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Original Article

# Serum proteomics in patients with diagnosis of abdominal aortic aneurysm

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**Abstract**

**Background:** Molecular mechanisms underlying abdominal aneurysm (AAA) formation and rupture are not well understood. Early detection and repair of AAA may reduce the high mortality rates associated with rupture. Serum proteomics allows the detection of alterations in the expression of proteins, guiding further studies on these target molecules as potential markers. Analysis of proteomic profile of asymptomatic patients with AAA allows the identification of reliable predictors or markers of disease presence or progression. **Methods:** A proteomics approach based on two-dimensional electrophoresis and mass spectrometry was used to compare serum proteomic profiles of patients with AAA who are candidates for surgical repair compared with healthy controls. We analyzed in parallel the proteomic profile of subjects with cardiac heart failure to discriminate these two pathologies, which show similar pattern of systemic inflammation process. **Results:** We identified in AAA subjects four serum proteins that show altered expression profile and that could be specifically linked to AAA pathology. We discuss the role of our identified proteins with their possible implications in disease outcome. **Conclusions:** This approach could provide an initial screening tool that may drive the basis for further research in the field of cardiovascular diseases. These results need to be validated in larger studies to find potential markers of AAA presence or progression to use in clinical settings. **Summary:** A proteomics approach was used to compare serum proteomic profiles of patients with abdominal aortic aneurysm who are candidates for surgical repair compared with healthy controls. Four serum proteins showed altered expression profile that could be correlated with the pathology. This approach could provide an initial screening tool that may drive the basis for further research in the field of cardiovascular diseases. 

*Keywords:* Abdominal aortic aneurysm; Proteomic analysis; Serum markers; Cardiac heart failure

## 1. Introduction

Abdominal aortic aneurysm (AAA) and its major complications represent a relevant clinical problem in vascular surgery because of its high morbidity and mortality rate [1], mainly related to the difficult predictability of aneurysm rupture. Additionally, diagnosis is often casual and improved strategies to identify patients with AAA need to be developed. Inflammation of the arterial wall, imbalanced expression of matrix-degrading metalloproteinases (MMPs) and their inhibitors, degenerative changes in elastin and collagen, and apoptosis of the smooth muscle cells of the media are claimed to play a major role in the pathogenesis of AAA enlargement and rupture [2]. The ongoing shift toward degradation of collagen and elastin is thought to be an important concept in the progression of aneurysms [3]. Therefore, the identification of reliable predictors or markers of aneurysm in asymptomatic patients represents a primary objective for the early detection and repair of AAA. In recent years [4], the study of the entire proteome by two-dimensional (2D) proteomics analysis has represented a reliable instrument for the identification of

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links among genes, proteins, and a specific disease [5]. Clinical proteomics is now on the verge of entering the hospital, similar to the field of metabolomics, which has now been established for clinical diagnosis in newborn screening. The field of clinical proteomics offers opportunities to identify new disease biomarkers in body fluids, cells, and tissues. These biomarkers can be used in clinical applications for diagnosis, stratification of patients for specific treatment, or therapy monitoring. Recently, Urbonavicius et al. [6] reported the differential expression of seven proteins in the aortic wall of ruptured and nonruptured aneurysms. The aim of the present study is the detection, through proteomics analysis, of molecular targets of AAA presence and progression for further identification of associated serum markers in serum of asymptomatic patients. However, a variety of concerns regarding the propensity for bias in multiplex profiling methods [7] and the inherent limitations of protein profiling [8] are still hampering the introduction of proteomics analysis at a broader level into the clinical setting. When using commonly used proteomics approach such as matrix-assisted laser desorption/ionization time of flight (MALDI-ToF), signals such as acute-phase responses or artifacts cannot be filtered out and may be identified as biomarkers. This represents an important issue especially when pathologies based on systemic inflammatory imbalance are intended to be studied. In this context, one of the main limitations of the proteomics analysis could rely in the lack of specificity of the molecular target identified as potential biomarker candidate. To handle this limitation in this study, we decided to add an additional study group composed of serum of patients with cardiac heart failure (CHF), another pathological cardiovascular condition whose pathogenesis is systemic inflammation and shares a number of biomolecular similarities with atherosclerosis and aneurysmatic disease. Flattening the variables related to inflammatory response in cardiovascular pathology, this strategy could help avoid biases of unspecificity, providing more realistic and reliable results. Additionally, it could provide a new insight in the pathogenic mechanisms underlying cardiovascular disease.

## 2. Materials and methods

### 2.1. Patient serum samples

The study population consisted of 20 asymptomatic patients admitted to our center for the treatment of AAA. Twenty healthy persons, matched for sex and age, screened by abdominal echography served as control (CTR) group. An informed written consent was obtained by all the participants, and the study was approved by the University Campus Bio-Medico Ethical Committee. The study conforms with "The Code of Ethics of the World Medical Association" (Declaration of Helsinki), printed in the *British Medical Journal* (July 18, 1964).

AAA patients were comparable for clinical conditions, comorbidities, and medications taken. Subjects with diabetes, pulmonary, cardiovascular, kidney, metabolic, autoimmune, or inflammatory diseases were excluded. Diagnosis was assessed by echography and confirmed by angio-computed tomography scans. Blood samples were obtained by venipuncture; the serum samples were separated by centrifugation within 1 h at 3300*g* for 15 min and subsequently stored at −80°C. Subjects' demographic characteristics are shown in Table 1. A similar age-matched group of patients with clinically and instrumentally ascertained diagnosis of CHF and without additional history of diabetes, pulmonary, vascular, kidney, metabolic, autoimmune, or inflammatory diseases were enrolled and underwent to the same blood harvesting procedures. In all the patients included in this group, the origin of heart failure was imputed to ischemic cardiac disease, and none of the patients had a history of surgical revascularization procedures.

The authors of this article have certified that they complied with the "Principles of Ethical Publishing" of the *International Journal of Cardiology* [9]

### 2.2. 2D electrophoresis

Serum sample (5 ml) was precipitated in acetone (1:3) overnight at −20°C, as previously described [10]. After centrifugation, the pellet was resuspended in Media I buffer (0.32 M sucrose, 0.1 mM $MgCl_2$, 0.1 mM EDTA. 10 mM Tris–HCl, pH 8.0) containing 1 mM phenylmethanesulfonylfluoride and 1 mg/ml aprotin. Protein determination was performed on the supernatant using the Coomassie Plus Pierce Protein Assay (Rockford, IL). Sample aliquots (300 μg protein) were depleted of the major serum proteins (albumin and IgGs) using PROT-BA depletion kit according to manufacturer instructions (Sigma-Aldrich). Protein concentration after albumin and IgGs depletion was determined again using the Coomassie Protein Assay (Pierce).

For 2D electrophoresis, 200 μg of proteins for each sample (20 AAA, 20 CHF, and 20 CTR) were dissolved in 200 μl of rehydration buffer (8 M urea, 20 mM dithiothreitol, 2.0% (w/v) CHAPS, 0.2% Bio-Lyte, 2 M thiourea, and bromophenol blue). Isoelectric focusing was performed at 300 V for 2 h linearly, 500 V for 2 h linearly, 1000 V for 2 h linearly, 8000 V for 8 h linearly, and 8000 V for 10 h rapidly. All the processes above were carried out at room

Table 1
Summary of subject's demographic data

| Clinical characteristics | CTR | AAA | CHF | *P* |
|---|---|---|---|---|
| Age (years) | 71±4 | 72±2 | 71±2 | NS |
| Sex (M/F) | 12/8 | 13/7 | 14/6 | NS |
| Weight (kg) | 68±5 | 71±3 | 69±4 | NS |
| Height (cm) | 165±9 | 162±6 | 163±5 | NS |
| Smoking status (Y/N) | 15/5 | 16/4 | 14/6 | NS |
| Aortic mean diameter, echography (cm) | 2.4±0.9 | 7.8±1.2 | 2.3±0.8 | .002 |