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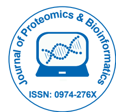

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Using Proteomics to Understand Abdominal Aortic Aneurysms: Where Are We?

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Abstract

Proteomics have been widely used to investigate potential biomarkers in a variety of diseases. Aortic aneurysm is an increasingly common vascular disorder with frequently fatal implications. However, there is no established diagnosis other than the one based on aneurysmal size. Despite widespread investigation, biomarkers for abdominal aortic aneurysms (AAA) development, progression and rupture are still lacking. A proteomic approach could reveal novel biomarkers. MEDLINE/PubMed and EMBASE were searched with the medical subject heading abdominal aortic aneurysm and keywords: size, rupture and expansion rate and proteomics. Studies investigating the association among AAA expansion, rupture or size with tissue, secretome and circulating biomarkers using proteomics were selected. Sixteen papers were identified. To summarize the data pointed out by these papers, we classified into functional categories, based on Gene Ontology, the proteins identified in tissue, secretome and in plasma/serum. This analysis highlighted that most of these proteins belong to categories related to cell adhesion, cytoskeleton, proteolysis, lipid metabolic process, blood coagulation, and acute phase response. We noted that some of these proteins correlated to AAA in all biological specimens. These proteins could be candidate biomarkers even if larger clinical studies are needed to evaluate their value as 'biomarkers', and to advance them to clinical use.

Keywords: Abdominal aortic aneurysm; Proteomics; Biomarkers

Introduction

Identification, by proteomic approach, of protein alterations specific to disease has been helpful in the clarification of the basic understanding of much pathology [1]. Therefore, proteomics may influence daily clinical practice by identifying biomarkers in body tissues or fluids of diagnostic, prognostic and therapeutic benefit [2,3]. Despite widespread investigation, strong biomarkers for abdominal aortic aneurysm (AAA) development, progression and rupture are still lacking. A proteomic approach could reveal novel potential markers. In this review, we report recent findings obtained applying proteomic approach to human AAA. AAAs affects 4%–8% of men and 1.5% of women over age 60 years and is usually asymptomatic until they reach a large size with the potential risk of rupture. In cross-sectional population studies of older men, the AAA prevalence varies from 3%-8% and it represents about 1% of causes of deaths in developed countries [4]. Ruptured AAAs represent an immediate emergency with a mortality rate as high as 80%–90% and with a 40–50% of deaths occurring before reaching the hospital. Common risk factors implicated in the growth and development of AAA include hypertension, hypercholesterolaemia, advanced age, male sex, smoking and genetic factors [5]. The size and the rate of expansion of the AAA are the major determinants for offering surgery. However, small AAAs do rupture, and some large AAAs would never have caused symptoms, if left unoperated. If we could predict which small AAAs are most likely to require later intervention, intervention could perhaps be offered earlier with less morbidity, mortality and fatal ruptures. Since elective surgical repair has mortality rates less than 5%, great interest exists for population screening that is currently based on ultrasonography (US), a highly accurate and non-invasive screening tool [6]. The diameter of the AAA reflects the magnitude of the degenerative process of the wall and this is a marker of rupture. Therefore, the need for additional strategies to refine cost effectiveness of screening programs is currently advocated [7]. AAA is a multifactorial disease. Some aneurysms may have a stronger genetic component while environmental factors, such as smoking, play a great role in others. Active investigations continue to identify markers other than size that would predict a risk of rupture and a field of growing interest is the search of potential prognostic biomarkers that may be identified in blood [8]. A biomarker is defined as a measurable cell, gene, or metabolic product that represents biologic processes in an organism at a given time. A current limitation relates to the fact that many biomarkers for AAA are not disease specific; most of them are also markers for atherosclerosis. Most studies have not assessed the validity of these markers as diagnostic tests for AAA because sensitivity and specificity appear to be inadequate when hypothesizing the use of one single biomarker for diagnosis.

This review could open up new possibilities into the application of proteins, found in more than one proteomic study considered, as plausible biomarkers in a future clinical practice.

Methods

Systematic literature search

MEDLINE/PubMed and EMBASE were searched with the medical subject heading abdominal aortic aneurysm and keywords: size, rupture and expansion rate and proteomics. In addition, reference lists were studied and manual searches performed. Studies, investigating the

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association of AAA expansion, rupture or size with circulating or tissue biomarkers and proteomics were selected. The search included articles published through January 2014. In this review, 16 articles accessible and available in English were included.

Results and Discussion

Circulating biomarkers for AAA size and expansion rate

Urbonavicius et al. [9] reviewed several potential biomarkers proposed for the progression of AAA. Of note, many are not disease specific and most are also markers for atherosclerosis. Few have potential clinical use. Serum Elastin Peptide (SEP) is one of the better biomarkers that can predict expansion and rupture. Plasmin-antiplasmin complexes (PAP) also may have clinical potential. PAP were measured in men with small AAA and it was found a significant, positive correlation with growth rate and a potential of predicting cases requiring surgery within the first five years, with both an optimal sensitivity and specificity at 83% [9]. Relationships between blood coagulation, the fibrinolysis system, and the morphology of aneurysms in patients with AAA indicate that the size and tortuosity of AAA are associated with blood levels of fibrinolytic factors such as D-dimer, fibrinogen/fibrin degradation products, and plasmin inhibitor-plasmin (PIP) complexes [10]. Moreover, AAA size was positively correlated with fibrinogen concentration [11].

Hellenthal et al. [12] investigated and quantified the relationship among several plasma candidate markers with aneurysm size. They reported that extracellular matrix (ECM) remodeling and the expression of markers of inflammation were related to AAA size.

Clinical proteomics

It is unlikely that the pathophysiology of a complex disorder, such as AAA, can be represented by a single biomarker. No one protein identified has the biologic plausibility to be used singularly as a biomarker for aneurysmal disease due to inadequate specificity. A more integrative approach that interrogates multiple pathways simultaneously appears most promising to achieve this task. By assessing a range of peptides and peptide fragments, proteomic studies have the potential to detect changes in many pathways involved in the pathogenesis of cardiovascular diseases [13]. The potential clinical applications of proteomics are of particular interest for this review. The proteomic approaches will probably represent a considerable advance by providing the investigators with the possibility of exploring hundreds of proteins at once and identifying new unforeseen biomarkers.

Even isolated cells are the result of the expression of thousands of genes, proteins and small molecules according to principles of structural and functional organization. Systems biology may be defined as an inter-disciplinary study field, which is mainly devoted to the analysis of the complex interactions that occur within biological systems [14,15]. In other words, Systems biology is the study of an organism, considered as an integrated and interacting network of genes, proteins and biochemical processes that give rise to life.

Proteomics studies of tissue, secretome and plasma/serum abdominal aortic aneurysm associated markers

A number of studies have appeared over the past few years that have applied a classical proteomic methodology to investigate AAA. The identification of any factors associated with greater rates of expansion or size of AAA may help to identify high-risk patients and prevent expansion and rupture of AAAs. Proteomic studies can be performed on a wide range of biological specimens, including whole-tissue samples, but also biofluids, cells and proteins secreted or released by cells or tissues. In this review, we summarize the present evidence for an association among tissue, secretome (the complete set of secreted proteins from a cell), and plasma/serum potential biomarkers and AAA size and expansion rate.

Tissue biomarkers and their association with AAA size, expansion rate and rupture

The aortic wall tissue: A major obstacle for applying proteomic analysis to vascular pathology is the heterogeneous cellular composition of AAA wall tissue. The normal artery contains three layers. The inner layer, tunica intima, is lined by a monolayer of endothelial cells in contact with blood and overlying a basement membrane. The middle layer, or tunica media, contains vascular smooth muscle cells (VSMCs) embedded in a complex extracellular matrix. The adventitia, the outer layer, contains mast cells, nerve endings and microvessels. Matrix changes: elastin and collagen. Elastin is one of the major components of the aorta, and experimental findings suggest that its degradation products, elastin peptides, also stimulate leukocyte recruitment and cytokine production. The cysteine, serine and metalloproteinase systems have been reported to be involved in the matrix degradation of the aortic wall, causing AAA. Plasmin is a common activator and could be involved in the pathogenesis of AAA by activating all three systems. Regarding the extracellular matrix proteins, fibrinogen increases the aggregation of platelets, endothelial cells, and leukocytes, which in turn, causes leukocyte and platelet activation and the release of mediators from the cells. Plasma fibrinogen concentrations have been reported to correlate positively with AAA size, and later development of large AAA in the Malmo Preventive Study [16]. However, Shindo et al. [17] observed no correlations between the AAA size, fibrinogen, and fibrin degradation products (FDP) in plasma of 43 patients who underwent the AAA repair. Vitronectin is an adhesive multifunctional glycoprotein in plasma and ECM. One of the main functions in the ECM is the binding of plasminogen activator inhibitor-1 (PAI-1) in order to stabilize the biological activity of this protease inhibitor, and thus protect matrix proteins against the degradation by proteases. The relationship between aortic diameter, aneurysmal expansion rate, and the expression of matrix-degrading metalloproteinases (MMPs) has been investigated. Vitronectin could modulate plasmin generation and subsequent arterial wall fragilization. A decreased level of vitronectin contained within the aneurysm wall prone to rupture or with large diameter relative to small aneurysms is consistent with a potential protective role of this glycoprotein in AAA stabilization. Changes in the interstitial collagens may predispose the aneurysm to rupture. Most of the collagen in the aortic wall is of type I and type III. Elevated level of circulating amino terminal propeptide of type III procollagen has recently been found to be associated with size, expansion rate of AAA, and the need for later repair. Cathepsins are strong candidates as key participants in aneurysm development. These elastolytic enzymes are strongly expressed in the AAA wall, whereas there is reduced expression of their endogenous inhibitor cystatin C.

Proteomics studies of aortic wall tissue associated with AAA size, expansion rate and rupture

In order to associate the protein composition of AAA wall tissue with the expansion rate and size, Urbonavicius et al. [18], by a proteomic approach, identified a number of proteins differentially regulated in AAA tissue and correlated with these two parameters. Among them, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), annexin A4 and a fragment of transforming growth factor-beta-induced protein ig-h3 were correlated with the expansion rate of AAA.

A significant negative correlation between the expansion rate and the protein expression was seen with apolipoprotein H, fibrinogen beta chain, apoA-I, albumin and immunoglobulin a-1 or 2. With respect to the size of AAA, they found that it was significantly negatively correlated with vitronectin and albumin. In addition, collagen a-3(VI) chain and vitamin D binding precursor was positively correlated and they may be related to the inflammatory or remodeling processes in the tissue or ECM. However, several of these proteins have been associated for the first time with the history of AAA. The formation of degenerative AAAs is associated with inflammation, upregulation of proteolysis, apoptosis, and matrix degradation [19]. To gain insight into early molecular changes leading to AAA development, Nordon et al. [20] investigated changes in the vascular tissue proteome, using inferior mesenteric vein (IMV) of patients with AAAs. Recent evidence suggested that AAAs is a local representation of a systemic disease of the vasculature. Morphologic and molecular changes, comparable to those found in the aneurysm wall, have been demonstrated in veins from patients with AAAs. Protein spots differentially expressed in AAA were identified using mass spectrometry. All proteins were localized to the vascular smooth muscle cells. The authors identified proteins involved in the oxidative stress defense and in the modulation of the response to inflammation [20]. These proteins resulted down-regulated in the vascular tissue of AAA patients. They reported that these changes might represent early events in AAA formation concluding that an increase of the expression of these proteins might offer a novel therapeutic avenue to inhibit AAA development. AAAs is characterized by pathological remodeling of the aortic ECM. However, besides the well-characterized elastolysis and collagenolysis, little is known about changes in other ECM proteins. Some proteomic studies on AAA focused on cellular changes without emphasis on the ECM. The study conducted by Didangelos et al. [21] was the first to employ a solubility-based extraction procedure to study the ECM composition in human AAA by proteomics. Their methodology led to the identification of novel candidate markers of pathological tissue remodeling and identified new proteolytic targets of MMP-12, one of the key proteinases in AAA. The destruction of the aortic connective tissue in aneurysms is driven by a severe inflammatory reaction and involves the excessive degradation of the aortic ECM. The proteomic analysis revealed novel changes in the ECM of AAA, including increased expression, as well as degradation, of collagen XII, thrombospondin 2, aortic carboxypeptidase-like protein, periostin, fibronectin and tenascin. AAA is often accompanied by aortic calcification [21]. Matsumoto et al. [22], by a quantitative proteomic approach with iTRAQ labeling in combination with nanoLC-MALDI-TOF/TOF-MS/MS, identified proteins that were significantly changed in calcified abdominal aortic aneurysms (CAAs) and calcified thoracic aortic aneurysms (CTAs) compared with those in adjacent normal aorta tissues. The prevalence of thoracic aortic aneurysms (TAAs) is only about one-third of that of AAAs. Structural heterogeneity of the aorta has been shown between the thoracic and abdominal aortic regions. This structural heterogeneity may contribute to the differences observed in the pathogenesis of AAAs and TAAs. The thoracic aorta has a thinner intima, thicker media, and more medial lamellar units than those of the abdominal aorta. In addition, the thoracic aorta has significantly higher elastin and collagen contents and a lower collagen-to elastin ratio than those of the abdominal aorta. In contrast to the abdominal region, atherosclerosis rarely presents in the thoracic region [22]. The proteomic analysis, presented by Matsumoto et al. [22], revealed 138 and 134 proteins differentially expressed in CAAs and CTAs in contrast to neighboring normal aorta tissues. Among them, the authors identified proteins already known to be involved in aneurysm formation and vascular calcification. Some of these proteins could be linked to specific biochemical pathways, such as the integrin signaling pathway, the blood coagulation pathway, the inflammation mediated by chemokine and cytokine signaling pathway, the glycolysis.

Modrego et al. [23] showed that, at the aneurysmal site, there are changes in the expression level of proteins associated with the cytoskeleton and with the glycolytic pathway. According to these authors, at the AAA site, the amount of some cytoskeleton-related proteins, such as microfibril-associated glycoprotein-4 isotype 1, annexin A5 isotype 1, and annexin A2, was reduced with respect to that found in the controls. Therefore, reduction of the amount of these proteins in AAA may promote the presence of intraluminal mural thrombus, which has been associated with AAA ruptures. In the proteomic map obtained by these authors, the expression level of most of the glycolytic enzymes was significantly reduced at the AAA site [23]. Recently, Molacek et al. [24] carried out a proteomic analysis of the AAA wall and compared the findings with those of non-dilated wall tissue, i.e. a relatively "healthy" aorta obtained during organ removal from heart-beating deceased organ donors. They highlighted significant differences in the proteomes of the aortic aneurysm tissue and the non-dilated aortas with atherosclerosis [24].

In order to summarize the proteomic data pointed out by the six cited studies, we listed and classified all the 203 identified proteins into functional categories based on Gene Ontology (Supplementary Table 1). The 203 proteins were clustered into 37 categories and most of them was related to the categories of cell adhesion, extracellular matrix organization, cytoskeletal organization proteolysis, blood coagulation, acute phase response and to categories associated to metabolic processes such as carbohydrate, lipid and hyaluronan metabolic process. Some of these proteins (cell adhesion proteins: vitronectin, microfibril-associated glycoprotein 4, vimentin; proteins related to energy production: apolipoprotein A-I, alpha enolase and the annexin A2) have been identified by several authors suggesting the idea of their possible role in the AAA development. To easily figure out what is the key literature of all these findings, the results are reported in Figure 1, in which it is shown the percentage of proteins identified by each author. Moreover, to highlight proteins that could be evaluated as potential biomarkers, we listed in Table 1 tissue proteins identified by more than one author. In this table, it is evident how many papers contributed to the protein identifications. This analysis pointed out that Didangelos et al. [21] provided about 63% of these identification followed by Matsumoto et al. [22] with about 36%. It is noteworthy that Didangelos et al. [21], unlike all other authors, have decided to perform the proteomic study on isolated ECM proteins instead of on whole- aortic wall tissue, overcoming the obstacle of tissue heterogeneity. Concerning Matsumoto et al. [22], the higher identification rate is probably due to the use of a quantitative proteomic approach with iTRAQ labeling in combination with nanoLC-MALDI-TOF/TOF-MS/MS.

Secretome biomarkers and their association with AAA size, expansion rate and rupture

The secretome: The Secretome includes all proteins that are secreted or released by cells or tissue in the extracellular compartment as consequence of the normal metabolism or in response to some stimuli. The interest of the characterization of the vascular cell secretome implicated in the AAA development is the detection of novel biomarkers that could be related with this pathology. Although certain key risk factors have been identified, a highly sensitive and specific diagnostic assay that could provide information on the extent, growth, stability, and type of AAA remains to be identified. Many authors have

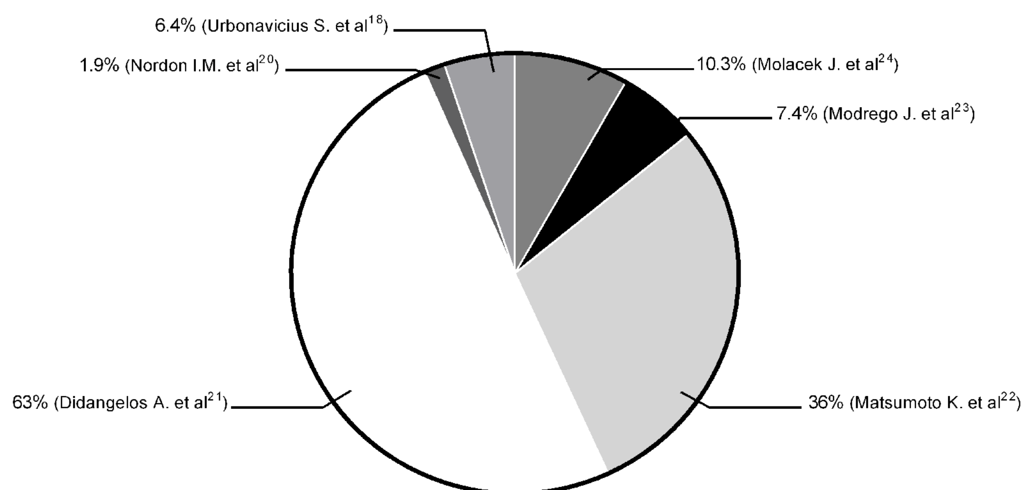

Figure 1: Pie chart depicts the percentage of proteins identified by each proteomic paper dealing with potential wall tissue biomarkers and their association with AAA, size, expansion rate and rapture. In parenthesis it is given the reference of the specific paper. The list of these proteins is reported in Supplementary Table 1.

recently reported a novel approach for the study of AAA secretome in the search of potential biomarkers. These strategies compare the secretome from normal and pathological arteries, using a differential proteomic approach.

Proteomics studies of secretome associated with AAA size, expansion rate and rupture

Martinez-Pinna et al. [25] analyzed, by a differential proteomic approach, proteins released by intra-luminal thrombus (ILT). ILT volume is highly correlated with AAA size, and has been associated with AAA growth and the incidence of cardiovascular events in AAA patients. Incubating different layers (luminal/abluminal) of the AAA ILT, they analyzed the proteins released. Several differentially expressed proteins involved in AAA pathological mechanisms (proteolysis, oxidative stress, and thrombosis) were identified by 1D-GE (one dimensional-gel electrophoresis) associated with liquid chromatography and mass spectrometry (LC–MS/MS). Among these identified proteins, peroxiredoxin-1 (PRX-1) was more released by the luminal layer compared with the abluminal layer of the ILT [25].

To further explore the pathophysiology of ILT in human AAA, by increasing the number of identified proteins, Martinez-Pinna et al. [26] analyzed ILT and wall conditioned media using liquid chromatography and tandem mass spectrometry. Global pathway analysis of identified peptides/proteins by Ingenuity software highlighted that complement system components were highly enriched in AAA tissue-conditioned media. In particular, they highlighted a decrease of systemic C3 concentration and activity in the later stages of AAA associated with local complement retention, consumption, and proteolysis in the thrombus that could induce polymorphonuclear (PMN) cell chemotaxis and activation, playing a detrimental role in AAA progression [26].

In order to increase the concentration of very low abundant proteins (those most difficult to identify), Moxon et al. [27] employed a non-specific hexapeptide ligand library (HLL) to deplete abundant proteins. They conducted a proteomic study, screening whole and HLL treated ILT explant secretions to identify potential ILT-derived markers for AAA.

From thrombus-conditioned media they identified 150 proteins and HLL treatment enabled the detection of 53 previously unseen polypeptides. Gene ontology analysis revealed high representation of platelet-secreted proteins. Thrombospondin-1 (TSP-1) and clusterin were selected and resulted negatively associated with AAA after adjusting for other risk factors. Assessment of circulating concentrations of two representative polypeptides suggests for the first time that the ILT selectively sequesters proteins rather than actively releasing them [27].

All the proteins identified by these three proteomic studies were listed in Supplementary Table 2. The proteins were classified into functional categories based on Gene Ontology terms related to their main biological function as reported in UniprotKB database (http://www.uniprot.org). The 311 proteins were clustered into 47 categories and most of them were related to the categories of cell adhesion, cytoskeletal organization, proteolysis, blood coagulation, complement activation, acute phase response, cell proliferation, regulation of transcription and to categories associated to metabolic processes. In Table 2, we reported secretome proteins identified by more than one author.

It is noteworthy that 33 proteins have been identified by several authors, suggesting the idea of their possible role in the AAA development. Among them, we can observe proteins involved in blood coagulation, complement activation and cell adhesion. It can be noted the presence of metabolic proteins such as inter-alpha inhibitor H4 involved in hyaluronan metabolic process, apolipoprotein A-I and E related to lipid metabolism and GAPDH for carbohydrate metabolism. We also found several acute phase response proteins and proteins associated to the transport category. Moreover, we highlighted proteins such as ceruloplasmin and hemopexin belonging to cellular cation homeostasis; hemoglobin (subunit alpha and beta) related to regulation of blood vessel size. In the pie chart represented in Figure 2, it is shown the percentage of proteins identified by each author. Concerning the role of each paper in the identification of these secreted proteins, it is noteworthy that the study of Martinez-Pinna et al. [26], provided about 83% of these identification. The high identification rate of this study [26] is probably due to the use of the two-dimensional liquid

Protein	Entry Name	Reference
GO:0007155 Cell adhesion		
Collagen alpha-1(I)	CO1A1_HUMAN	[21,22]
Collagen alpha-1[III]	CO3A1_HUMAN	[21,22]
Collagen alpha-1[XIV]	COEA1_HUMAN	[21,22]
Collagen alpha-2[I]	CO1A2_HUMAN	[21,22]
Collagen alpha-2[VI]	CO6A2_HUMAN	[21,24]
Collagen alpha-3[VI]	CO6A3_HUMAN	[18,21]
Dermatopontin	DERM_HUMAN	[21,22]
Lactadherin	MFGM_HUMAN	[21,22]
Microfibril-associated glycoprotein 4	MFAP4_HUMAN	[21,22,23]
Periostin	POSTN_HUMAN	[21,22]
Thrombospondin-1	TSP1_HUMAN	[21,22]
Vitronectin	VTNC_HUMAN	[18,21,22]
GO:0007160 Cell-matrix adhesion		
Fibulin-5	FBLN5_HUMAN	[21,22]
GO:0006629 Lipid metabolic process		
Apolipoprotein A-I	APOA1_HUMAN	[18,21,22]
Apolipoprotein A-IV	APOA4_HUMAN	[21,22]
Apolipoprotein B-100	APOB_HUMAN	[21,22]
Apolipoprotein E	APOE_HUMAN	[21,22]
GO:0006457 Protein folding		
Calreticulin	CALR_HUMAN	[18,21]
GO:0006508 Proteolysis		
Cathepsin D	CATD_HUMAN	[21,22]
GO:0030154 Cell differentiation		
Matrix Gla protein	MGP_HUMAN	[21,22]
GO:0008283 Cell proliferation		
Transforming growth factor-beta-induced protein ig-h3	BGH3_HUMAN	[18,21]
GO:0007010 Cytoskeletal organization		
Actin, alpha cardiac muscle 1	ACTC_HUMAN	[20,24]
Cysteine and glycine-rich protein 1	CSRP1_HUMAN	[22,24]
Filamin-A	FLNB_HUMAN	[22,23]
Tropomyosin β chain	TPM2_HUMAN	[22,23]
Vimentin	VIME_HUMAN	[20,21,23,24]
GO:0001525 Angiogenesis		
Annexin A2	ANXA2_HUMAN	[21,23,24]
GO:0006953 Acute-phase response		
Alpha 1 antitrypsin	A1AT_HUMAN	[22,24]
Serum amyloid P-component	SAMP_HUMAN	[21,22]
GO:0005975 Carbohydrate metabolic process		
Alpha enolase	ENOA_HUMAN	[22,23,24]
Biglycan	PGS1_HUMAN	[21,22]
Decorin	PGS2_HUMAN	[21,22]
Glyceraldehyde-3-phosphate dehydrogenase	G3P_HUMAN	[18,23,24]
Mimecan	MIME_HUMAN	[21,22]
GO:0007517 Muscle organ development		
Lumican	LUM_HUMAN	[21,22]
Transgelin	TAGL_HUMAN	[22,24]
GO:0006810 Transport		
Serum Albumin	ALBU_HUMAN	[18,24]
GO:0007596 Blood coagulation		
Annexin A5	ANXA5_HUMAN	[23,24]
Clusterin	CLUS_HUMAN	[21,22]
Fibrinogen beta-chain	FIBB_HUMAN	[18,22]
Fibrinogen gamma-chain	FIBG_HUMAN	[22,24]
GO:0006955 Immune response		
Ig alpha-2 chain C region	IGHA2_HUMAN	[18,24]

* UniProtKB/Swiss-Prot entry name (http://www.uniprot.org/)

Table 1: Tissue proteins identified by more than one author and associated with AAA size, expansion rate and rupture.

Protein	Entry Name*	Reference
GO:0007155 Cell adhesion		
Protein AMBP	AMBP_HUMAN	[25,26]
Thrombospondin-1	TSP1_HUMAN	[25-27]
GO:0030212 Hyaluronan metabolic process		
Inter-alpha [Globulin] inhibitor H4	B2RMS9_HUMAN	[26,27]
GO:0006629 Lipid metabolic process		
Apolipoprotein A-I	APOA1_HUMAN	[26,27]
Apolipoprotein E	APOE_HUMAN	[26,27]
Serum paraoxonase/arylesterase1	PON1_HUMAN	[26,27]
GO:0007010 Cytoskeletal organization		
Actin,cytoplasmic 1	ACTB_HUMAN	[26,27]
Gelsolin	GELS_HUMAN	[26,27]
GO:0006953 Acute-phase response		
Alpha-1-antichymotrypsin	AACT_HUMAN	[26,27]
Fibronectin	FINC_HUMAN	[26,27]
Haptoglobin	HPT_HUMAN	[25-27]
Prothrombin	THRB_HUMAN	[26,27]
Serum amyloid P-component	SAMP_HUMAN	[26,27]
GO:0005975 Carbohydrate metabolic process		
Glyceraldehyde-3-phosphate dehydrogenase	G3P_HUMAN	[25,27]
GO:0006810 Transport		
Serotransferrin	TRFE_HUMAN	[26,27]
Serum Albumin	ALBU_HUMAN	[26,27]
Transthyretin	A6XGL1_HUMAN	[26,27]
GO:0030003 Cellular cation homeostasis		
Ceruloplasmin	CERU_HUMAN	[26,27]
Hemopexin	HEMO_HUMAN	[25-27]
GO:0050880 Regulation of blood vessel size		
Hemoglobin subunit alpha	HBA_HUMAN	[26,27]
Hemoglobin subunit beta	HBB_HUMAN	[26,27]
GO:0007596 Blood coagulation		
Alpha 2 macroglobulin	A2MG_HUMAN	[26,27]
Antithrombin III	ANT3_HUMAN	[26,27]
Clusterin	CLUS_HUMAN	[26,27]
Complement component C9	CO9_HUMAN	[26,27]
Fibrinogen alpha-chain	FIBA_HUMAN	[25-27]
Fibrinogen beta-chain	FIBB_HUMAN	[26,27]
Fibrinogen gamma-chain	FIBG_HUMAN	[25-27]
Plasminogen	PLMN_HUMAN	[26,27]
GO:0006956 Complement activation		
Complement C4-A	CO4A_HUMAN	[25,26]
Complement C4-B	CO4B_HUMAN	[26,27]
Complement component C3	CO3_HUMAN	[25-27]
Plasma protease C1 inhibitor	IC1_HUMAN	[25,26]

* UniProtKB/Swiss-Prot entry name (http://www.uniprot.org/)

Table 2: Secretome proteins identified by more than one author and associated with AAA size, expansion rate and rupture.

chromatography coupled to tandem MS instead of a gel-based strategy performed in the other papers.

Plasma/serum biomarkers and their association with AAA size, expansion rate and rupture

Plasma/serum: The depth of proteome analysis is severely limited in complex samples with a wide dynamic range of protein abundance such as plasma/serum. Pre-fractionation of a sample divides it into fractions of reduced complexity, allowing improved detection of lower abundance proteins. However, smaller proteins may be part of larger protein complexes and hence the removal of proteins involved in complexes with high-abundance proteins such as albumin may compromises the search for disease biomarkers. The circulation half-life of low molecular weight plasma proteins is directly related to their binding affinity to high abundant carrier proteins. This observation has been supported by proteomic based studies that have detected a significant amount of low molecular weight proteins in specific carrier protein fractions (serum albumin).

Proteomics studies in plasma/serum associated with AAA size, expansion rate and rupture

We carried out a study on the plasma proteome of patients undergoing aortic aneurysm repair. The aim was to identify the key proteins involved in the pathogenesis of AAAs [28]. In this study we decided not to remove highly abundant proteins such as albumin and IgG since it would cause the removal of a broad range of other low mass

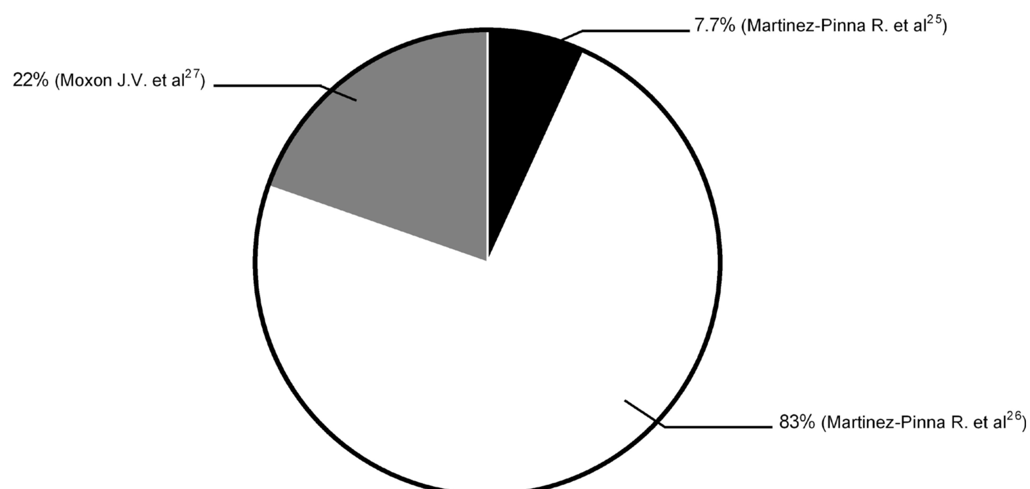

Figure 2: Pie chart depicts the percentage of proteins identified by each proteomic paper dealing with potential secretome biomarkers and their association with AAA, size, expansion rate and rapture**.** In parenthesis it is given the reference of the specific paper. The list of these proteins is reported in Supplementary Table 2.

and low abundant physiologically important regulatory and/or transient proteins. We confirmed a number of biomarkers associated with AAA that have been previously identified by various authors reported in this review [28]. We found a significant increase of a class of proteins such as fibrinogen, α1-antitrypsin and haptoglobin. The presence of these proteins in human AAA plasma is related to the inflammatory processes active in these subjects as reported by many authors. We also showed a negative correlation between the vitamin D-binding protein (DBP) and hemoglobin subunit β and AAA presence. DBP is very important for vascular remodeling and it may have an important role in the protection of vascular walls. In plasma, this protein reduces platelet aggregation and extends coagulation time.

Acosta-Martin et al. [29], using a data-independent shotgun proteomic strategy called Precursor Acquisition Independent From Ion Count (PAcIFIC), combined with spectral counting and isobaric tandem mass tags, identified 80 proteins as statistically differentially abundant between AAA and control patients. They used pooled human plasma samples of AAA and control patients depleted of the most abundant proteins. Among differentially abundant proteins, five of them were verified to be differentially up-regulated in plasma of AAA patients: adiponectin, extracellular superoxide dismutase, protein AMBP, kallistatin and carboxypeptidase B2 [29]. Several high-throughput multiplexed techniques have been recently developed to assess protein expression at a functional level. Array-based techniques offer the potential for analysis of entire protein networks through the use of specific antibodies. Ramos-Mozo et al. [30], analyzed 20 proteins in AAA plasma by an antibody array approach. However, the wide range of concentrations of plasma proteins represents a limitation to the use of protein arrays for high-throughput analysis. Among proteins with higher levels in AAA patients, they focused on insulin-like growth factor-binding protein 1 (IGFBP-1) due to the high significant differences observed between AAA patients and controls. This initial data was further verified in plasma samples of large AAA patients and controls matched by age, sex and risk factors, confirming the increased IGFBP-1 concentrations in plasma from large AAA patients [30]. Accumulating evidence indicates important roles for IGFBP-1 in metabolic homeostasis. Since AAA diameter is a surrogate marker of AAA growth rate and it is the clinical parameter used to the management of AAA patients, the authors studied its potential correlation with IGFBP-1; the results showed a positive correlation between IGFBP-1 and aortic size, which remains significant after analysis for traditional risk factors. Moreover, they have shown increased levels of IGFBP-1 in conditioned media of AAA wall compared to healthy aortic wall. Interestingly, proteolytic fragments of IGFBP-1 were observed in the thrombus conditioned media. The authors concluded that the proteolytic environment of AAA thrombus would favor the dissociation of IGFBP-1 from IGFBP-1/IGF-1 (insulin-like growth factor-1) complexes increasing the availability of IGF-1 to IGF-1 receptor in platelets, which could enhance platelet aggregation and promote thrombus formation [30]. A proteomics approach based on two-dimensional electrophoresis and mass spectrometry was used by Spadaccio et al. [31] to compare serum proteomic profiles of patients with AAA who are candidates for surgical repair compared with healthy controls. They identified in AAA subjects four serum proteins that showed altered expression profile and that could be specifically linked to AAA pathology. A recent research, conducted by Ehsan et al. [32], showed that surface enhanced laser desorption ionization time of flight (SELDI-TOF) can be used to study the plasma protein expression of AAA. They identified a plasma protein profile that could classify patients from a mixed population group. Multiple proteins (serum elastin peptides and plasmin-antiplasmin complexes, MMP-9, interferon-gamma, C-reactive protein, alpha 1-antitrypsin and lipoprotein, APC-PCI (Activated protein C-protein C inhibitor) complex and IL-6) have been suggested as biomarkers or risk factors for AAA. However, none has been independently validated, or checked for its potential clinical use [32]. After the removal of aneurysmal tissues in AAA, and thoracic aortic aneurysm (TAA), Satoh et al. [33] compared serum proteins showing different level of expression in post-surgery with those in pre-surgery, in order to identify potential serum biomarkers for AAAs and TAAs. They reported, for both AAA and TAA, an over-expression of acute-phase proteins and a down-expression of α-2-macroglobulin, gelsolin and kallistatin [33].

Protein	Entry Name*	Reference
GO:0030212 Hyaluronan metabolic process		
Inter-alpha-trypsin inhibitor heavy chain H2	ITIH2_HUMAN	[29,33]
GO:0006629 Lipid metabolic process		
Apolipoprotein A-I	APOA1_HUMAN	[28,31-34]
Apolipoprotein A-II	APOA2_HUMAN	[32,33]
GO:0006508 Proteolysis		
Complement factor B	CFAB_HUMAN	[28,29,34]
GO:0050873 Brown fat cell differentiation		
Leucine-rich alpha-2-glycoprotein	A2GL_HUMAN	[28,29,33,34]
GO:0007010 Cytoskeletal organization		
Gelsolin	GELS_HUMAN	[29,33]
GO:0006953 Acute-phase response		
Alpha 1 antitrypsin	A1AT_HUMAN	[28,32-34]
Alpha-1-acid glycoprotein 1	A1AG1_HUMAN	[32,33]
Alpha-1-antichymotrypsin	AACT_HUMAN	[29,33,34]
Alpha-2-HS-glycoprotein	FETUA_HUMAN	[28,29,33,34]
Haptoglobin	HPT_HUMAN	[28,31,32,34]
GO:0007601 Visual perception		
Retinol binding protein 4 [RBP4]	RET4_HUMAN	[33,34]
GO:0006810 Transport		
Serotransferrin	TRFE_HUMAN	[28,31,33,34]
Serum Albumin	ALBU_HUMAN	[32,34]
Vitamin D-binding protein	VTDB_HUMAN	[28,31]
GO:0030003 Cellular cation homeostasis		
Ceruloplasmin	CERU_HUMAN	[29,31,34]
Hemopexin	HEMO_HUMAN	[28,29,31]
GO:0007596 Blood coagulation		
Alpha 2 macroglobulin	A2MG_HUMAN	[28,32-34]
Clusterin	CLUS_HUMAN	[28,34]
Complement component C9	CO9_HUMAN	[29,33]
Fibrinogen alpha-chain	FIBA_HUMAN	[28,31,32,34]
Fibrinogen beta-chain	FIBB_HUMAN	[32,34]
Fibrinogen gamma-chain	FIBG_HUMAN	[28,32,34]
GO:0030195 Negative regulation of blood coagulation		
Kininogen-1	KNG1_HUMAN	[28,29]
GO:0010951 Negative regulation of endopeptidase activity		
Kallistatin	KAIN_HUMAN	[29,33]

* UniProtKB/Swiss-Prot entry name (http://www.uniprot.org/)

Table 3: Plasma/Serum proteins identified by more than one author and associated with AAA size, expansion rate and rupture.

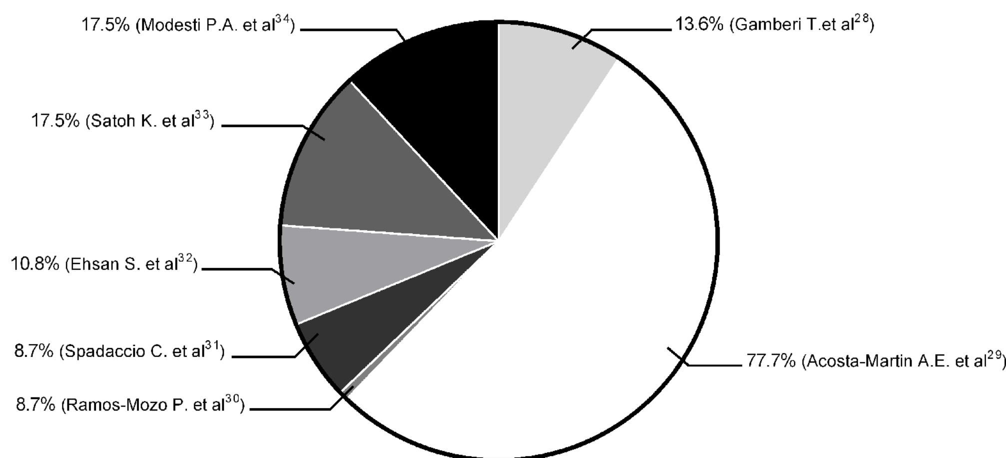

Figure 3: Pie chart depicts the percentage of proteins identified by each proteomic paper dealing with potential plasma/serum biomarkers and their association with AAA, size, expansion rate and rapture. In parenthesis it is given the reference of the specific paper. The list of these proteins is reported in Supplementary Table 3.

Protein	Entry Name	Reference
GO:0007155 Cell adhesion		
Vitronectin	VTNC_HUMAN	[18,21,22,27,29]
GO:0006801 Superoxide metabolic process		
Extracellular superoxide dismutase (Cu-Zn)	SODE_HUMAN	[21,26,29]
GO:0030212 Hyaluronan metabolic process		
Inter-alpha-trypsin inhibitor heavy chain H1	ITIH1_HUMAN	[22,27,29]
GO:0006629 Lipid metabolic process		
Apolipoprotein A-I	APOA1_HUMAN	[18,21,22,26-28,31-34]
Apolipoprotein A-II	APOA2_HUMAN	[22,26,32,33]
Apolipoprotein B-100	APOB_HUMAN	[21,22,26,29]
GO:0006953 Acute-phase response		
Alpha 1 antitrypsin	A1AT_HUMAN	[22,24,27,28,32-34]
Alpha-1-acid glycoprotein 1	A1AG1_HUMAN	[22,26,32,33]
Alpha-2-HS-glycoprotein	FETUA_HUMAN	[22,27-29,33,34]
Fibronectin	FINC_HUMAN	[21,26,27,33]
Haptoglobin	HPT_HUMAN	[22,25-28,31,32,34]
Serum amyloid P-component	SAMP_HUMAN	[21,22,26,27,31]
GO:0044419 Interspecies interaction between organisms		
Fibulin-1	FBLN1_HUMAN	[21,27,29]
GO:0005975 Carbohydrate metabolic process		
Glyceraldehyde-3-phosphate dehydrogenase	G3P_HUMAN	[18,23-25,27,29]
GO:0007517 Muscle organ development		
Transgelin	TAGL_HUMAN	[22,24,25,29]
GO:0006810 Transport		
Serotransferrin	TRFE_HUMAN	[24,26-28,31,33,34]
Serum Albumin	ALBU_HUMAN	[18,24,26,27,32,34]
Vitamin D-binding protein	VTDB_HUMAN	[18,26,28,31]
GO:0030003 Cellular cation homeostasis		
Ceruloplasmin	CERU_HUMAN	[21,26,27,29,31,34]
GO:0050880 Regulation of blood vessel size		
Hemoglobin subunit beta	HBB_HUMAN	[22,26,27,29]
GO:0007596 Blood coagulation		
Clusterin	CLUS_HUMAN	[21,22,26-28,34]
Complement component C9	CO9_HUMAN	[22,26,27,29,33]
Fibrinogen alpha-chain	FIBA_HUMAN	[22,25-28,31,32,34]
Fibrinogen beta-chain	FIBB_HUMAN	[18,22,26,27,32,34]
Fibrinogen gamma-chain	FIBG_HUMAN	[22,24-28,32,34]
Heparin cofactor 2	HEP2_HUMAN	[21,27,29]
GO:0006956 Complement activation		
Complement C1q subcomponent subunit C	C1QC_HUMAN	[22,27,29]
GO:0030195 Negative regulation of blood coagulation		
Keratin,type II cytoskeletal 1	K2C1_HUMAN	[24,27,29]
Kininogen-1	KNG1_HUMAN	[22,26-29]
GO:0006955 Immune response		
Ig alpha-1 chain C region	IGHA1_HUMAN	[18,25,29]

* UniProtKB/Swiss-Prot entry name (http://www.uniprot.org/)

Table 4: Tissue, secretome and plasma/serum proteins identified by at least two authors and associated with AAA size, expansion rate and rupture.

To determine the importance of inflammatory response in causing hemodynamic instability in the early postoperative period after AAA surgery, we performed a study to define the plasma proteomic changes after AAA repair [34]. We observed an enhanced thrombin expression at 6 h after declamping. Between 6 h and 36 h after AAA surgery, we viewed an apolipoprotein A-IV over-expression to produce significant protection against atherosclerosis. We proposed for the first time a possible role of thrombin in activation of inflammatory response in the early postoperative period after AAA surgery.

We listed in Supplementary Table 3 all the proteins (103) identified by these seven proteomic studies. As previously, the proteins were classified into functional categories based on Gene Ontology terms. These proteins were clustered into 33 GO categories. Most of them are proteins involved in acute phase response (such as alpha-1-antichymotrypsin, alpha 2-HS-glycoprotein, alpha-1-antitrypsin, haptoglobin) and in blood coagulation (such as fibrinogen alpha, beta, gamma chains and alpha-2-macroglobulin). We also found the lipid metabolic protein apolipoprotein A-I, proteins associated to proteolysis (complement factor B), to brown fat cell differentiation (leucine-rich alpha-2-macroglobulin), transport (serotranferrin and serum albumin) and cellular cation homeostasis (ceruloplasmin and hemopexin). It is noteworthy that some of these proteins have been identified by

several authors suggesting the idea of their possible role in the AAA development. Then, we listed the proteins, identified by more than one author, in Table 3. Moreover, in Figure 3, we reported the percentage of proteins identified by each author. This analysis pointed out that Acosta-Martin et al. [29] identified about 77% of plasma proteins. This high percentage of identification is probably due to the use of the data-independent shotgun proteomic strategy PAcIFIC [29].

Aortic wall tissue, secretome and plasma/serum proteins identified by more than one author and associated with AAA size, expansion rate and rupture

In order to highlight if the same protein is regulated in all biological specimens considered, we reported in Table 4 the proteins in common among tissue, secretome and plasma/serum. We found that 30 proteins were identified associated to AAA in tissue, secretome and plasma/serum. As evident from this table, several proteins are involved in acute phase response (such as alpha-1-antichymotrypsin, alpha 2-HS-glycoprotein, alpha-1-antitrypsin, haptoglobin), in blood coagulation (such as fibrinogen alpha, beta, gamma chains and alpha-2-macroglobulin) and lipid metabolic process (apolipoprotein A-I, apolipoprotein A-II, apolipoprotein B-100).

Some of these proteins have been previously correlated with the development of AAA. In particular, a very recent prospective study found that higher concentrations of 6 potential biomarkers including fibrinogen were strongly associated positively with AAA incidence [35]. Among proteins of acute-phase response, we found haptoglobin (Hp) that was associated with occurrence of AAA by several other studies [36,37]. In particular, Pan et al. [38] demonstrated that plasma Hp concentration was elevated in patients with AAA, particularly those with the Hp 2-2 phenotype. They suggested that if the results of this study were confirmed and extended by a further larger scale study, Hp concentration among with Hp2-2 phenotype might be potential biomarkers of AAA development and could be used clinically in association with other biomarkers [38]. Concerning proteins belonging to lipid metabolic process, we found apolipoproteins. ApoA-I and ApoB were previously found to be associated with AAA [39]. Moreover, Liu et al. [40], using animal model, demonstrated that dyslipidemia is implicated in abdominal aortic aneurysms (AAAs) in humans and angiotensin (Ang) II-infused mice. This study determined effects of major lipoprotein classes on AngII-induced AAAs using multiple mouse strains with dietary and pharmacological manipulations. ApoB-containing lipoproteins contribute to augmentation of AngII-induced AAA in male mice. However, unlike atherosclerosis, AAA occurrence was not correlated with increases in plasma apoB-containing lipoprotein concentrations.

We can conclude that this analysis pointed out several potential AAA biomarkers, but their clinical value is yet to be established. Future research should focus on the most relevant AAA markers, and how they could be used clinically.

Conclusion

Several potential biomarkers for the progression of AAA have been investigated by proteomic approach in whole-tissue samples, secretome and plasma/serum. In this review, we classified all the identified proteins into functional categories based on Gene Ontology terms related to their main biological function. This analysis pointed out that most of these proteins belong to categories related to cell adhesion, cytoskeletal organization, proteolysis, lipid and hyaluronan metabolic process, blood coagulation, acute phase response. However, most show either no correlation or a weak correlation with the clinical course of AAA. No one of proteins identified in these studies has the biologic plausibility to be used singularly as a biomarker of AAA disease due to inadequate specificity.

This review points out that the 30 identified proteins associated to AAA in all biological specimens, combined with clinical factors, could be used as clinical predictive markers for abdominal aortic aneurysms (AAA) development, progression and rupture. However, this hypothesis requires investigation in carefully designed population-based studies. Another limitation relates to the fact that many biomarkers for AAA are not disease specific; most of them also are markers for atherosclerosis. AAA is a multifactorial disease; some aneurysms may have a stronger genetic component while environmental factors, such as smoking, play a greater role in others.

Moreover, in an attempt to find a set of suitable AAA biomarkers it must be taken into account also non-protein targets such as miRNA. Indeed, some recent studies clearly demonstrated the roles of miRNAs in the development of AAA [41-44]. Zampetaki et al. [45] examined miR-195, a member of the miR-15 family. The authors performed proteomic analysis of the secretome of murine aortic smooth muscle cells, after miR-195 manipulation, confirming that miR-195 regulates numerous ECM elements such collagens (COL1A1, COL1A2, and COL3A1). It is noteworthy that these proteins were found to be associated to ECM damage in AAA by the cited proteomic study of Didangelos et al. [21]. These findings strengthen the idea of integration of set of different biomarkers (proteins among with microRNAs) as predictive of AAA development. Thus, further studies are needed to more firmly establish which specific proteins or fragments do participate.

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