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REVIEW

New Emerging Biomarkers for Bone Disease: Sclerostin and Dickkopf-1 (DKK1)

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Abstract

A healthy skeleton depends on a continuous renewal and maintenance of the bone tissue. The process of bone remodeling is highly controlled and consists of a fine-tuned balance between bone formation and bone resorption. Biochemical markers of bone turnover are already in use for monitoring diseases and treatment involving the skeletal system, but novel biomarkers reflecting specific biological processes in bone and interacting tissues may prove useful for diagnostic, prognostic, and monitoring purposes. The Wnt-signaling pathway is one of the most important pathways controlling bone metabolism and consequently the action of inhibitors of the pathway such as sclerostin and Dickkopf-related protein 1 (DKK1) have crucial roles in controlling bone formation and resorption. Thus, they might be potential markers for clinical use as they reflect a number of physiological and pathophysiological events in bone and in the cross-talk with other tissues in the human body. This review focuses on the clinical utility of measurements of circulating sclerostin and DKK1 levels based on preanalytical and analytical considerations and on evidence obtained from published clinical studies. While accumulating evidence points to clear associations with a number of disease states for the two markers, and thus, the potential for especially sclerostin as a biochemical marker that may be used clinically, the lack of standardization or harmonization of the assays still hampers the clinical utility of the markers.

Keywords Sclerostin · DKK-1 · Dickkopf-1 · Bone turnover markers · Bone formation · Bone resorption · Osteoporosis

Introduction

A healthy skeleton depends on a continuous renewal and maintenance of bone tissue. The human skeleton is a multifunctional organ that undergoes continuous remodeling which includes the coordinated and harmonized activities of multicellular components. The main activities involved in the remodeling process are osteoblastic bone formation and bone resorption performed by osteoclasts. These activities result in the release of proteins and protein metabolites by osteoblasts and osteoclasts, which reflect different aspects of the dynamic bone remodeling process. They are, therefore, referred to as bone turnover markers [1, 2].

Bone is an endocrine organ that maintains its metabolic activity throughout life. It has metabolic, mechanical, and protective functions. Bone metabolism is characterized by the ability to remodel already existing bone tissue, maintaining the normal bone structure and the growth of the skeleton, depending on the age and stage of development. The storage of minerals such as calcium, phosphorus, magnesium, and the maintenance of homeostasis of these minerals are among the numerous metabolic functions of bone.

Bone turnover markers can be evaluated by measuring products of the synthetizing and enzymatic activities of osteoblasts and osteoclasts and the bone matrix elements that are released to the peripheral circulation during formation and resorption. In addition to the traditional bone turnover markers such as N-terminal pro-peptide of procollagen type I (PINP) and C-terminal cross-linking telopeptide of type I collagen (CTX), newer markers reflecting specific

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pathways in the regulation of the individual activities have been explored. These include periostin, cathepsin-K, sclerostin, dickkopf-1 (DKK1), receptor activator of nuclear factor kappa-B ligand (RANKL), fibroblast growth factor 23 (FGF-23), klotho, sphingosine-1-phosphate, and microRNAs. Exploring these newer biochemical and molecular markers will potentially help us to predict risk groups for bone-related diseases in early stages. However, their relationship with fracture risk has still not been fully elucidated as well as the predictive value for many of these is still not established. Thus, the clinical utility of these markers as diagnostic, prognostic, or monitoring tools during treatment still needs to be studied.

Osteocytes are central in the coordination of bone remodeling and focusing on markers related to the regulation of osteocyte activities may prove useful clinically as diagnostic, prognostic, or monitoring markers for bone diseases. The aim of this review is, therefore, to review the clinical utility and analytical and preanalytical specifics of two markers involved in regulating bone metabolism, sclerostin, and DKK1.

The Wnt/β-catenin Pathway

One of the main regulators of bone remodeling is the Wnt signal transduction pathway. It is the most important regulator of bone formation. The Wnt/β-catenin pathway is vital in normal bone homeostasis and has an important role in mediating the signal that couples bone formation with resorption primarily by regulating cell growth, differentiation, and apoptosis (Fig. 1). It has a major role in stimulating osteoblast proliferation and regulates osteogenesis through multiple mechanisms [3].

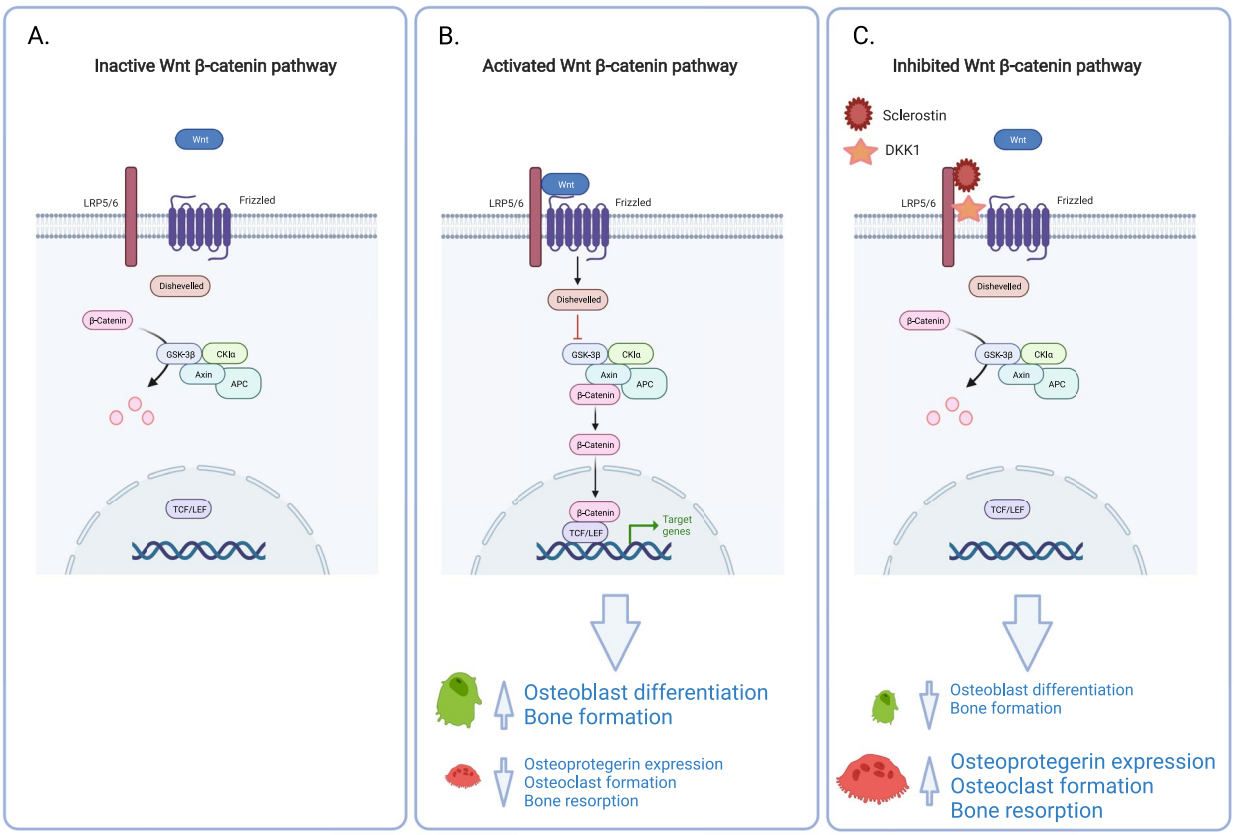

Fig. 1 The figure shows the canonical Wnt/β-catenin pathway. **A** The inactivated Wnt/β-catenin pathway, β-catenin, is phosphorylated by GSK3 at specific serine/threonine residues, where it is degraded and does not reach the nucleus. **B** The activated pathway where Wnt, Frizzled protein, and LRP5/6 co-receptor are assembled into a complex, GSK3 is inactivated leading to inhibition of degradation of β-catenin which subsequently activated the transcription and translation of target genes important for osteoblast differentiation and osteocyte formation. **C** In the presence of Wnt inhibitors sclerostin and DKK1, Wnt binding to LRP5/6 and Frizzled protein is blocked, resembling the inactivated pathway. This leads to lack of activation of bone formation. Please see text for additional details. GSK-3β, glycogen synthase kinase 3 β; TCF/LEF, T-cell factor/lymphoid enhancer factor. Created with BioRender

The Wnt-signaling pathway involves two signaling cascades: canonical (Fig. 1) and non-canonical Wnt/β-catenin pathways with the non-canonical pathway are divided into the planar cell polarity signaling pathway (Wnt/PCP) and the Wnt/Ca2 + signaling pathway [4].

The Wnt/β-catenin pathway is expressed in many human tissues and represents a developmentally, highly conserved signal transduction pathway. The canonical pathway regulates various cellular activities during embryonic development and adult homeostasis, such as stem cell renewal and cell fate determination [5, 6]. Furthermore, it can induce differentiation, stimulate proliferation, inhibit apoptosis of osteoblasts as well as cell migration and adhesion (Fig. 1) [7, 8]. In bone tissue, the canonical pathway is mainly regulated by osteocytes that are bone cells formed when active osteoblasts become embedded in the bone matrix. As a regulator of skeletal remodeling and in addition to secrete sclerostin, osteocytes have a complex communication network in response to both mechanical and hormonal pathways. They communicate with osteoblasts and osteoclast via signaling molecules including sclerostin, dkk-1, and Wnt axis.

In a mineralized matrix as well as controlling osteoblast regulation by inhibiting apoptosis, classical Wnt-signaling pathway can also control osteoclast differentiation [9]. In addition to inhibition of osteoclast activity, it stimulates osteoprotegrin synthesis indirectly (Fig. 1) [10]. Osteocytes both act as locally at bone environment and function as an endocrine cell. Besides their endocrine functions as insulin secretion in the pancreas, phosphate reabsorption from kidney and skeletal muscle function, they can also balance bone mass, shape, and size [11]. Inhibition of the β-catenin canonical pathway causes bone resorption and contributes to the dynamic balance of bone metabolism, while dysregulation may lead to various osteoarticular diseases [5].

Wnt genes encode lipid-modified glycoproteins, acting as ligands for their cognate-frizzled receptors. The co-receptor of the β-catenin-dependent Wnt-signaling pathway is a single-pass transmembrane protein called low-density lipoprotein receptor-related protein 5/6 (LRP5/6). It has an extracellular domain consisting of four repeating units that is an important regulatory site which binds of Wnt proteins. Wnt proteins are hydrophobic molecules which make them unstable and insoluble [3, 12, 13].

After the formation of the trimeric complex of the transmembrane Frizzled receptor together with its co-receptor LRP6/5 and Wnt, the canonical Wnt pathway is activated. Binding of a Wnt ligand to the Frizzled receptor induces phosphorylation of LRP5/6, which forms a docking site for AXIN, inactivating the glycogen synthase kinase 3 (GSK3). Subsequent sequestration of AXIN prevents the destruction of the complex and allows stabilization and nuclear translocation of non-phosphorylated β-catenin by the downstream-signaling cascade for intracellular actions. β-catenin is protected from proteasomal degradation. In the nucleus, β-catenin displaces repressors and activates the TCF/ LEF transcription factor complex resulting in pro-osteogenic gene expression [14–17].

Inhibitors of the Wnt-Signaling Pathway

Wnt signaling is antagonized at multiple levels and regulated by different inhibitors. The first step of inhibition consists of various secreted Wnt inhibitor cell signaling molecules such as sclerostin and DKK1. They inhibit activation of Wnt signaling by blocking the binding between Wnt and specific cell surface receptors (LRP5/6 and Frizzled), thus, inducing the degradation of β-catenin and hindering LRP5/6 phosphorylation. DKK1 acts as a bipartite inhibitor that interacts with the N- and C-terminal regions of LRP5/6. The binding of DKK1 on LRP-6 also named as functional DKK1 [18–20]. These inhibitors are expressed and secreted within the bone microenvironment and regulate bone formation and resorption resulting in changes in bone mass. They are involved in the PTH signal transduction pathway, and SCL is downregulated by PTH [21, 22]. Moreover, an anti-sclerostin antibody, romosozumab, has recently been approved for clinical use as the most potent anabolic treatment for osteoporosis [23].

Sclerostin is a soluble antagonist of the canonical Wnt-signaling pathway. It is primarily secreted by osteocytes as a 22-kDa glycoprotein and has anti-anabolic actions in bone. Sclerostin-mediated inhibition of the pathway leads to increased bone resorption, inhibition of bone formation, and growth through tandem interaction with two LRP6 ectodomains [24]. As the production and actions were initially thought to be limited to the skeleton, it appeared to be an attractive target for therapy [25]. However, recent studies showed that also osteoblasts, hepatocytes, and vascular smooth muscle cells are sources of sclerostin [26, 27] and targeting sclerostin may, therefore, have other non-skeletal effects. Its expression is regulated by cytokines, mechanosensors, and endocrine factors [28]. Different studies suggested that the expression of sclerostin in osteocytes, osteoblastic cells, and mesenchymal stromal cells can be inhibited by estrogen [10, 29, 30].

Dickkopf proteins (DKK1–4) are soluble proteins belonging to the family of secreted glycoproteins. DKK1 is the most extensively studied subtype in bone and has been classified as one of the most potent extracellular Wnt inhibitors [12, 14, 17]. DKK1 is expressed by mature osteoblasts and osteocytes, and it regulates bone homeostasis [31].

Preanalytical Considerations

The clinical utility of a bone turnover marker depends on (a) whether the marker truly reflects the physiological or pathophysiological process that we want to monitor and

(b) whether reliable and reproducible measurements of the specific marker can be done. In the first place, ideal BTMs should be bone specific, predict the risk of fracture, allow evaluation of treatment efficacy, measured with a standardized method, and show low biological variation. In addition, the sample should be easily collected and suitable for automated measurement. Thus, for at potential marker, both preanalytical and analytical issues must be considered for both the clinician and the laboratory professional, and many clinical studies have shown discordant results possibly due to preanalytical and analytical issues.

Our knowledge related to the preanalytical variation of sclerostin and DKK1 is relatively limited as few studies have addressed this, especially in relation to the optimal sample handling. The available information is presented in Table 1 (sclerostin) and Table 2 (DKK1). More studies investigating the preanalytical variation and requirements for the two markers are highly warranted.

Analytical Considerations of Sclerostin and DKK-1 Assays

Sclerostin Assays

Sclerostin in serum or plasma is measured by immunoassays. A number of commercial assays are available and the most frequently used are listed in Table 3. No harmonization

Table 1 Preanalytical recommendations for measurement of sclerostin in humans

	Preanalytical factor	Preanalytical consideration	Comment	References
Sample handling	Sample type	Serum or plasma (EDTA)	Heparin as anti-coagulant might interfere with the binding of sclerostin to proteins and antibodies used in some assays. However, some manufacturers recommend use of heparin plasma	
	Storage	−70°C	No studies investigating the long-term stability are available	
	Transport	?		
	Stability	Plasma stable within 4 h after collection		[124]
	Freeze/thaw cycle	?		
Controllable biological variation	Circadian rhythm	None (healthy young men) None (older men and women) Minor rhythmicity (patients with diabetes and healthy individuals > 50 years)	Discrepant results in different studies may be a result of different populations or different assays used, but sclerostin does not seem to have any major rhythmicity	[125] [126] [127]
	Fasting/food intake	?	Glucose intake affects levels and effects of food cannot be excluded. Fasting recommended	
	Physical activity	Yes	Levels are positively correlated with level of physical activity	[47]
	Menopausal status	Yes	Pre-menopausal levels are lower than post-menopausal	[42]
	Seasonal variation	Yes	Sclerostin levels are higher during winter and lower during fall	[46]
Uncontrollable biological variation	Sex	Yes	Men have higher levels than women Boys have slightly higher levels than girls	[37, 38, 40, 41] [44]
	Age	Yes	Sclerostin levels increase with increasing age in adults	[41, 43]
	Fractures	?		
	CKD	Yes		[32, 105, 128]
	Medications	Yes	Acute effects of glucocorticoids decrease circulating sclerostin levels	[73, 74]

Table 2 Preanalytical recommendations for measurement of DKK1 in humans

	Preanalytical factor	Recommended handling	Comment	References
Sample handling	Sample type	Serum or plasma depending on the assay	As platelets are major contributors to circulating levels of DKK1 which is released upon activation of clotting, one study recommends only to use serum	[129]
	Storage	-70 °C	No studies investigating the long-term stability are available	
	Transport	?		
	Stability	?		
	Freeze/thaw cycle	?		
Controllable biological variation	Circadian rhythm	No		[126]
	Fasting/food intake	?		
	Physical activity	No		[47, 57]
	Menopausal status	No		[48]
	Seasonal variation	No		
Uncontrollable biological variation	Sex	No		[48]
	Age	No		[48]
	Fractures	?		
	CKD	No clear relationship	Minor associations in most studies One study found decreasing DKK1 levels with decreasing GFR	[118–122] [123]
	Medications	?	No effect of glucocorticoids	[74]

or standardization of assays have yet been done. Antibodies used in the immunoassays detect different epitopes of the sclerostin molecule which contributes to the large discrepancies seen between the different assays (Table 3). Not only levels of sclerostin in serum or plasma are hugely different between the individual assays, but there is also an absence of systematic bias between the assays indicating that assays determine different fragments of the sclerostin molecule making it difficult to compare [32]. The Biomedica assay seems to detect both intact sclerostin and sclerostin fragments in addition to other proteins with as similar structure to sclerostin as levels were detected in patients with sclerosteosis even if no levels were expected in these patients [33]. In addition, within the same assay, differences in levels between serum and plasma were seen though the degree of variation differed between different assays [34–36]. This indicates that antibodies used in the different assays detect varying degrees of free and protein-bound forms of the sclerostin-protein complex.

Newer assays targeting well-defined epitopes are now available such as the Bioactive Sclerostin ELISA from Biomedica [37] and the automated sclerostin assay for the Liaison XL from DiaSorin measuring "intact" sclerostin [38]. The latter uses antibodies directed against both the C- and N-terminal parts of the protein and a very high correlation between measurements in serum and EDTA-plasma samples were demonstrated. This suggests that the variation seen in older assays may be avoided in newer assays using antibodies specific for the intact sclerostin molecule. Finally, it has been claimed that as the R&D assay gives the lowest sclerostin values, this would mean that it only measures the intact molecule [39]. However, there are still large differences in levels measured using the R&D assay and the DiaSorin assay so it is still not clear which assay most reliably measures the biologically intact sclerostin protein. Analytical characteristics are listed in Table 4.

DKK1 Assays

As for sclerostin, a number of immunoassays are commercially available for DKK1. All of these are manual assays and no harmonization nor standardization have been done. While many assays exist, only two have been used consistently in published, clinical studies (Table 5). No direct comparison between the assays have been published, but ranges determined in healthy individuals suggest variation in levels between the two assays (Table 8). While no evaluation of differences between serum and plasma has been published for the Biomedica assays, clear differences in DKK1 levels are demonstrated among serum, heparin, and EDTA plasma when measured with the R&D Systems assay (Table 8). No information on epitope is available for the two assays (Table 6).

Table 3 Commercially available assays for measurement of serum/plasma sclerostin

Manufacturer	Assay	Methodology	Measurand	Analytics	References
Biomedica	Human Sclerostin ELISA	Sandwich ELISA	Epitope not defined	Manual	Biomedica validation data report
Biomedica	Bioactive Sclerostin ELISA	Sandwich ELISA	Epitope is in loop 2 of the sclerostin molecule, which is the binding site for the LRP5/6 complex. All sclerostin molecules including potential fragments containing this receptor-binding region can be detected	Manual	Biomedica validation data report
DiaSorin, Italy	Sclerostin	Chemiluminiscence immunoassay	Sclerostin (intact). Epitope is a 16-mer located within the N-terminal tail (Gln1 to Ser56) of sclerostin	Automated	DiaSorin assay IFU and [38]
MesoScale	96-well MULTI-ARRAY® Human Sclerostin Assay	Electro-chemiluminiscence immunoassay	Epitope not defined	Manual	
R&D Systems	Quantikine ELISA Human SOST Immunoassay	Solid-phase sandwich ELISA	Epitope not defined	Manual	R&D systems package insert
TECOmedical	Sclerostin TECO® High-sensitive EIA	ELISA	Epitope not defined for the current assay. A previous version of the assay (discontinued in 2013) recognized the amino terminus and mid-region of the sclerostin molecule	Manual	TECOmedical package insert

Table 4 Analytical characteristics of commercially available assays for sclerostin

Assay	Measuring range (pg/mL)	LOD/LLOQ (pg/mL)	Intra-assay CV	Inter-assay CV	Sample material
Biomedica	72–5400	LOD: 72 LLOQ: < 169	< 7%	< 10%	Serum, plasma (EDTA, citrate, heparin)
Biomedica Bioactive	43–7200	LOD: 43 LLOQ: 29	< 1%	< 5%	Serum, plasma (EDTA, citrate) Heparin disturbs binding of detection antibody
Diasorin	50–6000	LOD: 20 LLOQ: 50	< 2.5%	< 5%	Serum, EDTA plasma
MesoScale		LOD: 0.9 LLOQ: N.A	< 6%	< 10%	Information not available in IFU. [71, 130]
R&D Systems	31–2000	LOD: 1.7 LLOQ: N.A	< 3%	< 11%	Serum, plasma (EDTA, heparin). Citrate has not been validated for use
TECOmedical	59–2970	LOD: 9 LLOQ: 59	< 5%	< 5%	Serum, plasma (EDTA, heparin)

Table 5 Commercially available assays for measurement of serum/plasma DKK1

Manufacturer	Assay	Methodology	Measurand	Analytics	References
Biomedica	DKK-1 ELISA	Sandwich ELISA		Manual	Manufacturer IFU
R&D Systems	Quantikine ELISA Human DKK-1 immunoassay	Sandwich ELISA		Manual	Manufacturer IFU

Reference Values of Sclerostin and DKK1

Sclerostin Reference Values

Reference values for sclerostin have been established in several studies covering populations of different ethnicities in several geographical regions (Table 7). Also, ranges in healthy individuals are provided from most manufacturers in their IFU, though relatively little information about the characteristics of the donors contributing to the manufacturer-established reference ranges is provided. Most studies are based on relatively small numbers of participants affecting the validity of the estimated reference intervals. Moreover, as levels of sclerostin differ between the different assays, the established reference ranges are highly variable and individual measurements should be interpreted in relation to the reference range of the particular assay and matrix type used for measuring the sample.

In general, circulating sclerostin levels are higher in men than in women [37, 38, 40, 41] independent of the assay used. Also, pre-menopausal sclerostin levels are lower than post-menopausal levels [42], and sclerostin levels increase with increasing age [41, 43]. In children, boys seem to have slightly higher circulating sclerostin levels [44] while age, height, weight, and BMI do not seem to influence the reference ranges in children and adolescents [45]. Finally, sclerostin levels vary with season, as levels are higher during winter and lower during fall [46], and sclerostin is positively correlated with the level of physical activity [47].

In summary, reference intervals have been published for the different sclerostin assays, but most are based on relatively small populations. As sclerostin levels depend on both the assay used, matrix in which it is measured and biological factors such as sex, age, menopausal status and time of year, and specific reference ranges are warranted if measurements should be validly evaluated for the individual patient.

DKK1 Reference Values

Very few studies have established reference ranges for circulating DKK1 in humans. Manufacturer-provided reference ranges are given in Table 8. One single study determined reference ranges in both pre- and post-menopausal women and men with levels significantly higher than the ranges provided by the manufacturer. DKK1 levels were not affected by age and sex [48] and are not correlated with level of physical activity [47]. Additional studies establishing DKK1 reference intervals are highly warranted.

Clinical Applications of Measuring Sclerostin and DKK1

The use of Wnt Inhibitors in Osteoporosis and Fracture Prediction

Because of the direct regulatory function of sclerostin on bone turnover, it has been proposed as an ideal marker for bone metabolism and predictor of fracture risk. Intuitively, as Wnt inhibitors inhibit bone formation that the effect on the skeleton would be decreased bone mass. However, although some conflicting results have been published, most studies demonstrated that serum sclerostin levels were positively associated with increased BMD levels, both in post-menopausal women [10, 49] and in patients with chronic kidney disease [50, 51]. In contrast, studies addressing the association between circulating sclerostin levels and fracture risk have provided less clear correlations between circulating sclerostin levels and fracture incidence.. In some studies, serum sclerostin was found to be a strong and independent risk factor for higher fracture incidence in women [52, 53], while others found no association between baseline serum sclerostin and fracture incidence in post-menopausal women [49]. Finally, other studies found that high sclerostin levels in older men and women were associated with lower fracture risk [54, 55]. No similar association was found for DKK1 [55], nor did a study investigating the association between a common genetic variation in the *DKK1* gene find an association between the genetic variation and bone mineral density or bone turnover markers in a cohort of young adult men [56].

In patients immobilized for 6 months or longer due to stroke, increased circulating levels of sclerostin were found when compared to non-immobilized controls [57]. The elevated levels correlated positively with increased bone

Table 6 Analytical characteristics of commercially available assays for serum/plasma DKK1

Assay	Measuring range (pg/mL)	LOD/LLOQ (pg/mL)	Intra-assay CV	Inter-assay CV	Sample material
Biomedica	32–4103	LOD: 44 LLOQ: 32	< 3%	< 3%	Serum
R&D Systems	31–2000	LOD: 16 LLOQ: 31	< 5%	< 8%	Serum, plasma (EDTA, heparin). Citrate has not been validated for use

Table 7 Reference intervals (95%) in healthy individuals for commercially available assays for serum/plasma sclerostin

Manufacturer/ assay	Sample matrix	95% RI Sclerostin (pg/mL)	Sex	Age group	Geographical region/ ethinicity	References
Biomedica	Serum	169–1453	All ($n=411$)	N.A	N.A	IFU
	Serum	GM: 600 GM: 714	Women ($n=443$) Women ($n=1,803$) GM values are given for 10-year interval age groups	35-45y 20–79y	Saudi Arabia	[43]
	Serum	89–1,917 1–1,773	Men ($n=173$) Women ($n=273$)	18–90y 18–90y	Tobago, African decent	[40]
	Serum	Median (app.): 510 IQR: 380; 625 Median (app.): 430 IQR: 290; 550	Boys ($n=70$) Girls ($n=70$)	6–21y	US, primarily white	[44]
Biomedica Bioactive	Serum EDTA plasma Citrate plasma	281–3,226 657–5,081 432–3,719	All ($n=32$) All ($n=24$) All ($n=24$)	N.A	N.A	IFU
	Serum	Median: 1,154 IQR: 857; 1,580 Median: 1,386 IQR: 914; 2,061	Women ($n=175$) Men (n$n=61$)	Median: 43y IQR: 37; 50 Median: 49y IQR: 41; 56	Austria	[37]
Diasorin	Serum	139–663 139–921	Women ($n=265$) Men ($n=271$)	21–97y 21–97y	Northern USA, primarily caucasian origin	[38]
MesoScale	Serum	14–66	All ($n=77$)	20–77y	N.A	[71, 130]
R&D Systems	Serum EDTA plasma Heparin plasma	52–296 172–680 211–759	All ($n=35$) All ($n=35$) All (n = 35)			
TECOmedical	N.A	208–754 247–913 348–934	Pre-menopausal women ($n=20$) Post-menopausal women ($n=19$) Men ($n=10$)			IFU
	Serum	361–1,165 578–1,500	Pre-menopausal women ($n=77$) Post-menopausal women ($n=61$)	29–40y 59–70y	Chinese-American and White	[42]
	Serum	200–1,000 (no differences with age)	Children, adolescents ($n=424$, where of $n=221$ boys)	0.1–21y	Germany, Caucasian	[45]

95% reference intervals (RI) were calculated from published information on mean, SEM, SD, number of participants and 95% CI. If RI could not be calculated from the published information, range or median/inter quartile range (IQR) is given in the table. GM: Geometric mean

Table 8 Reference intervals (95% RI) and median (range) in healthy individuals for commercially available assays for serum/plasma DKK1

Manufacturer/assay	Sample matrix	95% RI DKK-1 (pg/mL)	Sex	Age group	Reference
Biomedica	Serum	874 (129–1800)	N.A. ($n=51$)	N.A	IFU
	Serum	95% RI: 1355–5785 95% RI: 340–6300	Post-menopausal women ($n=52$) Pre-menopausal (n = 123)	20-65y	[48]
R&D Systems	Serum EDTA plasma Heparin plasma	2513 (1357–5240) 630 (172–1499) 424 (151–865)	N.A. ($n=36$)	N.A	IFU

resorption measured as CTX and negatively with decreased bone formation, while no differences between groups were found for serum DKK1. This confirms the role of sclerostin in the adaptation of bone turnover to changes in mechanical loading where sclerostin production increases during unloading with stimulation of bone resorption and inhibition of bone formation leading to pathophysiological bone loss.

While interesting associations between circulating sclerostin levels and different bone parameters have been demonstrated and important information can be gained when using the marker in clinical studies, the marker is not yet suitable for clinical use.

The Use of Wnt inhibitors in Diabetes

Diabetes is associated with an increased risk of fractures. However, this is not explained by changes in bone mineral density but patients with diabetes have low bone turnover measured as decreased levels of serum CTX and PINP. Several studies have shown that patients with both type 1 diabetes (T1DM) and type 2 diabetes (T2DM) have elevated circulating levels of sclerostin, which was confirmed by a meta-analysis [58]. Also, a recent study in post-menopausal women investigating the expression of *SOST*, the gene regulating sclerostin production showed that women with T2DM had higher expression of *SOST* in bone tissue from the femoral head as compared to healthy women [59]. Thus, the high sclerostin levels may be an important contributing factor to low bone turnover seen in patients with diabetes as the high sclerostin levels may suppress bone formation and consequently bone turnover resulting in reduced remodeling and renewal of bone tissue. This may subsequently result in hypermineralization of old, non-remodeled bone tissue seen as normal to high BMD in patients with T2DM. However, it is not known whether the high sclerostin levels are the cause of the reduced bone turnover or secondary to other changes to glucose or bone metabolism seen in T2DM.

Sclerostin has also emerged as a potential regulator of glucose homeostasis. A recent study investigated the association of sclerostin with different measures of glucose metabolism. Overall, circulating levels of sclerostin were not associated with insulin secretion, insulin sensitivity, or prediabetes in healthy men. However, acute hyperinsulinemia suppressed serum sclerostin [60]. Other studies have reported conflicting associations between serum sclerostin and markers of glucose metabolism where both an inverse association with fasting plasma glucose levels [61] and no association with markers of glycemic variability [62] were found. Moreover, in children with T1DM, a negative correlation between serum sclerostin and HbA1c was found [63], and in obese children, a negative correlation between serum sclerostin and HOMA-IR was shown [64].

For DKK1 only, few studies have investigated the association with diabetes. However, they have consistently demonstrated that children and adolescents with T1DM have elevated levels of serum DKK1 compared to healthy children [65, 66]. Moreover, adult T2DM patients have increased DKK1 levels, though there was no correlation with glycemic control or duration of diabetes [67].

Thus, sclerostin seems to be involved in the low bone turnover found in diabetes, but its role as a regulator of glucose metabolism is less evident. While highly important as a biomarker for studying the mechanisms underlying the development of diabetes-associated bone disease, the clinical utility of determination of serum sclerostin levels is virtually unexplored. Therefore, its use in the clinical setting of either diagnosing or monitoring diabetes or diabetic bone disease is currently not relevant though it holds promising potential in the future. The same is the case for DKK1.

Wnt Inhibitors and Inflammatory Diseases

Sclerostin has been suggested to be involved in both regulation of bone resorption and the pathogenesis of ankylosing spondylitis and rheumatoid arthritis, yet the results of the studies have been conflicting. A recent systematic review and meta-analysis have evaluated relevant studies and found no differences in circulating sclerostin levels in neither patients with ankylosing spondylitis nor in patients with rheumatoid arthritis when compared to healthy controls [68]. In contrast, another meta-analysis found significantly elevated levels of DKK1 in patients with ankylosing spondylitis, but not in patients with rheumatoid arthritis [69]. The markers have no current utility in the clinical setting.

Wnt Inhibitors and Other Hormone-Related Diseases

Parathyroid hormone suppresses sclerostin expression and studies have shown that patient with increased levels of circulating PTH such as in primary hyperparathyroidism, sclerostin levels correlated inversely with PTH [70, 71]. Moreover, serum sclerostin levels normalized shortly after parathyroidectomy and faster than most of the other bone turnover markers measured.

Glucocorticoid excess has detrimental effects in the bone tissue and leads to a rapid bone loss and increased risk of fragility fractures. It is mainly characterized by a decrease bone formation and a few studies have investigated the serum levels of sclerostin in patients with endogenous hypercortisolism. Yet, the available studies have found inconsistent results. One study found higher circulating sclerostin levels, but not DKK1 levels in patients with Cushing's syndrome than in healthy controls [72] while another study found lower levels of sclerostin in the patients as compared

with the controls [73]. In the latter study, sclerostin levels increased after surgical treatment of the disease with subsequent biochemical remission. In support of the negative effect of glucocorticoids on serum sclerostin levels, as study examined the acute effects of glucocorticoids on levels of serum sclerostin and a number of bone turnover markers. During the first 96 h after glucocorticoid administration, serum levels of both sclerostin and PINP decreased while levels of CTX increased. No significant effects on DKK1 were seen [74], though a positive correlation between DKK1 and CTX was found. Thus, while sclerostin seems to be downregulated during glucocorticoid excess the clinical utility of this relationship is not yet determined though it might prove useful in the future.

Wnt Inhibitors as Markers in Cancer-Related Bone Disease

Accumulating evidence indicates that the Wnt-signaling pathway is involved in the development and progression of both osteoblastic and osteolytic bone metastases [75, 76] and inhibitors of the signaling pathway may, therefore, be potential diagnostic or prognostic biomarkers of metastasis. Yet, only few studies have addressed the potential use of sclerostin and DKK1 as biomarkers of metastasis. One study compared the levels of circulating sclerostin between patients with bone metastases from prostate cancer, patients with Paget's disease and healthy controls. Both prostate cancer patients and Paget's disease patients had increased sclerostin levels compared to the healthy controls, but levels of sclerostin could not distinguish between prostate cancer patients and patients with Paget's disease [77]. While prostate cancer metastases are primarily osteoblastic, metastases originating from breast, lung, and renal cancers are primarily osteolytic. One study of patients with renal cell carcinoma, sclerostin levels were compared between patients with localized disease, patients with bone metastases and patients with visceral metastases. No differences between the three groups were found, indicating that serum sclerostin is not a useful marker for identifying patients with metastatic disease [78]. Another study examined the levels of serum sclerostin and DKK1 in patients with early breast cancer and found that breast cancer patients had higher levels of both markers than healthy controls and patients with benign breast tumors [79]. Finally, a longitudinal study compared the serum levels of sclerostin and DKK1 in patients with bone metastases from primarily breast and lung cancer and levels in patients with malignant disease but without metastases [80]. Not only did patients with metastases have higher serum sclerostin values, area under the curve (AUC) for the receiver operating characteristics (ROC) curve for diagnostic sensitivity shows good performance with AUC close to 0.9.

Multiple myeloma is a clonal neoplasm of plasma cells. More than 80% of the patients develop osteolytic lesions because of increased osteoclastic bone resorption [81]. Circulating levels of sclerostin and DKK1 are increased in patients with myeloma compared with healthy controls and patients with monoclonal gammopathy of unknown significance (MGUS)[82–84]. Also, the level of DKK1 was positively correlated with the presence and number of lytic lesions [82, 85, 86]. In patients responding to chemotherapy, both markers decreased while non-responders, levels remained stable during treatment [83, 87, 88]. Finally, both markers seemed to increase early before a relapse of the disease [87, 89, 90].

Thus, compelling evidence demonstrates that patients with bone involvement of malignant diseases have increased circulating levels of both sclerostin, and in some cases DKK1, further and better powered studies addressing the diagnostic and prognostic potential of these markers are warranted to fully explore and document the clinical utility of sclerostin and DKK1 as markers in malignant disease. However, the already published studies show a great potential for the two markers in this indication.

Wt Inhibitors as Biomarkers of Vascular Disease/ Calcification

Vascular calcification is common in a number of chronic diseases including diabetes, CKD (please see section below), and rheumatoid arthritis and is a marker of cardiovascular morbidity and mortality [91, 92]. Cumulating evidence points to a role for the Wnt-signaling pathway in multiple processes involved in atherosclerosis. A number of studies have investigated the association between levels of Wnt-signaling inhibitors and different cardiovascular events in a number of clinical conditions including post-menopausal osteoporosis, chronic kidney disease in both dialyzed and non-dialyzed patients, rheumatoid arthritis, and T2DM. However, results have been inconsistent with studies showing both positive [93–95] and inverse [96] associations between circulating levels of sclerostin and all-cause mortality, positive [97, 98], and inverse [99] associations between serum sclerostin and carotid intima media thickness, and associations between serum sclerostin and aortic artery calcification [100–102]. Moreover, a meta-analysis based on the published literature did not find any association between serum sclerostin and neither risk of all-cause mortality, cardiovascular mortality, nor cardiovascular events [103], but concluded that most studies were relatively small and heterogeneous in study design. In addition, different sclerostin assays were used further contributing to the heterogeneity of the studies. Thus, while sclerostin is clearly involved in the pathogenesis of vascular calcification, its potential for use

as a biomarker for cardiovascular disease has not yet been clearly demonstrated.

Influence of CKD on Sclerostin and DKK-1 Levels

Sclerostin and CKD

Chronic kidney disease (CKD) involves abnormalities of both mineral metabolism and bone turnover, also called CKD metabolic bone disease (CKD-MBD) which subsequently results in vascular calcifications and calcifications in soft tissues [104]. Several studies have investigated the role of sclerostin in CKD-MBD and in the associated vascular calcifications and the potential of serum sclerostin measurements as a biomarker of bone and vascular changes. Circulating sclerostin levels have been shown to increase when GFR decreases [105], though it is not clear whether this is due to reduced renal clearance or increased osteocytic production or whether it comes from extraskeletal sources [106]. In non-CKD individuals, sclerostin is cleared hepatically and with no renal elimination [107], but no studies were found examining renal clearance in CKD patients.

Several studies have demonstrated that circulating sclerostin levels are negatively correlated with biochemical parameters of bone turnover such as PTH concentrations [49, 71, 108] and markers of bone formation [109, 110]. Sclerostin was also negatively correlated with histomorphometric bone indices of bone formation in CKD patients over a broad spectrum of disease stages [111], hemodialysis patients [112], and peritoneal dialysis patients [113]. However, the ability to distinguish between patients with high and low bone turnover was low [112] and sclerostin was not superior to bone-specific alkaline phosphatase as a measure of bone turnover [114]. A few studies have examined the correlation between serum sclerostin levels and bone mineral density (BMD). These generally found a positive correlation between circulating sclerostin and BMD both in hemodialysis patients [93, 109], in peritoneal analysis patients [51], and in patients with end-stage renal disease [115]. Finally, in a longitudinal study with hemodialysis patients, high sclerostin levels were predictive of one-year bone loss [116].

Although interesting correlations between sclerostin and different clinical and paraclinical parameters have been found in cohorts of CKD patients, the sometime discrepant results warrant a discussion of alternative explanations. Recent studies have demonstrated that the correlates and determinants of sclerostin are highly dependent of the assay used for measuring sclerostin. In a cohort of 91 patients with CKD undergoing hemodialysis, sclerostin results differed significantly depending on whether samples were measured using the Biomedica or the TECOmedical assay [117]. In another study by Delanaye et al. in 82 non-dialysis patients referred for GFR measurement and 39 hemodialysis patients, large discrepancies were found between sclerostin levels measured on four different assays (Biomedica, TECOmedical, R&D Systems, and MesoScale) [32]. Also, when correlating a number of different biological variables such as age, height, GFR, and PTH with sclerostin, either all, some, or none of the variables were correlated with sclerostin depending on the assay used. Finally, the effect of one single hemodialysis session on sclerostin levels was assessed and highly varying reduction ratios between 25 and 46% were found [32]. In another cohort of 68 end-stage kidney disease patients, serum sclerostin levels were measured on four different assays (DiaSorin, TECOmedical high sensitivity, Biomedica, and R&D Systems) and correlated with measurements of bone sclerostin and bone histomorphometry [39]. Again, large discrepancies were seen between sclerostin levels from the different assays. However, serum sclerostin levels from all 4 assays correlated moderately with the number of sclerostin-positive lacunae in bone biopsies from the patients. In 3 out of 4 assays, serum sclerostin correlated negatively with both histomorphometric parameters of bone formation and biochemical serum parameters of bone formation (bone-specific alkaline phosphatase and PINP) as well as parathyroid hormone (PTH). Only the DiaSorin assay did not correlate with the histomorphometric and biochemical formation parameters. For all 4 assays, serum sclerostin correlated with clinical parameters such as age, BMI and residual renal function [39]. Thus, while sclerostin may be a potential biomarker of bone turnover in patients with CKD as it correlates inversely with the bone formation parameters, it is currently difficult to use clinically due to the discrepancies in sclerostin levels between assays. Only when we have a reference method for such as mass spectrometry, the true potential of using sclerostin as a biomarker for bone turnover in CKD patients will be fully uncovered.

DKK1 and CKD

In contrast to sclerostin, only relatively few studies have examined correlations of DKK1 with clinical parameters and disease characteristics in patients with CKD-MBD. Most of these studies report no association between circulating DKK1 levels with neither sex nor age and only minor correlations with declining renal function [118–122]. Moreover, no correlations with BMD or bone histomorphometry were demonstrated [112]. However, most studies have used estimated GFR (eGFR) determination, but a recent study examined the association between directly measured GFR and circulating DKK1 levels. They found DKK1 to be associated with PTH levels and that DKK1 levels decreased with decreasing GFR [123]. Further studies are needed to

determine the usefulness of DKK1 as a biomarker of CKD-MBD and very little, if any, data are available on the analytical aspects of DKK1 in patients with CKD.

Conclusion

While studies have shown that both circulating levels of sclerostin and DKK1 are associated with a range of clinical parameters and outcomes and that the inhibitors in some cases can help understand the underlying pathophysiological mechanisms regulating bone turnover, the clinical utility of the two markers is currently very limited. This is due to a number of issues. First, there is currently no standardization nor harmonization of assays making the comparison of results between the individual studies complicated as the different assays possibly measure different molecules or metabolites as the primary antibodies in the assays measure different epitopes. Second, very little information on the preanalytical requirements of the samples is available. This carries a large risk of bias as the sample handling in the published studies has not been standardized. These two aspects undoubtedly contribute to the large discrepancies between studies. Next, many of the studies have relatively low power to fully examine the associations between levels of sclerostin and DKK1 and the clinical outcomes we want to detect/predict. Finally, most of the studies have not addressed the clinical utility of the markers with appropriate statistical methods such as calculation of predictive value of the markers, calculation of appropriate cut-offs with ROC-analyses, etc. Taken together, the clinical utility of sclerostin and DKK1 is currently very limited though it might be promising, given the issues with standardization/harmonization of assays are solved, more detailed information on preanalytical requirements are provided, and additional prospective studies sufficiently powered for investigation of the markers with relevant, clinical endpoints.

Declarations

Conflict of interest Aylin Sepinci Dincel and Niklas Rye Jørgensen declare that they have no conflict of interest relevant to this manuscript.

Human and Animal Rights No permissions for human or animal experiments have been sought and no informed consents from patients have been obtained as this is irrelevant as the current study is a review of existing literature.

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