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High serum levels of sclerostin and Dickkopf-1 are associated with acute ischaemic stroke

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ABSTRACT

Background and aims: Sclerostin and Dickkopf-1 (Dkk-1) are potent antagonists of Wnt signalling and might therefore play important roles in cardiovascular disease. We investigated whether serum sclerostin and Dkk-1 levels are associated with acute ischaemic stroke and specific stroke subtypes.
Methods: Serum levels of sclerostin and Dkk-1 were measured by ELISA on day 1 and on day 6 after stroke in 62 patients with large artery atherosclerotic (LAA) stroke, on day 1 after stroke in 62 age- and gender-matched patients with small-artery occlusion (SAO) stroke and on admission in 62 healthy controls. Stroke severity was determined based on the National Institutes of Health Stroke Scale (NIHSS) and by measuring stroke volume on diffusion-weighted imaging. Outcome was measured by the modified Rankin Scale (mRS) on day 90.
Results: Compared with controls, serum sclerostin and Dkk-1 levels were significantly higher in both patients with LAA stroke and with SAO stroke, and no difference was detected between the stroke subtypes. Sclerostin and Dkk-1 levels remained stable between the first and sixth day after stroke in the patients with LAA stroke. Receiver operating characteristic curve analysis was used to evaluate sclerostin and Dkk-1 as markers of a high risk of stroke and produced area under curve values of 0.773 and 0.776. Adjusted logistic regression showed that serum sclerostin and Dkk-1 levels remained as independent markers of stroke. No correlations were found between sclerostin or Dkk-1 levels and stroke severity or stroke outcome.
Conclusions: High serum levels of sclerostin and Dkk-1 are associated with acute ischaemic stroke.

1. Introduction

Increasing evidence has demonstrated the important roles of the Wnt signalling pathways in the regulation of atherosclerosis-related processes, including vascular inflammation [1,2], proliferation [3,4], angiogenesis [2,5], and calcification [6,7], all of which indicating the potential relationship between Wnt signalling pathways and atherosclerosis.

β-Catenin is a central component in the canonical Wnt pathways. Wnt ligands bind to the Frizzled receptor and the co-receptors low-density lipoprotein receptor-related proteins 5 and 6 (LRP-5 and LRP-6), which stabilize the structure of β-catenin and induce its translocation into the nucleus to regulate the transcription of target genes [8]. These processes are regulated by multiple secreted antagonists, including sclerostin and Dickkopf-1 (Dkk-1), which prevent the formation of the Wnt-Frizzled-LRP5 complex by binding to the LRP5/6 co-receptors or to LRP5 itself, respectively [9].

Sclerostin and Dkk-1 are primarily expressed and secreted by osteocytes and other terminally differentiated cells embedded within mineralized matrices [10,11]. Besides, the expression of these two antagonists has been detected in many human tissues, such as the heart and lung, as well as in cancerous tissue [10,12,13]. Moreover, some studies have reported that increased expression levels of sclerostin and Dkk-1 have been detected in calcified aortas or carotid plaques [1,14].

Several clinical studies have explored the relationships between sclerostin or Dkk-1 and surrogate markers of atherosclerosis (such as abnormal artery intima-media thickness, plaques and

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calcifications) in humans. However, the role of sclerostin in atherosclerosis remains controversial. Some studies have demonstrated a positive correlation between sclerostin levels and parameters related to atherosclerosis [15], whereas other studies have shown a negative correlation [16] or no correlation [17,18]. Similarly, studies investigating the impact of Dkk-1 on atherosclerosis have generated conflicting results, showing either direct or inverse correlations between the levels of Dkk-1 and surrogate markers of atherosclerosis [7,16–19]. These discrepancies might result from heterogeneity or clinical differences between the different cohorts. However, the common theme among the above studies is that the mechanisms by which these two Wnt pathway inhibitors function in atherosclerosis are complex and require further exploration.

Therefore, the aims of this study were to evaluate serum levels of sclerostin and Dkk-1 on day 1 and on day 6 after stroke in patients with large artery atherosclerotic (LAA) stroke. We also enrolled age- and gender-matched patients with small-artery occlusion (SAO) stroke and healthy subjects as a reference cohort and a control cohort, respectively. The serum levels of sclerostin and Dkk-1 in these individuals were measured on day 1 after stroke or on admission. The patients were also subjected to a 90-day follow-up.

2. Materials and methods

2.1. Study subjects

The present study is in accordance with the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Medical Ethics Committee of Taizhou Hospital, written informed consent was obtained from each subject included in the study.

From June 2014 to January 2015, a total of 62 patients with first-time large artery atherosclerotic (LAA) stroke were consecutively recruited from the Department of Neurology at Taizhou Hospital, Zhejiang, China. The following inclusion criteria were applied: free-living prior to the incident, first-time stroke, admission within 24 h of symptom onset, and no thrombolysis or interventional therapy. The following exclusion criteria were applied: any history of stroke (including ischaemic stroke, intracerebral haemorrhage, or subarachnoid haemorrhage); any forms of coronary heart disease; severe arrhythmias; ischaemic peripheral arterial disease; severe hepatic, renal, or thyroid diseases; chronic inflammatory, autoimmune or haematologic diseases; abnormal platelet count; history of tumours or recent surgery; or treatment with medications that might affect bone metabolism (including hormones, thiazides, thiazolidinediones, bisphosphonates, vitamin D preparations, atorvastatin, calcium supplements or antiresorptive therapy) within six months.

In addition to the above-mentioned patients, 62 age-matched (blocks of 5 years) and gender-matched patients with first-time small-artery occlusion (SAO) stroke who were admitted within 24 h after symptom onset during the study period were included as a reference cohort; consecutive inclusion was used whenever possible. These patients were subjected to the same inclusion/exclusion criteria as described above. Moreover, 62 age-matched and gender-matched healthy individuals were recruited as a control group. These patients were recruited during physical check-ups, and all of them were free of the exclusion criteria. A flow chart of the study design is shown in Fig. 1.

2.2. Determination of presence, volume, severity and prognosis of ischaemic stroke

All the patients with acute stroke were confirmed by MRI with diffusion-weighted imaging (DWI) and underwent at least one angiographic study [MR angiography (MRA) or CT angiography (CTA)], carotid ultrasound examination, transthoracic echocardiography, and electrocardiogram. LAA stroke and SAO stroke were defined according to TOAST (Trial of Org 10172 in Acute Stroke Treatment) criteria [20]. At least two neurologists reviewed the clinical features and diagnostic test results before giving the subtype classifications.

Infarct volume was evaluated based on initial DWI lesion volume. An experienced neuroradiologist blinded to the clinical presentations and laboratory examinations of the patients calculated the infarct volume. The infarct volume was calculated as the sum of every slice infarction area multiplied by the slice thickness.

Stroke severity was assessed using the National Institutes of Health Stroke Scale (NIHSS; scores range from 0 to 42, with higher scores indicating greater deficits) [21] on admission for all patients. Moreover, we assessed the NIHSS scores on day 6 after stroke for the patients with LAA stroke who also had second blood samples available on day 6.

Outcome evaluation was performed on day 90 after stroke by a neurologist blinded to the clinical presentations and examinations using the modified Rankin Scale (mRS). mRS scores ≤ 2 and > 2 corresponded to good and poor outcomes, respectively.

2.3. Baseline clinical data collection

Demographic data (age, sex and race), conventional vascular risk factors (hypertension, diabetes mellitus, hyperlipidaemia, smoking

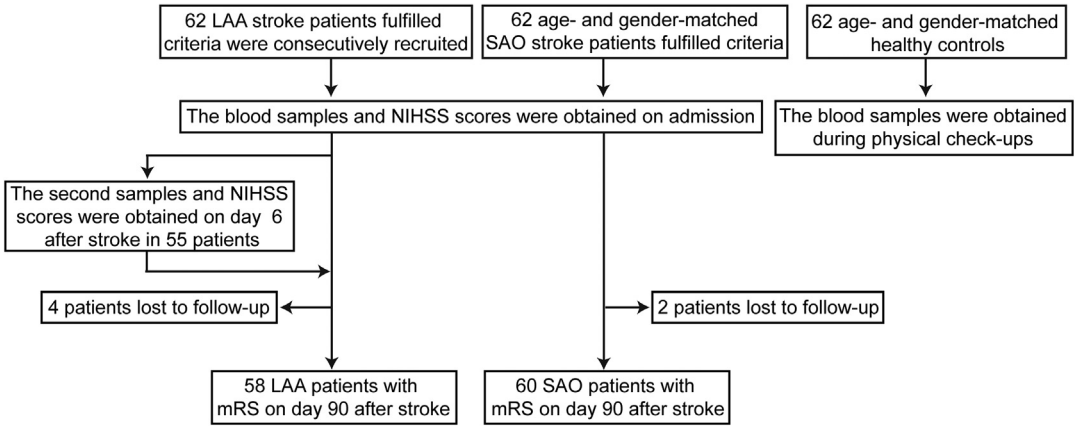

Fig. 1. Study flow chart. LAA stroke, large artery atherosclerotic stroke; SAO stroke, small-artery occlusion stroke; NIHSS, National Institutes of Health Stroke Scale; mRS, modified Rankin Scale.