Chronic kidney disease and vascular remodelling: molecular mechanisms and clinical implications

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ABSTRACT

CKD (chronic kidney disease) is a severe and complex disease with a very high prevalence of CV (cardiovascular) complications. CKD patients are exposed to haemodynamic disturbances in addition to severe metabolic abnormalities that lead to a specific form of arterial remodelling, which contributes to the development of CV disease. Arterial calcification is a major event in the arterial remodelling process and is strongly linked to mineral metabolism abnormalities associated with CKD. Arterial remodelling is not limited to arterial calcification and modifications in arterial wall composition are also observed. Activation of the RAS (renin–angiotensin system), ET-1 (endothelin-1), endothelial dysfunction, oxidative stress and ADMA (asymmetric ω-NG,NG-dimethylarginine), as well as the anti-aging molecule Klotho, are implicated in this process. The present review details the mechanisms involved in arterial calcification and arterial remodelling associated with CKD, and provides the clinical consequences of large and small artery stiffness and remodelling in CKD patients.

INTRODUCTION

CKD (chronic kidney disease) is a highly prevalent condition, affecting ∼10% of the population in Europe and North America. CKD is a particularly severe disease, mostly due to the high prevalence of CV (cardiovascular) complications. Patients with CKD stage 4 (see Table 1 for CKD stages) are more likely to die from CV disease than from kidney failure [1]. Indeed, approximately 50% of patients with ESRD (end-stage renal disease) die from CV causes [2,3]. The association between CV disease and renal disease is also present at moderate

Key words: angiotensin II, arterial calcification, arterial stiffness, Klotho, pulse wave velocity, remodelling, phosphorus.

Abbreviations: ACE, angiotensin-converting enzyme; ACEi, ACE inhibitor; ADMA, asymmetric ω-N\textsuperscript{G},N\textsuperscript{G}-dimethylarginine; AngII, angiotensin II; ApoE, apolipoprotein E; ARB, AngII type 1 receptor blocker; BMP, bone morphogenetic protein; BP, blood pressure; CAC, coronary artery calcium; Cbfα1, core-binding factor α-1; CKD, chronic kidney disease; CRP, C-reactive protein; CUA, calcific uraemic arteriolopathy; CV, cardiovascular; DDAH, dimethylarginine dimethylaminohydrolase; ESRD, end-stage renal disease; ET-1, endothelin-1; FGF, fibroblast growth factor; FGFR, FGF receptor; Gas6, growth arrest-specific gene 6; GFR, glomerular filtration rate; eGFR, estimated GFR; IGF, insulin-like growth factor; IL, interleukin; LPK, Lewis polycystic kidney; LV, left ventricular; MAPK, mitogen-activated protein kinase; MDRD, Modification of Diet in Renal Disease; MI, myocardial infarction; mKlotho, membrane-bound Klotho; MMP, metalloproteinase; Mx2, msh homeobox 2; NOS, NO synthase; PI3K, phosphoinositide 3-kinase; Pit-1, type III sodium-dependent phosphate co-transporter; PKB, protein kinase B; PTH, parathyroid hormone; PTHrP, PTH-related peptide; PWV, pulse wave velocity; RANK-L, receptor activator of nuclear factor κB; RAS, renin–angiotensin system; ROS, reactive oxygen species; SOD, superoxide dismutase; TNF, tumour necrosis factor; TRPC-1, transient receptor potential channel-1; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor; VSMC, vascular smooth muscle cell.

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Arterial calcification: an active and regulated process

Arterial calcification is associated with major disturbances of mineral metabolism in CKD, such as hyperphosphataemia, hyperparathyroidism and vitamin D deficiency (Figure 1). Interestingly, other biological disorders classically associated with CKD, such as inflammation and oxidative stress, also play a role in the calcification process. Arterial calcification is an active and regulated process in which vascular cells may acquire osteoblastic functions [19]. The calcified lesions contain osteoblasts, osteoclasts, trabeculae and numerous proteins regulating calcification, such as osteopontin, bone sialoprotein and alkaline phosphatase [20]. The expression of these proteins in the inferior epigastric artery of CKD patients has been correlated with the severity of the histological calcification score [21].

In arterial calcification, the crystallisation step implicates lipid vesicles which could be either mineralizing matrix vesicles (100 nm in diameter) extruded by viable VSMCs (vascular smooth muscle cells) or apoptotic bodies (200 nm in diameter) of dying cells [22,23]. In cultured VSMCs, apoptosis occurs before the first signs of calcification [24]. Vascular calcification is strongly reduced when VSMCs are cultured in the presence of a broad spectrum inhibitor of caspasases, such as ZVAD-fmk [N-benzyloxycarbonyl-Val-Ala-Asp-(O-methyl)fluoromethane] [24], implying a role for caspase-stimulated apoptosis in the pathogenesis. The membrane integrity of apoptotic bodies appears to be essential for calcium accumulation, since the calcification process is blocked by membrane permeabilization with the detergent NP-40 (Nonidet P40) [24].

Table 1: Stages of CKD

<table>
<thead>
<tr>
<th>Stage</th>
<th>eGFR (ml/min per 1.73m²)</th>
<th>Evidence of kidney damage</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>≥90</td>
<td>Albuminuria or structural renal abnormalities</td>
</tr>
<tr>
<td>2</td>
<td>60–89</td>
<td>Albuminuria or structural renal abnormalities</td>
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<tr>
<td>3</td>
<td>30–59</td>
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<td>4</td>
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<td>5</td>
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stages. Using the database of a large healthcare provider in Northern California, it has been demonstrated that an inverse linear relationship exists between GFR (glomerular filtration rate) estimated with the MDRD (Modification of Diet in Renal Disease) formula [4] and all-causes of death, CV events and hospitalization, after adjustment for age, sex, race, co-existing illness and socio-economic status [5]. One explanation for this very high CV risk derives from the observation that CKD patients are not only exposed to traditional CV factors, but also to non-traditional factors, including mineral metabolism disturbances, anaemia, ADMA (asymmetric ω-NG,NG-dimethylarginine), activation of sympathetic tone, inflammation and oxidative stress. The poor prediction of CV risk using classical measures, such as the Framingham equation, in this population illustrates this feature of CKD. Indeed, when Framingham equations were used to calculate the CV risk in patients with CKD from the ARIC (Atherosclerosis Risk in Communities) and CHS (Cardiovascular Health Study) trials, the prediction of cardiac events was very low, forecasting only 13.9 and 4.8 % of events over 10 years in men and women respectively [6]. In the present review, we detail the key mechanisms accounting for the high CV risk in CKD patients, with a focus on arterial remodelling and stiffening in CKD and the clinical implications.
Arterial remodelling and renal failure

At a molecular level, the key factors promoting vascular calcification include morphogens that activate osteogenic BMP (bone morphogenetic protein) and Wnt signalling pathways [20]. BMP-2 and BMP-4 induce osteogenic differentiation of VSMCs through induction of transcription factors, such as Cbfa1 (core-binding factor α-1), osterix and Msx2 (msx homeobox 2) [25]. BMP-2 is also implicated in VSMC-induced apoptosis and in the loss of regulation of matrix Gla protein, a negative regulator of arterial calcification [25].

In an autopsy study that characterized the intima and media calcified lesions in CKD patients, compared with patients with coronary artery disease without kidney dysfunction, Gross et al. [26] showed that both groups had identical numbers of intima-associated calcified plaques, although CKD patients had increased numbers of media-associated calcified lesions in the coronary arteries. In addition, the calcified plaques observed in CKD patients were very distinct in terms of morphology, extent of calcification and inflammatory character [26]. In CKD, the calcified lesions contained more calcium, and the expression of pro-calcific proteins, such as osteocalcin, was higher, whereas the expression of calcification inhibitors, such as fetuin A, was decreased [26]. At the level of inflammatory markers, calcified lesions from CKD patients had enhanced macrophage infiltration and CRP (C-reactive protein) expression, as well as intense C5b deposition [26].

Role of phosphorus in arterial calcification

Abnormalities in phosphorus metabolism are critical determinants of the CV complications associated with CKD [27]. Large epidemiological studies in CKD stages 3 and 4 have shown that increasing serum phosphorus levels are associated with increased mortality risk even when phosphorus levels are in the ‘normal’ range [28]. The association between serum phosphorus levels and CV disease is also observed in the general population. Indeed, Dinhgra et al. [29] have demonstrated that LVMI [LV (left ventricular) mass index] and new-onset congestive heart failure were associated with phosphorus levels even in individuals with an eGFR >90ml/min per 1.73m². In dialysis patients, a strong association exists between serum phosphorus levels and arterial calcification [30,31]. This association is also reported at moderate CKD stages. In 439 patients with CKD stage 3 from the Multi-Ethnic Study of Atherosclerosis (mean eGFR, 51 ml/min per 1.73m²), each 1 mg/dl (0.32 mmol/l) increase in serum phosphorus was independently associated with a 21 % \( (P = 0.002) \) greater prevalence of coronary artery calcification [32]. This robust association was confirmed in the general population in 3014 individuals from the CARDIA (Coronary Artery Risk Development in Young Adult) study where patients with serum phosphorus levels > 3.9 mg/dl (1.26 mmol/l) had a 52 % greater risk of coronary artery calcification after 15 years of follow-up [33]. Interestingly, in a longitudinal...
follow-up of 883 patients from the Spokane Heart Study, who underwent evaluation of coronary artery calcification, CV risk factors and laboratory testing every 2 years for 6 years, coronary artery calcification increased during the follow-up and serum phosphorus level was one of the determinants of progression [34].

Despite the strong link between large arterial stiffness and arterial calcification, the association between serum phosphorus level and aortic stiffness is not found consistently. The Multi-Ethnic Study of Atherosclerosis reported an association between serum phosphorus level and arterial stiffness, estimated from the ankle brachial index, but did not find an association with central pulse pressure, an indirect marker of stiffness [35]. Other investigators have not found an association between serum phosphorus levels and arterial stiffness, when the latter was estimated with carotid-femoral PWV [36,37].

In rodent models of CKD, high-phosphorus diets induce arterial calcifications [38-40], and dietary phosphate restriction prevents kidney disease progression and calcification [41]. In cultured human VSMCs, phosphorus induces the expression of two key factors for osteoblastic differentiation, osteocalcin and Cbfa1 [42]. The phosphate-induced phenotypic change in VSMCs depends on activity of Pit-1 (type III sodium-dependent phosphate co-transporter). Thus, in vitro, pharmacologic blockade of sodium/phosphate co-transport [42,43] or siRNA (small interfering RNA) knockdown of Pit-1 [44] inhibits high phosphorus-induced calcification and expression of calcification regulatory proteins such as Cbfa1 and osteopontin. However, Pit-1-mediated phosphate transport is not the only pathway involved since VSMC calcification is not completely blocked by inhibitors of sodium/phosphate co-transport [42,43]. High phosphate levels stimulate the secretion of BMP-2 in VSMCs, which, in addition to its major role in calcification, increases the expression of Pit-1 [45]. Recent experimental studies provide new mechanisms by which phosphate induces vascular calcification, namely via its ability to form calcium phosphate deposition passively, independent of transport through Pit-1 [46]. Indeed, Villa-Bellosta et al. [46] have demonstrated that calcium-phosphorus deposition is primarily a passive phenomenon that occurs when the abundance of calcification inhibitors is low. This step is followed by an active process where calcium-phosphorus deposits initiate expression of specific osteogenes, such as Bmp2, Cbfa1, Msx2 and Osx (osterix), and transdifferentiation of VSMCs into osteoblast-like cells [46].

In addition to its effect on VSMC differentiation, phosphorus induces VSMC apoptosis. This leads to the formation of apoptotic bodies that play an important role in the initiation and the amplification of the calcification process through their ability to concentrate and crystallize calcium [47]. Phosphate induces apoptosis of cultured human aortic VSMCs via a mechanism involving a decrease in the expression of Gas6 (growth arrest-specific gene 6) [48]. Finally, phosphate also acts as an inhibitor of monocyte/macrophage differentiation into osteoclast-like cells. Indeed, in the presence of RANK-L (receptor activator of nuclear factor κB) and M-CSF (macrophage colony-stimulating factor), human peripheral blood mononuclear cells differentiate into osteoclast-like cells. This process is inhibited by phosphate in a dose-dependent manner via a mechanism involving the inhibition of the RANK-L-induced JNK (c-Jun N-terminal kinase) and Akt activation. Interestingly, the effect of phosphate on osteoclast differentiation is inhibited by a blocker of sodium-dependent phosphate co-transport [49].

In summary, elevated phosphate is associated with vascular calcification in CKD patients. Experimental data indicate that phosphate plays a role at each step of the calcification process, inducing apoptosis, osteoblastic differentiation of VSMCs and prevention of monocyte/macrophage differentiation into osteoclastic-like cells.

Role of FGF (fibroblast growth factor)-23, Klotho and PTH (parathyroid hormone) in arterial calcification

FGF-23, Klotho and PTH are also implicated in phosphorus regulation and arterial calcification. FGF-23 is produced by osteocytes and osteoblasts in bone and acts at the level of the kidney, mostly through its receptor [FGFR (FGF receptor) 1c], which requires Klotho as a co-factor. PTH and FGF-23 reduce the expression of the sodium-dependent phosphate co-transporters NPT2a [solute carrier family 34 (sodium phosphate), member 1] and NPT2c [solute carrier family 34 (sodium phosphate), member 3] in the proximal tubule [50], which in turn decreases phosphate reabsorption. In CKD, plasma FGF-23 is increased even at early stages [51]. Indeed, an increase in FGF-23 expression is observed in bone biopsies from CKD patients as early as stage 2 [52]. Epidemiological studies have shown that FGF-23 is independently associated with poor kidney function in diabetics [53] and CKD patients from the HOST (Homocysteinemia in Kidney and End Stage Renal Disease) study [47], and with all-cause of mortality and CV outcomes [54,55], as well as initiation of dialysis [55]. In addition, FGF-23 is independently and positively associated with aortic calcification [56] and coronary calcification [57] in ESRD patients. In a longitudinal follow-up of ESRD patients, however, FGF-23 was independently and negatively associated with the progression of aortic calcification [58]. The discrepancies between these studies highlight the complexity of the mechanisms involved. FGF-23 is theoretically a ‘protective’ molecule against hyper-phosphataemia. However, during CKD progression, a resistance to the effects of FGF-23 is observed, leading to increased FGF-23 plasma concentrations. In
addition, it is difficult to separate the effects of FGF-23 from the effects of the phosphorus disorder itself. In order to illustrate this point, the phenotype of mice deficient in FGF-23 is informative. These mice have elevated serum levels of phosphate, calcium, and 1,25-dihydroxyvitamin D₃, as well as vascular calcification and early death. Interestingly, interventions aimed at reducing hyperphosphatemia, such as dietary phosphate or vitamin D restriction, or knockout of genes critical to vitamin D action or renal phosphate reabsorption [59–61] reverse the phenotype. By contrast, transgenic mice overexpressing FGF-23 have low BP (blood pressure), hypophosphatemia, and an impairment of vascular reactivity, a phenotype rescued by high dietary phosphate [62]. In CKD, the levels of plasma FGF-23 are very high, but are unable to normalize hyperphosphatemia [51].

Considering the epidemiological data, one could hypothesize that FGF-23 exerts a direct function on the vessel wall since the associations with CV disease are independent of serum phosphorus levels and kidney function [63]. Interestingly, in human carotid artery specimens, FGF-23 is detected in calcified segments, along with SOX-9 [SRY (sex determining region Y)-box 9], collagen type II, cathepsin-K and fetuin-A. Indeed, FGF-23 is localized to the cytoplasm of VSMCs, suggesting the possibility of local synthesis [64]. Further support for a direct effect of FGF-23 on arterial calcification derives from experiments in mice with moderate CKD fed on a high-phosphate diet. Serum phosphorus levels do not increase, whereas serum FGF-23 is elevated. In this model, FGF-23 levels (but not serum phosphorus) were significantly associated with arterial calcification [39]. In summary, these descriptive studies provide arguments supporting the concept that FGF-23 may exert a direct stimulatory effect on arterial calcification in addition to its role in phosphorus regulation. However, functional studies are required to formally assess the role of FGF-23.

Klotho is a transmembrane and secreted protein involved in aging. The KL (Klotho) gene encodes two proteins via alternative splicing: mKlotho (membrane-bound Klotho) and sKlotho (secreted Klotho). A third form of Klotho (cKlotho) is a cleavage product of the extracellular domain of mKlotho [65]. In humans, Klotho is mainly expressed in the kidney and the secreted form predominates over the transmembrane form [66]. Klotho-deficient mice and FGF-23-deficient mice exhibit a similar phenotype that includes arterial calcification [67]. Within the kidney, Klotho acts as an obligatory co-receptor for FGF-23. Thus these receptors are named mKlotho–FGFR1c, mKlotho–FGFR3c and mKlotho–FGFR4 [68,69]. Whether FGF-23 interacts with Klotho in the proximal tubule, where sodium-phosphate co-transporters are localized, or acts at the level of the distal convoluted tubule, where Klotho is mainly expressed, is still controversial. Klotho expression in the kidney decreases with CKD progression and this is associated with resistance to the effects of FGF-23, which might contribute to elevation of circulating FGF-23 and plasma phosphate levels. In addition to its role in phosphorus regulation, recent evidence suggests that Klotho is directly involved in regulating VSMC signalling responses. In cultured VSMCs, Klotho suppressed sodium-dependent uptake of phosphate and mineralization induced by high phosphate and preserved cell differentiation [67]. The decrease in Klotho expression during CKD might contribute to the increase in serum phosphate levels and arterial calcification.

PTH is another major regulatory protein implicated in the mineral and bone disease associated with CKD. Conflicting results have been published regarding the relationship between all-cause mortality and PTH levels in ESRD, and findings are lacking in pre-dialysis patients [70]. With respect to CV mortality, a significant positive association with serum PTH levels has been found in most epidemiological studies [70]. However, this positive association is weaker than that observed between CV mortality and serum phosphorus [70]. In addition, controlling PTH levels to within targets recommended by K/DOQI (Kidney Disease Outcomes Quality Initiative) does not necessarily improve the CV risk in patients with ESRD [71]. Despite these epidemiological observations that suggest a limited role for PTH in determining CV outcomes in CKD, in experimental systems PTH has an impact on vascular structure and function. Receptors for PTH [PTH receptor 1 (PTHr1) and PTHrP (PTH-related peptide)] have been identified in cultured VSMCs [72], and conflicting results have been reported regarding the effects of PTH on vascular calcification. Some studies provide evidence for a beneficial effect in the context of osteoporosis treatment with PTH. For example, Jonu et al. [73] have shown that PTH-(1–34) inhibits VSMC calcification in a dose-dependent manner. In addition, administration of PTH-(1–34) to diabetic LDLR (low-density lipoprotein receptor)-deficient mice inhibits valvular and vascular calcification [74]. Other studies have not found an effect of PTH on vascular calcification in vitro [75]. In rats with CKD who underwent parathyroidectomies and were fed on low-, normal- or high-phosphorus diets, Neves et al. [76] reported that all rats on PTH replacement therapy developed intense aortic medial calcification, regardless of the dietary phosphorus content. In summary, the epidemiological and experimental findings regarding the role of PTH in vascular calcification associated with CKD emphasizes the complexity of the mechanisms involved and the need for further experimental and clinical studies.

**Role of vitamin D in arterial calcification**

Vitamin D deficiency has been observed in CKD from early stages to ESRD [77,78]. Epidemiological studies have shown an inverse association between vitamin D
levels and mortality in the general population [79] and in CKD patients [80]. In ESRD, vitamin D levels are inversely associated with aortic stiffness and endothelial function [81] and with aortic calcification [82]. The effect of vitamin D in the calcification process is complex. Vitamin D deficiency might influence vascular calcification indirectly via stimulation of secondary hyperparathyroidism. In rodents, it is well demonstrated experimentally that supraphysiological doses of 1,25-dihydroxyvitamin D induce vascular calcification [83].

VSMCs possess the vitamin D receptor, and also express 1α hydroxylase to transform 25-hydroxyvitamin D into the active form 1,25-dihydroxyvitamin D [84]. The active form of vitamin D enhances VSMC proliferation through a VEGF (vascular endothelial growth factor)-mediated pathway [84] and migration via activation of the PI3K (phosphoinositide 3-kinase) pathway [85]. In vitro, the active form of vitamin D, calcitriol, induced VSMC calcification through its effect on PTHrP secretion [86]. However, the dosages used in these in vitro studies were relatively high and the physiological relevance is unclear. The active form of vitamin D has also been found to be a negative regulator of the RAS (renin–angiotensin system), which could have a beneficial effect on the CV system [87]. In addition, vitamin D levels have been associated with the state of inflammation in patients with Type 2 diabetes [88] and in CKD [89]. Thus, in patients with Type 2 diabetes, a randomized controlled trial showed that improving vitamin D status was associated with a reduction in systemic inflammatory markers [88]. In CKD, although clinical trials are still needed, observational studies reveal an improvement in inflammatory status associated with efficient correction of vitamin D deficiency [90]. In vitro, vitamin D inhibits the human monocyte/macrophage pro-inflammatory cytokine production through the MKP-1 [MAPK (mitogen-activated protein kinase) phosphatase-1 pathway] [91]. As described below, inflammation plays an important role in the calcification process in CKD and vitamin D might therefore play a protective role against vascular calcification through its anti-inflammatory effects.

In CKD patients, retrospective studies have shown a protective effect of 1α-hydroxyvitamin D3 supplementation on vascular calcification [92]. Historical cohorts also indicate a survival advantage of selective vitamin D receptor activators [93], but these findings still require confirmation in randomized controlled trials. In this regard, the PRIMO (Paricalcitol Capsule Benefit in Renal Failure-Induced Cardiac Morbidity) study assigned CKD patients (stage 3 and 4) to receive oral paricalcitol (2 μg/day) or placebo for 48 weeks, but did not show any difference in cardiac structure or function between the two groups of patients [94]. Episodes of hypercalcaemia were more frequent in the paricalcitol group.

**Role of inflammation and oxidative stress in arterial calcification**

Aside from parameters of mineral metabolism, inflammation and oxidative stress play important roles in the process of vascular calcification. CKD is associated with an increase in inflammatory markers, both circulating and in the arterial wall, and oxidative stress markers increase due to an imbalance between excessive production of free radicals and insufficient antioxidant mechanisms [95]. The importance of oxidative stress in the calcification process is illustrated in studies performed on Sprague–Dawley rats with CKD induced by adenine-rich chow. These rats exhibit arterial calcification and increased expression of oxidative stress markers such as 8-hydroxydeoxyguanosine and 4-hydroxynonenal in plasma and in the aorta. In this model, arterial calcification is prevented by administration of tempol, an SOD (superoxide dismutase) mimetic [96]. In vitro, macrophage-derived cytokines, such as IL (interleukin)-1β, IL-6, IL-8, TNF (tumour necrosis factor)-α, IGF (insulin-like growth factor)-1 and TGF (transforming growth factor)-β, induce an osteogenic transformation of VSMCs [97,98]. In diabetic models, emerging evidence indicates that adventitial inflammatory mediators play a role in the acquisition of a osteochondrocytic phenotype [99]. In addition, BMP-2 and BMP-4 are released by endothelial cells and adventitial microvasculature in response to inflammatory cytokines or ROS (reactive oxygen species) [100]. BMPs are key factors in the initiation of arterial calcification and act through the up-regulation of paracrine Mxs-2/Wnt signalling [100].

Inflammatory cells also participate in the calcification process via the release of proteolytic enzymes, cathepsins or MMPs (metalloproteinases; MMP-2 and MMP-9) [101]. MMP-2 and MMP-9 are secreted by inflammatory cells, such as macrophages, or by VSMCs. In a mouse model of CKD induced by partial nephrectomy, a high-phosphate diet stimulated elastin turnover and phenotypic changes in VSMCs, followed by increases in MMP-2, MMP-9 and cathepsin S, leading to arterial calcification [102]. In a rodent model of elastin calcification, MMP-2 and MMP-9 were localized close to the calcified lesions, and this was dependent on the activities of MMPs since it was inhibited by BB-1101, an MMP inhibitor [103]. Furthermore pre-treatment of elastin fibres by aluminium chloride, which makes elastin fibres resistant to MMP degradation, inhibits elastin calcification [104]. Moreover, aortae from MMP-2- and MMP-9-deficient mice are resistant to calcium chloride-induced calcification of elastin fibres, further supporting a role for these MMPs in arterial calcification [105].

As suggested by Pai et al. [102], cathepsin S appears to play an important role in elastin fragmentation and calcification. In combined ApoE (apolipoprotein E)-deficient/cathepsin S-deficient mice (ApoE−/−/cats−/−) with CKD secondary to partial nephrectomy, aortic
calcification and osteogenic and elastolytic activity were decreased, compared with ApoE−/−/cats+/+ mice with CKD. Cathepsin S-associated elastin breaks were also less frequently observed in the double-knockout mice [102]. In addition, in ApoE−/−/cats+/+ mice with CKD, immunoreactive cathepsin S co-localized with osteogenic near-infrared signals [102]. Elastin fragmentation leads to the production of elastin-derived peptides, which can bind the ELR (elastin–laminin receptor), and exerts biological effects. In cultured VSMCs, elastin-derived peptides induce the expression of bone proteins such as Cbfa1, osteocalcin and alkaline phosphatase [106]. These observations therefore underline the importance of MMPs and cathepsin S-induced elastolysis in the arterial calcification process [107].

MECHANISMS IMPLICATED IN ARTERIAL REMODELLING IN CKD

Arterial calcification is only one component of the remodelling process associated with CKD. Qualitative and quantitative changes in the composition of the arterial wall also influence the functional and geometric properties of large and small arteries in CKD patients. Interestingly, these abnormalities are not solely driven by increases in BP associated with CKD. For example, LPK (Lewis polycystic kidney) rats develop hypertension by 6 weeks of age, renal dysfunction by 12 weeks of age and ESRD by 18–24 weeks of age [108]. Compared with Lewis rat controls, LPK rats at 12 weeks of age exhibit increased aortic stiffness that is associated with arterial calcification and medial thickness [109]. In this model, Ng et al. [109] clearly demonstrated that increases in aortic stiffness are independent of resting BP, since at each BP level studied LPK rats have significantly higher aortic PWV. Indeed, the composition of the arterial wall from LPK rats is very distinct, with a decrease in elastin content and an increase in collagen content. In addition, aortic calcium content is significantly increased in LPK rats compared with Lewis rat controls [109]. These structural abnormalities (and not only arterial calcification) contribute to the increase in aortic stiffness, independent of BP level. In the Dahl/rapp salt-sensitive rat, a model of hypertension and hypertensive renal disease, salt administration is associated with VSMC proliferation in the aorta and renal arterioles and a progressive luminal narrowing, suggesting that VSMC proliferation is an early event in the vascular disease associated with CKD [110]. At the level of the small arteries, CKD is associated with eutrophic inward remodelling and endothelial dysfunction in 5/6 nephrectomized rats [111]. The mechanisms leading to arterial remodelling and endothelial dysfunction during CKD involve other pathways, including activation of the RAS, ET-1 (endothelin-1), decreased Klotho expression, ADMA, inflammation and oxidative stress, and these will be discussed below.

Role of the RAS

CKD is associated with a sustained activation of the RAS, which plays a major role in disease progression and in CV remodelling. AngII (angiotensin II) exerts various effects at the level of the CV system, including a potent vasoconstrictor effect that increases vascular resistance, a remodelling effect with hypertrophy and fibrosis, and an inflammatory and pro-oxidant effect, as extensively described and reviewed [112,113]. Through its effects on oxidative stress, AngII is also implicated in the arterial calcification process associated with CKD. Indeed, administration of an ARB (AngII type 1 receptor blocker) to male New Zealand White rabbits fed on an atherogenic diet prevented arterial calcification [114].

The major role of AngII in the remodelling process in CKD has been uncovered in several studies. In the 5/6 nephrectomy rat model, the ARB losartan normalized the relaxation response to acetylcholine and the morphology of resistance arteries [111,115]. In the LPK rat model, ACEi [ACE (angiotensin-converting enzyme) inhibitor] treatment was associated with a significant improvement in PWV in a BP-independent fashion. At the level of the aorta, ACE inhibition decreased elastin degradation and improved the collagen/elastin ratio [116]. The effect of RAS blockade on arterial remodelling and CV prognosis has been extensively studied in hypertensive patients. Fewer studies have addressed this issue in CKD patients, although there have been numerous studies focused on the effects of RAS blockade on CKD progression [117]. In the FOSIDIAL (Fosinopril in Dialysis) study, 397 ESRD patients were included and randomized to either the ACEi fosinopril (5 mg titrated to 20 mg daily) or placebo. After adjustment for potential confounding variables, no significant benefit of fosinopril on CV events was observed in the intent-to-treat analysis, although the study was limited by a relatively small sample size [118]. A secondary analysis of the SAVE (Survival And Ventricular Enlargement) trial, which was designed to evaluate the effect of the ACEi captopril on CV mortality in post-MI (myocardial infarction) patients with an LVEF (LV ejection fraction) <40% showed that CKD was associated with an increase in all CV events after an MI and that randomization to captopril resulted in a reduction of CV events irrespective of baseline kidney function [119]. In the HOPE (Heart Outcome and Prevention Evaluation) trials, renal insufficiency was associated with increased risk for CV events, and the ACEi ramipril reduced the incidence of CV events in patients with and without renal insufficiency [120]. Even if these secondary analyses appear promising, trials specifically designed in CKD populations are still needed to properly assess the effect of RAS blockade on arterial...
remodelling and CV prognosis. In small interventional trials in ESRD patients, ACEi therapy decreased aortic PWV in a BP-independent manner [121,122].

Role of ET-1

Expression of the potent vasoconstrictor ET-1 is stimulated in experimental [123] and human [124] CKD and increases with the progression of the disease. Evidence suggests that the increase in ET-1 contributes to both the arterial remodelling associated with CKD and the increase in CV risk. ET-1 is mainly synthesized by endothelial cells, but is also produced by VSMCs and epicardial cells. ET-1 acts through ETA (ET-1 type A) and ETB (ET-1 type B) receptors. ETA receptors are mainly expressed in VSMCs, whereas ETB receptors are mainly present in endothelial cells and, to a lesser extent, in VSMCs. Activation of ETA receptors leads to vasoconstriction, whereas activation of ETB receptors results in vasodilation via generation of prostacyclin and NO [125]. Experimental findings have shown that ET-1 overexpression at the level of the endothelium is associated with a hypertrophic remodelling, a decrease in endothelial function and an increase in oxidative stress [126]. In uraemic rats, ET-1 and ETA receptor expression are increased in the vascular wall, whereas ETB receptor expression is decreased [127]. In the rat remnant kidney model, the hypertrophic remodelling of mesenteric arteries is prevented by ETA receptor blockade [128].

ET-1 induces an inflammatory response and increased oxidative stress in the vascular wall, which causes vascular remodelling and endothelial dysfunction [129]. ET-1 exerts effects on cell proliferation, migration and contraction, and induces extracellular matrix components and growth factors [130]. At the molecular level, ET-1 receptor activation stimulates several signalling pathways, including MAPKs and PI3K/PKB (protein kinase B) [131]. An intermediary role for CaMKs (calcium/calmodulin-dependent protein kinases), PKC (protein kinase C) and receptor and non-receptor protein tyrosine kinases in triggering the activation of MAPK and PI3K/PKB signalling in response to ET-1 has been suggested [131].

In addition to its own direct effects on the CV system, links exist between ET-1 and the RAS. Thus, in cultured endothelial cells in vitro, AngII stimulates ET-1 release [132,133]. Furthermore, in the rat remnant kidney model Larivi ère et al. [133a] have shown that the ARB losartan administration causes a decrease in ET-1 concentration in the thoracic aorta and pre-glomerular arteries.

Interventional trials in humans with CKD and hypertension have demonstrated that treatment with ETA receptor blockers has a beneficial effect on BP, but serious secondary effects have also been observed. In a recent interventional trial involving patients with proteinuric CKD, the ETA receptor blocker sitaxsentan significantly improved BP and arterial stiffness [134]. However, a larger clinical trial in diabetic nephropathy [the ASCEND (A Study of Cardiovascular Events in Diabetes) trial], involving use of the predominant ETA receptor antagonist avosentan, was terminated prematurely after a median follow-up of 4 months because of an excess of CV events in the avosentan group, mostly due to congestive heart failure. In patients with resistant hypertension, the use of the selective ETA receptor antagonist darusentan was associated with significant lowering of BP, although oedema or fluid retention was observed in 27% of patients given darusentan compared with only 14% treated with placebo [135]. These studies therefore highlight the importance of caution in targeting the endothelin system in patients with CKD and vascular disease.

Role of endothelial dysfunction, inflammation, oxidative stress and ADMA

Endothelial dysfunction is an early step in atherosclerosis and is also associated with the remodelling process that leads to an increase in arterial stiffness. Indeed, a close inverse relationship between flow-mediated vasodilation at the level of the brachial artery and arterial stiffness has been shown in hypertensive patients [136]. In healthy volunteers, Bellien et al. [137] demonstrated that arterial stiffness is regulated by the endothelium through the release of both NO and the cytochrome-related EDHF (endothelium-derived hyperpolarizing factor). Endothelial dysfunction has been demonstrated in patients with CKD in both large [138] and small [136] arteries, and is associated with poor CV [139] and kidney [140] outcomes in this population. Reduced bioavailability of NO is one of the main factors involved in CKD-associated endothelial dysfunction and is mainly due to low-grade inflammation, oxidative stress and ADMA, as detailed below. Hormonal factors have also been implicated. Thus a decrease in testosterone levels in male patients with CKD has been associated with endothelial dysfunction and a heightened risk of future CV events [141]. Finally, serum phosphorus level is also a determinant of endothelial function [142]. In randomized controlled trials in CKD patients, phosphate-lowering therapies improved endothelial function at the level the brachial artery [143,144].

Circulating microparticles have recently been linked to endothelial dysfunction associated with CKD. Microparticles are defined as vesicles (between 100 nm and 1 μm diameter) budded from the outer membrane of cells on their activation or as part of cell apoptosis. Amabile et al. [145] reported that circulating microparticles were increased in ESRD patients compared with healthy subjects. The presence of endothelial microparticles was significantly associated with a decrease in endothelial function and, in vitro, endothelial microparticles from
ESRD patients decreased endothelial NO release. In a rodent model of hypertension, endothelial microparticle release was induced by AngII via stimulation of NADPH oxidase, ROS and Rho-kinase targeted to lipid rafts [146]. In that study, Burger et al. [146] also demonstrated that endothelial microparticles had a direct effect on endothelial cells themselves, stimulating further ROS production and inflammatory responses.

A growing body of evidence suggests that the reduction in kidney function in both mild [147] and advanced [148] CKD may be associated with an inflammatory response and an increase in oxidative stress. Oxidative stress plays an important role in the vascular remodelling process in CKD since arterial remodelling and endothelial dysfunction of mesenteric arteries in the 5/6 nephrectomized rat model is prevented by the administration of tempol [132]. Low-grade inflammation, characterized by an increase in markers, such as ultra-sensitive CRP, IL-6 and TNF-α, has been reported in CKD [149] and is associated with increased mortality risk [125]. Low-grade inflammation has been associated with endothelial dysfunction in patients with CKD at any stage [150–152], including patients with renal transplantation [150]. Low-grade inflammation also plays a role in the calcification process as has been described above. In addition, inflammation stimulates MMP release, associated with modified proteoglycan composition and cell infiltration around the vasa vasorum, leading to arterial ischaemia. The role of inflammation in arterial remodelling is perhaps best illustrated in inflammatory diseases, such as rheumatoid arthritis, where increased arterial stiffness has been documented [153]. Interestingly, anti-TNF-α therapy has been shown to reduce aortic stiffness in these patients to a level comparable with that of healthy individuals [153].

ADMA blocks the active sites on NOS (NO synthase) and thereby decreases NO synthesis. Thus, in cultured endothelial cells and isolated human blood vessels, ADMA administration significantly inhibits NOS and reduces NO generation [154]. Acute ADMA administration in humans significantly decreases flow-mediated dilation, a marker of endothelial dysfunction [155]. ADMA is released after post-translational methylation of proteins containing methylated arginine residues [156]. ADMA is a product of the enzyme PRMT1 (protein arginine methyltransferase type 1) and is synthesized by numerous cell types, including endothelial cells [156]. The elimination of ADMA mainly involves metabolic degradation by the enzyme DDAH (dimethylarginine dimethylaminohydrolase) and to a lesser extent renal excretion. Circulating ADMA levels are markedly increased in humans with ESRD and, in moderate CKD, ADMA levels progressively increase with the decrease in eGFR [157]. ADMA levels are independently associated with all-cause mortality and CV events in patients on haemodialysis [158] and in CKD stages 3–5 [159]. Ravani et al. [160] also showed that plasma ADMA levels predicted mortality in CKD patients independently of traditional and non-traditional risk factors, and that an increase in plasma ADMA of 0.1 μmol/l was associated with a 20% increase in death or ESRD [160]. In CKD, a strong association exists between plasma ADMA levels and CV remodelling parameters including intima-media thickness [161,162] and LVH (LV hypertrophy) [163]. In addition, in a cohort of 224 ESRD patients, ADMA levels have been shown to correlate to sympathetic activity, as measured by noradrenaline (norepinephrine) concentrations [164]. These observations emphasize the role of ADMA in the vascular remodelling associated with CKD, mostly via promoting endothelial dysfunction.

AngII, oxidative stress and ADMA are tightly associated, as reviewed recently by Wilcox [165]. Rats infused with AngII had increased oxidative stress, endothelial dysfunction at the level of mesenteric arterioles and increased vascular ADMA levels. All of these changes were prevented by tempol, suggesting that AngII-mediated ROS production contributes to enhanced ADMA levels [165]. Cultured rat preglomerular VSMCs transfected with p22phox have increased NADPH oxidase activity, enhanced activity and expression of protein arginine methyltransferase, and reduced activity and protein expression of DDAH and CAT1 (cationic amino acid transferase 1), resulting in increased cellular levels of ADMA [165]. Taken together, these results suggest that ROS and ADMA form a tightly coupled amplification system.

**Role of Klotho in the vascular remodelling process**

Beside its role in arterial calcification, Klotho is involved in the arterial remodelling process associated with CKD. In Klotho-deficient mice, the vasodilatory response to acetylcholine is reduced and this is reversed by parabiosis between wild-type mice and deficient mice, suggesting a role for Klotho in the circulating form of Klotho [166]. In addition, adenovirus-mediated Klotho gene delivery in Otsuka Long-Evans Tokushima Fatty rats (that exhibit hypertension, obesity, severe hyperglycaemia and hypertriglyceridaemia) improves vascular endothelial dysfunction, increases NO production, reduces elevated BP, and prevents medial hypertrophy and perivascular fibrosis [167]. Klotho protects cultured endothelial cells from oxidative stress-induced apoptosis [168], and inhibits insulin/IGF-1 signalling [169]. As a consequence of its effects on insulin/IGF-1 signalling, the expression of MnSOD (manganese SOD) expression is increased. This in turn facilitates removal of ROS and confers oxidative stress resistance [169]. In Klotho-deficient mice, Kusaba et al. [170] have demonstrated endothelial hyperpermeability, with increased apoptosis and a down-regulation
of cadherin expression. The mechanisms involve an increase in VEGF-mediated internal calcium influx and hyperactivation of calcium-dependent proteases [170]. Klotho binds directly to both VEGFR (VEGF receptor)-2 and TRPC-1 (transient receptor potential channel-1) and the complex is internalized in response to VEGF stimulation, thus regulating VEGF/TRPC-1-mediated calcium influx to maintain endothelial permeability [170]. These observations underline the role of Klotho as a protective factor at the vascular level. In CKD, a decrease in Klotho expression is associated with a decline in this vascular protective effect.

ARterial REMODELLING IN CKD PATIENTS: CLINICAL SIGNIFICANCE

Arterial remodelling associated with CKD
In 1843, Toynbee and Bright [171] noticed that CKD was accompanied by severe CV remodelling with arterial and cardiac hypertrophy. The characteristics of the arterial remodelling associated with CKD and its clinical significance has been extensively studied in recent years, driven by the development of non-invasive methods, including high-resolution echotracking systems and aplanation tonometry. The first studies involving ESRD patients reported increases in carotid and aortic stiffness and arterial enlargement compared with healthy subjects [172]. In cross-sectional studies, several groups have shown that the increase in aortic stiffness starts as early as CKD stage 2 and increases with progression to stages 3 and 4 [37,173–175]. In addition to arterial stiffening, the arterial ancillary study of the NephroTest cohort has provided interesting findings about remodelling parameters. Compared with healthy and hypertensive subjects, a maladaptive outward remodelling was described in patients with CKD stages 2–5 [37]. The maladaptive outward remodelling has also been associated with proteinuria in the Hoorn study, which is a population-based cohort [176]. The follow-up of this cohort for a mean of 3.1 years allowed an accurate description of the evolution of arterial remodelling during CKD progression [177]. Interestingly, aortic stiffness was stable, whereas carotid stiffness significantly increased during follow-up. The maladaptive outward remodelling progressed with thinning of the carotid artery (−22 μm/year) and enlargement leading to a progressive increase in circumferential wall stress [177], following Laplace’s law. After renal transplantation, conflicting results have been reported regarding the progression of aortic stiffness [178–180]. The discrepancies between these clinical studies could be due to donor characteristics that may influence the structure and function of recipient large arteries. Indeed, in a previous study, Delahousse et al. [181] have shown that donor age is the main determinant of changes in aortic stiffness in recipients within the first year post-transplantation, independent of BP, eGFR and recipient age. Interestingly, at the level of the carotid artery, the remodelling parameters improved after renal transplantation, with a significant decrease in carotid diameter and circumferential wall stress [182].

Few studies have examined the remodelling of subcutaneous resistance arteries in CKD. Recently, Luksha et al. [183] have shown that arterial distensibility of subcutaneous resistance arteries was reduced in ESRD patients, compared with an age-matched control group, although the wall/lumen ratios and wall cross-sectional areas were not significantly different despite higher BP values [183].

Clinical significance of arterial remodelling in CKD
Alteration of large artery structure leading to increased arterial stiffness is associated with target organ damage in the heart, kidney and brain through secondary microvascular damage [184]. Arterial stiffness is defined by the ability of the artery to distend in response to increased pressure following the classical pressure–volume relationship. The increase in aortic stiffness limits the ability of the aorta to receive the stroke volume during LV ejection and this leads to a selective increase in systolic BP, resulting in high pulse pressure. Increased aortic stiffness also has an impact on the timing of the forward and reflected waves. With increased PWV, the reflected waves return earlier, having an impact on the central arteries during systole rather than diastole, amplifying aortic and ventricular pressures during systole, and reducing aortic pressure during diastole. All of these events contribute to increased central pulse pressure, which induces small artery remodelling and rarefaction, leading to a vicious cycle of further increases in mean BP and large artery stiffness [185]. In accordance with these observations, increases in aortic [12] and carotid [13] stiffness in ESRD are strongly predictive of all-cause mortality. Conversely, improvement of aortic stiffness has been associated with improved prognosis (Figure 2A). These findings support the conduct of interventional trials aimed at improving aortic stiffness in ESRD patients. The arterial ancillary study of the NephroTest cohort confirmed that, at moderate stages of CKD (stage 2–5), aortic stiffness is independently associated with all-cause mortality [186]. In addition, in a cohort of 512 renal transplant recipients, aortic stiffness and increased wave reflections were independent predictors of CV events [187].

Kidney function is very sensitive to large artery function. Indeed, the renal circulation is characterized by a relatively low resistance in the afferent arteriolo and a relatively high resistance in the efferent arteriolo, designed to maintain glomerular hydrostatic pressure.
Figure 2  Clinical significance of arterial remodelling in CKD


Protective mechanisms exist to counteract increases in pulse pressure, such as the myogenic response and tubuloglomerular feedback. However, in pathological situations, such as hypertension, dysregulation of these mechanisms is observed [188]. In accordance with these observations, central pulse pressure and aortic stiffness are independently associated with the resistive index of the kidney and with proteinuria in hypertensive patients [189]. In the arteriolar ancillary study of the NephroTest cohort, central pulse pressure was independently associated with CKD progression, as was circumferential wall stress (Figure 2B) [177].

ARTERIAL CALCIFICATION AND REMODELLING IN CKD: THERAPEUTIC ASPECTS

Phosphorus and calcium play central roles in the calcification process in CKD patients, justifying interventional trials aimed at reducing phosphate level without increasing calcium intake. Randomized controlled trials comparing sevelamer, an aluminium- and calcium-free phosphate binder, have been conducted with conflicting results on vascular calcification. The first randomized open controlled trial was conducted by Chertow et al. [190] in 200 haemodialysis patients, comparing the effects of sevelamer (average dose 6.5±2.9 g/day) to calcium acetate (average dose 4.3±1.9 g/day) on coronary artery and thoracic aorta calcification as assessed by electron beam tomography. After a follow-up period of 52 weeks, the median absolute calcium score of coronary arteries and thoracic aorta increased significantly in the calcium treatment group, but not in the sevelamer group [190]. A beneficial effect of sevelamer on coronary artery calcification was also reported in two randomized controlled trials involving haemodialysis patients with a follow-up between 12 and 18 months [191]. However, in the study conducted by Block et al. [191], it is interesting to note that in patients with baseline CAC (coronary artery calcium) scores > 30, the progression of CAC was not different between the two study groups. The BRiC (Bone Remodelling and Calcification) study, which included 101 haemodialysis patients, showed no difference in CAC progression between the calcium and sevelamer groups [192]. Interestingly, when phosphate-lowering therapies were tested in addition to intensive lipid-lowering therapy, no difference was seen in terms of progression of CAC between calcium acetate and sevelamer, as reported in the CARE-2 (Calcium Acetate Renagel Evaluation-2) study [193]. In conclusion, the small size of these studies and the conflicting results emphasize the need for large interventional trials looking at vascular calcification in addition to hard clinical end points.

Another therapeutic approach may involve the use of vitamin D receptor activators. Despite encouraging experimental results in rodent models of CKD [194,195], randomized controlled trials are needed to define the effect of vitamin D receptor activators on vascular calcification in CKD patients. 25-Hydroxyvitamin D deficiency by itself has been associated with arterial calcification in CKD patients, although the efficacy of vitamin D supplementation on vascular calcification still requires confirmation.

Calcimimetic agents act on the calcium sensing receptor and reduce PTH levels without affecting calcium intake. Thus calcimimetics are potential candidates for the treatment of vascular calcification. The ADVANCE study, a randomized study to evaluate the effects of cinacalcet plus low-dose vitamin D against a flexible dosage of vitamin D alone on vascular calcification in haemodialysis patients, did not show a significant difference in CAC scores between the two groups after a follow-up of 52 weeks [196]. However, a relative reduction in aortic valve calcification was observed in the cinacalcet arm [196].
**Figure 3** Arterial remodelling associated with CKD

A normal blood vessel in cross-section is shown on the left-hand side of the Figure, and effect of CKD on vascular structure is depicted on the right-hand side. CKD patients are exposed to traditional CV risk factors, including age, hypertension, overweight, diabetes, dyslipidaemia and smoking, and non-traditional CV risk factors, such as mineral metabolism disorders, inflammation, oxidative stress, RAS activation, ET-1, ADMA and microparticles. All of these factors participate in arterial remodelling associated with CKD, including arterial calcification (depicted as dark streaks in the media), alteration of extracellular matrix composition and VSMC proliferation and apoptosis.

Recently, the effect of vitamin K2 supplementation was tested in 53 CKD patients in an interventional non-placebo-controlled trial. Vitamin K2 supplementation for 6 weeks induced a dose- and time-dependent decrease in circulating dephosphorylated-uncarboxylated matrix Gla protein, uncarboxylated osteocalcin and uncarboxylated prothrombin. These results encourage the design of interventional trials aimed at decreasing vascular calcification by vitamin K supplementation [197].

**CONCLUSIONS**

CKD is a complex condition characterized by exposure not only to traditional CV risk factors, but also to risk factors that arise due to the reduction of GFR and accumulation of uraemic toxins (Figure 3). Arterial calcification is one of the main features of the vascular remodelling process associated with CKD that occurs even at early stages. Arterial calcification and remodelling of the arterial wall lead to increased arterial stiffness, which has a strong predictive value for mortality in CKD. The change in arterial structure associated with CKD is also characterized by a maladaptive outward remodelling, which has clinical significance since it is associated with CKD progression and all-cause mortality. Many metabolic disturbances associated with CKD, such as oxidative stress, activation of the RAS and impaired NO production, have been implicated in the arterial remodelling process. Interventional clinical trials that target these risk factors and focus on arterial remodelling and CV outcomes are needed to guide the optimal treatment of the patients.

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