Bone xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Full Length Article

Sclerostin measurement in human disease: Validity and current limitations☆

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ARTICLE INFO

Article history:
Received 27 June 2016
Revised 6 October 2016
Accepted 10 October 2016
Available online xxxx

Keywords:
Sclerostin
ELISA
Validation

ABSTRACT

Sclerostin a potent regulator of bone formation, is an antagonist of the Wnt-signaling pathway. The advent of assays to measure circulating sclerostin has enabled research to be performed with the aim to understand the potential role of circulating sclerostin as a pathophysiological marker in a variety of clinical settings. At this time, however, assays to measure circulating sclerostin are still relatively new and have not demonstrated consistent internal agreement in addition to which there are differences between serum and plasma levels. Nevertheless, measurement of sclerostin in the circulation has the potential to reflect the dynamics of bone formation with particular reference to situations in which osteocytes, the major source of circulating sclerostin, may be perturbed. Because of technical uncertainties regarding sclerostin assays that are currently available, circulating sclerostin measurements should be interpreted cautiously with attention to reference ranges for each assay and whether or not the measurement is made in serum or plasma.

1. Introduction

Sclerostin is a soluble glycoprotein product of the *SOST* gene. Although *SOST* mRNA is widely expressed, particularly during embryogenesis in many tissues including heart, aorta, cartilage, bone marrow, liver, pancreas, kidney and bone, current data suggest that in humans, sclerostin is mainly a product of the *SOST* gene present in osteocytes [1–4]. The link between sclerostin and its regulation of bone formation was highlighted by the discovery of two rare high bone mass syndromes, van Buchem's disease and sclerosteosis both of which are characterized by a defective sclerostin gene and absence or profound reduction of its product, sclerostin [5,6].

The complete elucidation of the signaling pathway utilized by sclerostin is still incomplete but progress has been made. Initially, it was thought that since sclerostin presents an amino acid sequence similar to the DAN family of glycoproteins, it would act as an antagonist of BMP signaling similar to the proteins of the DAN family [3,7]. Later, however, it was demonstrated that sclerostin's actions on BMP signaling were non-classical and rather weak [1,8]. Recent, more compelling findings indicate that sclerostin is a regulator of the canonical osteoanabolic Wnt/β-catenin pathway. This pathway plays an important role in osteoblast differentiation, proliferation and survival [8–12].

Early attempts to measure sclerostin focused on detecting its mRNA expression rather than measuring directly the actual glycoprotein. Subsequently, direct detection of sclerostin included immunochemistry and Western-blot analyses, which were an improvement but laborious [13–15]. A major step forward in the measurement of sclerostin was the development of sensitive and specific immunoassays such as ELISAs to measure circulating serum and plasma levels. The development of these assays led to clinical assessment of sclerostin concentrations in numerous clinical situations, the validity of which was enhanced by showing that there were significant correlations between sclerostin measurements in serum and sclerostin levels in bone marrow (determined by custom-made single-plex Luminex kits - Millipore/Linco, St. Charles MO) [16].

2. Sclerostin measurements in human subjects

Findings of increased bone mass in sclerosteosis and van Buchem disease in the absence of sclerostin in addition to sclerostin's antagonist role of Wnt signaling, initiated multiple studies focused on explaining the role of this glycoprotein in physiological and pathological conditions [17]. With regard to the assays themselves, the clinical disorders in which there is no circulating sclerostin provided a validation methodology for the assays in that such individuals who have no circulating sclerostin should not have any measureable circulating levels by immunoassay. It was shown by Lierop et al. that circulating sclerostin was undetectable in patients with sclerosteosis but, as expected, was

☆ Disclosure summary: Aline G Costa – Nothing to disclose; Serge Cremers – Nothing to disclose; John P. Bilezikian – Nothing to disclose.

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http://dx.doi.org/10.1016/j.bone.2016.10.012
8756-3282/

measurable in heterozygous carriers for the disease and in healthy controls [6].

The development of assays to measure circulating sclerostin enabled human studies to be conducted in a large number of diseases. Sclerostin commercial assays, in addition to a few non-commercially available assays and laboratory-generated 'in-house' assays, have been used in a growing list of publications, mostly focused on understanding the role of circulating sclerostin as a pathophysiological marker of metabolic bone disease [18–26].

In the aggregate, the results of these studies seem to demonstrate that circulating levels of sclerostin are higher in men than in pre- or postmenopausal women. Sclerostin levels are higher in postmenopausal women (not taking hormone replacement therapy) than in premenopausal white women. Circulating sclerostin levels are lower in primary hyperparathyroidism, a state of parathyroid hormone (PTH) excess in comparison to hypoparathyroidism, a state of PTH deficiency, and in euparathyroid controls. This observation is consistent with the idea that PTH is an antagonist of sclerostin secretion. In settings of reduced mechanical stimuli, such as in disuse osteoporosis, sclerostin levels are higher than ambulatory subjects [19,27–30]. Additional data show that the circulating concentration of sclerostin can be influenced by estrogen administration, body mass index and physical activity [31–33]. Recently, sclerostin was shown to be differentially associated with bone turnover markers in Chinese-American women when compared with Caucasian-Americans, raising the possibility of race as a variable that influences sclerostin secretory dynamics [34]. Moreover, circulating sclerostin has been shown to be high in Type 2 diabetes, Paget's disease and in subjects with bone metastases [35–37]. In ankylosing spondylitis, sclerostin levels appear to vary according to disease activity [38]. Although the mechanisms are still obscure, some data suggest a potential link between the osteoanabolic Wnt-signaling pathway, bone disease and vascular calcifications in chronic kidney disease (CKD). In this regard, sclerostin has been advanced as a potential marker for morbidity and mortality in CKD patients [39–43].

3. Sclerostin assays

Three commercially available assays have been utilized in most of the clinical studies to date: an ELISA from Biomedica (Biomedica Gruppe, Wien, Austria), an ELISA from TECOmedical (TECOmedical group, Sissach, Switzerland, distributed by Quidel in the US) and an electrochemiluminescence immunoassay (ECLIA) from MesoScaleDiscovery (MSD, Gaithersburg, Maryland, USA) (MSD). A third ELISA to measure circulating sclerostin is manufactured by R&D systems [44]. These assays all differ in their set-up, antibodies used and subsequent performance. It is therefore likely that discrepancies in the outcome of studies that would be expected to be internally consistent [44–49] could be explained by the use of different assays.

Sclerostin is a glycoprotein characterized by 190 residues forming a 3-loop structure around a cysteine knot with long C- and N- termini [3,50]. The 2nd loop has been identified as the binding site for monoclonal antibodies, and the binding site for LRP5 receptor is on the 3rd loop [51]. Sclerostin is classically considered to be a monomeric protein, but recent data have raised the possibility that circulating sclerostin has a dimeric configuration [52]. The commercially available assays utilize different antibodies, and except for the MSD assay and a previous ELISA version by TECOmedical (Sclerostin TECO® kit), there are no data showing to which sclerostin epitope(s) the antibodies used in these assays bind. Therefore, it is not clear if these assays are measuring the whole sclerostin molecule, fragments or a combination of both. With epitope mapping, Lierop et al. [53] demonstrated that the MSD assay is likely to detect the whole sclerostin molecule, with the sclerostin antibodies used in this assay binding to various epitopes that span the entire molecule from the N-terminus to the C-terminus. Specifically, the capture antibody recognizes 2 binding sites at the N- and C-termini. The detection antibody recognizes 3 binding sites at the 3rd loop as well as at the N- and C-termini. The same group demonstrated that the Biomedica assay when directly compared with the MSD assay detected fragments of the sclerostin molecule while measurements performed with MSD assay detected only the whole sclerostin molecule [53]. Dimerism and the formation of protein complexes could affect sclerostin recognition by the antibodies present in the assays [45,46]. As noted above, a standard of validity for these assays is whether the assay detects "sclerostin" in the circulation of patients who have a genetic absence of the protein. The Biomedica assay detected sclerostin in the serum of these patients while the MSD assay did not, raising the hypothesis that the Biomedica assay may cross-react with circulating proteins that are similar in structure to sclerostin. While there are no data on the possible cross-reactivity with sclerostin fragments for the ELISA from TECOmedical, the findings that the Sclerostin TECO® kit (discontinued kit) reads higher sclerostin concentrations as compared to MSD assay may imply that this assay also detects fragments, similar to the Biomedica assay [19,46] (Table 1). Such differences may have other explanations such as lack of standardization of the

Table 1
Comparison of commercially available sclerostin assays.[a]

Manufacturer	Assay name	Assay type	Primary/secondary antibody	Intra- and inter-assay CVs	Standard range/LOD (pmol/l)	Assay observed normal range (pmol/l)
Biomedica	Sclerostin ELISA	ELISA	Polyclonal/monoclonal	<7%; <10%	0–240 pmol/l 3.2 pmol/l	Healthy individuals (n = 411) 24.14
TECO	Sclerostin TECO®[b]	ELISA	Polyclonal/monoclonal	<5%, <10%	11–176 5.7	Premenopausal women (n = 60) = 24.64 ± 5.72 Postmenopausal women (n = 60) = 30.6 ± 8.80 Men (n = 18) = 32.56 ± 11.44 All subjects (n = 138) = 26.65 ± 8.38
TECO	Sclerostin TECO® high sensitive	ELISA	Polyclonal/monoclonal	<5%, <5%	2.2–132 0.4	Premenopausal women (n = 20) = 21.12 ± 6.16 Postmenopausal women (n = 19) = 25.52 ± 7.48 Men (n = 10) = 28.16 ± 6.6
MSD	Human sclerostin kit	ECL	Polyclonal/polyclonal	6%, 10%	0.1–440 0.1	NA by the assay manufacturer Undetectable in 19 patients with sclerosteosis [5,6] Patients with van Buchem disease (n = 13) = mean 8.0 pg/mL (95%CI 4.9–11.0 pg/mL) Healthy controls (n = 77) = mean 40.0 pg/mL (95%CI 34.5–41.0 pg/mL) [58]
R&D Systems	Human SOST immunoassay	ELISA	Monoclonal/polyclonal	≤2%, <11%	1.4–88 0.1	Serum (n = 35) = 6.47 ± 2.68 EDTA plasma (n = 35) = 18.74 ± 5.59 Heparin plasma (n = 35) = 21.34 ± 6.03

Abbreviations: CV, coefficient of variation; LOD, lower limit of quantification; ECL, electro-chemiluminescence assay; ELISA, enzyme-linked immunosorbent assay; TECO, TECO Medical Group; MSD, meso scale discovery; R&D Systems, NA, not available in the assay insert.

[a] Adapted from Clarke et al. [17] and

[b] Discontinued in 2013.

Please cite this article as: A.G. Costa, et al., Sclerostin measurement in human disease: Validity and current limitations, Bone (2016), http://dx.doi.org/10.1016/j.bone.2016.10.012