitamin K-dependent gamma-carboxylated protein found in bone and dentine	Inconclusive/conflicting results	
Sclerostin	Glycoprotein inhibitor of Wnt signalling	Inconclusive/conflicting results	

CKD, chronic kidney disease; ESRD, end-stage renal disease.

osteoprotegerin (*Table 1*).[8,9] Yet, as the authors show in this study, advancing age alone is already an independent risk factor for higher severity of VC in the ESRD population (OR 1.066, $P = 0.002$). More importantly, in the absence of effective treatments for VC, the presence of VC by radiographic examination is itself merely a biomarker (for cardiovascular risk) at this time. Further, in the CKD/ESRD population, VC is often already incidentally identified through imaging performed for other indications (e.g. chest radiographs or computed tomographic scans to evaluate shortness of breath). When seen on such studies, its current clinical value is not as a treatment target, but rather as a marker for a patient at increased cardiovascular risk. Thus, is the current quest for a biomarker for VC instead a search for a biomarker by proxy? This question is particularly important given recent claims that some types of VC may actually be protective.[10]

Nonetheless, the journey towards this miRNA/mRNA biomarker pair by the authors of this study is certainly impressive. And while the diagnostic value of the biomarkers remains to be seen, especially given the existing straightforward radiographic approaches, their work may lead to progress towards the ultimate goal of determining conclusively the best way to treat VC itself.

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