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Plasma concentrations of growth arrest specific protein 6 and the soluble form of its tyrosine kinase receptor Axl as markers of large abdominal aortic aneurysms

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Abstract

Objective: The tyrosine kinase receptor Axl is expressed in the vasculature and Gas6 is the ligand. The extracellular part of Axl (sAxl) can be found in circulation. The aim of this study was to determine plasma concentrations of Gas6 and sAxl in patients with abdominal aortic aneurysms (AAA) and to evaluate if Gas6 and sAxl can be used as biomarkers.

Design and methods: Immunoassays for sAxl and Gas6 were used to investigate plasma from AAA patients. Patients with large ($n=123$) or small AAA ($n=122$) were compared with healthy controls ($n=141$).

Results: Gas6 correlated positively and sAxl correlated negatively with AAA size. As a consequence, the calculated Gas6/sAxl ratios correlated even better to AAA size.

Forty percent of all patients with a large AAA had higher Gas6/sAxl ratio than any in the control group.

Discussion: The Gas6/Axl system might be involved in AAA pathogenesis, and the Gas6/sAxl ratio may be useful as a biomarker.

Keywords: Abdominal aortic aneurysm; ELISA; Gas6; Axl; RTK; Vitamin K

Introduction

Abdominal aortic aneurysm (AAA) is a disease of the abdominal aorta weakening the vessel wall, resulting in increased vessel diameter, and making the vessel more prone to rupture. Ruptured AAA is currently the 15th leading cause of mortality among men in the United States [1]. The mechanisms governing AAA growth and rupture are not fully elucidated, but heredity, smoking, age, hypertension, and high cholesterol have been shown to increase the risk of AAA formation [2]. Inflammation, as reflected by increased plasma concentrations of fibrinogen, IL-6, C-reactive protein [3], and elevation of matrix metalloproteinases [4] has also been suggested to be involved in the pathogenesis of AAA [5]. Biomarkers that could differentiate between stable aneurysms and aneurysms prone to growth and subsequent rupture have been extensively sought for, as they would be of great clinical relevance [1]. However, no marker has yet been identified as "the marker of choice" to use for screening or follow up.

Receptor tyrosine kinases (RTKs) and their ligands are crucially important for the functional integrity of the vasculature [6,7]. Axl is a member of the TAM family of RTKs, consisting of Tyro3 (Sky), Axl and Mer, and it is expressed in endothelium, vascular smooth muscle cells and fibroblasts of the vessel wall [8,9]. The expression of Axl is up-regulated in response to vascular injury being primarily located in the cells of the neointima, suggesting that Axl may be a mediator of vascular smooth muscle migration and proliferation [10]. Axl is stimulated by Gas6 (product of the growth arrest specific gene 6) that was originally found as a protein expressed by growth arrested fibroblasts [9,11,12]. Axl is phosphorylated in response to Gas6 binding and the activation of Axl results in antiapoptotic and prosurvival effects, mainly due to involvement of the PI3 kinase and Akt

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Table 1
Comparison of patients with and without AAA investigated for Gas6 and sAxl concentration.

	Large AAA		Small AAA		Healthy individuals	
		Range		Range		Range
Number	123		122		141	
% Male	87		75		51	
% Smokers	28		31		10	
% Hypertensive	70		74		52	
Age in years	75	55–87	73	55–87	67	67–78
BMI (kg/m^2)	25	14–39	25	15–39	27	17–39
SBP (mm Hg)	140	85–200	140	100–200	140	100–190
Gas6 (ng/mL)	13.5	5.4–32.2	11.7	5.2–28.9	11.9	4.6–23.8
sAxl (ng/mL)	26.0	13.4–64.2	29.0	17.1–64.4	32.1	21.2–59.8
Gas6/sAxl	0.49	0.25–1.54	0.39	0.21–1.02	0.36	0.08–0.55

The median value and the range are given for all variables. BMI, body mass index; SBP, systolic blood pressure.

pathways [9,12]. Gas6 stimulation can rescue serum-starved fibroblasts and vascular smooth muscle cells from apoptosis [13–16]. Gas6 also has growth promoting activity, inducing proliferation in normal and malignant cells expressing Axl [9,17,18]. The Axl receptor has been shown to be expressed in a wide range of tissues and cell lines [8,9,19]. Overexpression of the Axl receptor is found in many different human cancers [20] and has been demonstrated to be of importance for growth of human gliomas [21] and gastric cell cancer [22].

Axl is a transmembrane protein, but the extracellular part of the Axl receptor tyrosine kinase can be shedded from cells, resulting in a soluble receptor. In mice, this process is dependent on the ADAM10 enzyme, but the shedding mechanism for the human sAxl has not been elucidated [23].

The Axl ligand Gas6 is a member of the vitamin K-dependent protein family, Gas6 being homologous to the anticoagulant protein S [11,12,18]. Gas6 is expressed in many cell types, including endothelial cells, vascular smooth muscle cells and fibroblasts [11]. However, expression is low in the liver, explaining the low concentration of Gas6 (approximately 0.2 nM) in plasma [24–26]. The affinity between Gas6 and the Axl receptor is in the subnanomolar range, suggesting that Gas6 and sAxl in plasma may form a complex [27,28].

The physiological importance of Gas6 and the TAM receptors have been studied in genetically modified mice models. Gas6$^{-/-}$ mice are found to be resistant to both arterial and venous thrombosis models [29,30]. Similar results were obtained with Axl/Tyro3/Mer$^{-/-}$ mice [31]. Additional studies in mice have suggested that Gas6 is important for endothelial activation, as Gas6$^{-/-}$ animals showed impaired ICAM up-regulation, as well as decreased adhesion by immune cells and platelets after exposure to tumor necrosis factor alpha [32].

The aim of this study was to elucidate whether the disease process of AAA are related to circulating concentrations of Gas6 and sAxl and if Gas6 and sAxl are useful as biomarkers for AAA.

Material and methods

Study design

The Vascular Center in Malmö serves an area with 750,000 inhabitants. AAA patients visiting the center 2002–2006 for planned AAA operations or for routine ultrasonographic surveillance of AAA were included in this study. Blood sampling and data collection was done before any AAA

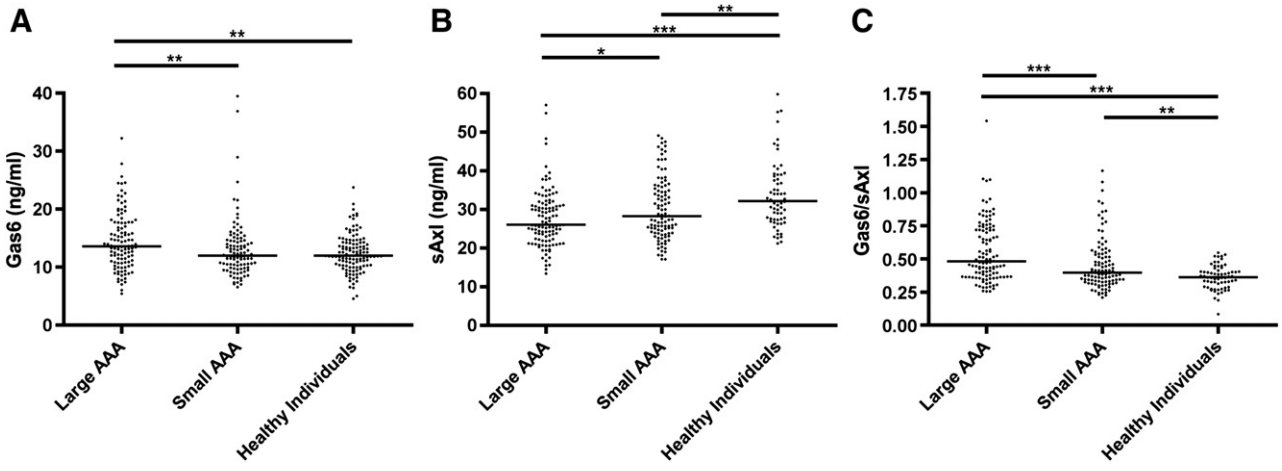

Fig. 1. Gas6 and sAxl concentrations and Gas6/sAxl ratios in AAA patients. (A) Gas6 plasma concentrations in patients with large AAAs, small AAAs and healthy controls. sAxl plasma concentrations (B) and Gas6/sAxl ratios (C) in the same groups. The p values in the graph are defined *$p<0.05$, **$p<0.01$, and ***$p<0.001$ using the Mann–Whitney test.