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The Need for Novel Biomarkers of Abdominal Aortic Aneurysm Disease

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SUMMARY

Abdominal aortic aneurysm (AAA) is a complex disease defined as a pathologic dilatation of the infrarenal aorta that is often accompanied by significant superimposed atherosclerosis, inflammation and thrombosis. AAAs are a significant cause of morbidity and mortality in the United States and worldwide. While some patients may present with lower back pain, abdominal pain or pulsatile abdominal masses, identification of small aneurysms are dependent mostly upon incidental ultrasound evaluation. Furthermore, there remains no good way to predict which small aneurysms will grow to critical size lesions and at what pace. Thus, a significant number of patients are left with long, often stressful, follow-up periods that may be unnecessary.

Basic science and clinical studies have added great insight into the pathophysiology of AAAs. However, detailed understanding of the underlying mechanisms of AAA development and expansion are still incomplete. Thus, the major clinical challenges in disease management include the absence of 1) effective non-surgical therapies to prevent progression of early stage disease and 2) adequate means to monitor disease activity and to guide suppressive medical therapies.

Serum biomarkers have proved useful in assessing the risk in several disease states including cancer and coronary artery disease. In addition, a number of inflammatory markers have shown correlations to vascular disease status or complications including AAAs, although their utility for individual patients remains unknown.

Strategies that combine novel biomarker data (e.g., circulating proteins, genetic polymorphisms, imaging data) with known clinical risk factors should aid in this endeavor.

Key words: **abdominal aortic aneurysm, atherosclerosis, biomarker**

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INTRODUCTION

An abdominal aortic aneurysm (AAA) is defined as a pathologic thinning of the vessel wall that is often accompanied by significant superimposed atherosclerosis and thrombosis. Despite considerable variations in histopathologic appearance of AAAs, they typically involve some degree of inflammatory response that may involve all layers of the vessel wall and significant destruction of the medial elastic lamellae and other supporting connective tissues. While AAAs are common and often lethal, the underlying mechanisms of formation are not well understood. Equally important, there are not adequate means to rapidly stratify the risk of aneurysm development, progression, or ultimately, a rupture.

The problem of abdominal aortic aneurysms

An abdominal aortic aneurysm (AAA) is defined as a pathologic dilatation of the infrarenal aortic diameter >30 mm. AAAs are a significant cause of morbidity and mortality in the United States and worldwide (1). They account for approximately 9.000-30.000 deaths per year, 150.000 inpatient hospitalizations per year, and 30.000 open repairs per year in the United States (2,3). Patients with AAAs can present with back pain, abdominal pain, or a pulsatile abdominal mass. However, since the majority of AAAs are asymptomatic and the definition of AAA is not uniformly applied, it is difficult to determine the true prevalence of this disease process. Data from clinical studies encompassing different populations suggest that the prevalence ranges 2.4-16.9% in men while it is 0.5-2.2% in women (3).

The most feared clinical consequence of AAA development and progression is acute rupture. Although this clinical scenario is rare, AAA rupture caries a mortality of 80% (4). Interestingly, 60% of patients with AAAs die of other cardiovascular causes, such as myocardial infarction or stroke, suggesting a relationship between AAAs and atherosclerosis (5). The risk factors for development of AAAs are age (>65 years old), male sex, cigarette smoking, hypertension, a family history of AAAs in a male first degree relative, and presence of atherosclerosis in other vascular beds (5). Since most patients with AAAs do not have symptoms, there has been much debate over whether population screening strategies using ultrasound or other imaging modalities are cost-effective and reduce the morbidity and mortality associated with AAAs. The most recent United States Preventive Task Force recommendations conclude that screening ultrasonography should be performed in men aged 65-75 years with a history of smoking (6).

Once detected, monitoring AAAs for progression and need for surgical intervention is essential. The diameter of the aorta normally increases gradually with age and the rate of expansion is proportional to the aortic diameter. In the setting of AAAs, the average rate of expansion is 1-4 mm per year, close to the detection limits of conventional ultrasound exams (1). Predictors of AAA growth include aortic diameter at diagnosis and active smoking. Some studies have demonstrated that progression is also related to hypertension and age (4, 5). Although insight into the underlying mechanisms of disease has been gleaned from idealized animal models, no specific medical therapy has been shown to slow the progression of AAA expansion in humans to date. The risk of AAA rupture clearly increases with enlargement of the aortic diameter; however, the diameter at which the risk of acute rupture becomes higher than the risk of repair (indicating the need for open or endovascular intervention) is the subject of debate (4).

Based on observational studies evaluating the risk of rupture in inoperable patients, the consensus is that AAAs exceeding a diameter of 55 mm should be repaired provided that the risk of rupture is greater than that of repair (1, 5). Similarly, careful surveillance of AAAs with regular imaging is indicated until AAA diameter is greater than 55 mm or the rate of expansion is more than 10 mm per year. Trials evaluating early elective surgery versus surveillance found no mortality benefit with early surgery; however, one trial did show some late benefit in the early surgery group which was attributed to lifestyle modifications (6).

The relationship between atherosclerosis and AAAs

The causal relationship between atherosclerosis and AAAs is controversial although the strong association implies a connection (7). There is a significant overlap in risk factors for atherosclerosis and AAAs suggesting the local and systemic environmental stimuli generated from these risk factors result in these pathological vascular processes. In fact, one of the risk factors for AAAs is the presence of atherosclerosis in other vascular beds. AAAs are commonly observed in areas of atherosclerosis and they share histological features, such as inflammatory infiltration of the media, thrombosis, and neovascularization. Given the benefit of lifestyle changes, such as smoking cessation, lipid management, regular aerobic exercise, to cardiovascular disease, as well as the pathophysiological overlap between atherosclerosis and AAA development and progression, further examination of the role of lifestyle modifications on the natural history of AAAs is warranted.

In addition to genetic predilection, three major mechanisms for AAAs have been proposed:

- proteolytic degradation of aortic connective tissue,
- immune and inflammatory processes, and
- local biomechanical forces (2).

AAA formation and progression is a complex vascular remodeling process that involves the degradation

and synthesis of extracellular matrix components. The proteolytic destruction of the media and supporting adventitia occur through the degradation of elastin and collagen by cysteine proteases, matrix metalloproteinases (MMPs), and serine proteases. Studies on human aneurysm tissue suggest that small amounts of the serine protease neutrophil elastase are present whereas MMPs and cysteine proteases are both abundant. These elastases and collagenases can be derived from intrinsic components of the vessel wall, such as vascular smooth muscle cells (VSMC) or fibroblasts, or can be elaborated by infiltrating macrophages. Serine proteases, such as tissue - plasminogen activator (tPA) and urokinase - plasminogen activator (uPA), are produced by macrophages and can activate the MMPs. MMP-2, MMP-9, and MMP-12 degrade elastin while MMP-1 and MMP-13 dissolve collagen; all of these MMPs are found in increased amounts in human AAA tissue (2). The cysteine proteases, cathepsin S and cathepsin K, are produced by VSMC and macrophages and degrade elastin.

Given the clear importance of macrophages, inflammatory and immune responses play a critical role in AAA pathogenesis. Chronic transmural inflammation with lymphocytes and macrophages is a prominent histological feature of AAAs. It is believed that these infiltrating cells elaborate a variety of inflammatory cytokines, such as tumor necrosis factor-α (TNFα), interferon-γ (IFNγ), interleukin-1β (IL-1 β), IL-6, IL-8, and IL-10, that have local and systemic effects that increase matrix turnover and support chemotaxis (2). Some scientists believe that infiltration of immune cells is due to molecular mimicry resulting in automimmune aortic antigens perpetuating the progression of AAAs. Others have postulated that oxidative stress appears to play a role in the inflammatory milieu, as levels of superoxide radicals and the expression of antioxidant species in human AAA tissue differ markedly from control aortic tissue.

AAA and obstructive atherosclerotic plaques occur preferentially in the infrarenal abdominal aorta while the thoracic aorta is protected. Regional susceptibility to these disease processes are thought to be due to differences in vessel composition, structure, biology, humoral milieu, and hemodynamic enviroment. In particular, distinct biomechanical forces, such as disturbed flow and increased wall stress, predispose regions of the vasculature to early atherosclerotic lesion formation which some believe contributes to the development of AAAs. Abnormal hemodynamic conditions in the abdominal aorta subsequent to major limb amputation and chronic spinal cord injury are thought to contribute to the increased risk of AAA disease in these populations. The molecular basis of underlying biomechanical force-induced vascular remodeling remains to be defined although in vitro work has identified a variety of potential mechanisms including local oxidative stress. Reactive oxygen species (ROS) associated with oxidative stress promote the upregulation and activation of proteolytic MMPs, increase expression of proinflammatory transcription factors, chemokines and cytokines, and stimulate apoptosis. Given the significant evidence for these processes in AAA development and expansion, novel efforts for the diagnosis and monitoring of AAAs should include strategies to identify markers of inflammation and proteolytic degradation.

Current evidence for genetic influences in AAA disease predilection

In 1977, Clifton (8) described a family in which three brothers were affected by AAA disease, thereby proposing for the first time a potential genetic component. Since then, several studies have compared the family histories of AAA patients and compared them to those of controls and found increased incidence within families. For example, Baird and colleagues compared relatives of both AAA patients and controls (undergoing cataracts surgery) (9). After interviewing the subjects, they found that 4.4% of the siblings of AAA patients had aneurysms while only 1.1% of controls had been diagnosed. However, they subsequently performed ultrasound screening and found that 19% of siblings of AAA patients had aneurysms compared to 8% of controls. A subsequent study by Fitzgerald et al. produced similar findings in that 22% of male siblings of AAA patients had ultrasound-verified aneurysms (10). Segregation studies have been mixed in their conclusions with both dominant and recessive models possible. The lack of Mendelian inheritance of AAA disease argues against a single gene defect. Indeed, most investigators suggest a multigenic model of predisposition that is influenced by known environmental factors such as smoking and hypertension. The genetic underpinnings are likely composed of common genetic variants with small effect and rare sequence variants with relatively larger effects.

Genome-wide linkage studies of AAA have identified a locus on chromosome 19. Using 400 DNA markers Van Vlijmen-van Keulen and colleagues investigated three families with 13 affected individuals identified by referral to hospitals in the Netherlands (11). Similarly, Shibamura et al. used 405 microsatellite markers to assess 86 affected individuals in 36 families from 9 different countries (12). Thus both studies assessed relatively small numbers of individuals with low density mapping by comparison to whole genome mapping currently being carried out in other diseases. In addition, several genome - wide association studies have identified loci at 9p21 and 9q33 to be associated with AAA disease (13, 14). However, since the human genome contains regions of strong linkage disequilibrium, a disease - associated locus may encompass several genes and multiple tightly associated polymorphisms, making it difficult to pinpoint the causal variant by association mapping. Moreover, in many instan-

ces, SNPs show the most significant disease association map to genomic regions with no obvious function, thus providing a few clues as to how causal variants affect the disease gene.

Another approach adopted in an attempt to identify genes important in AAA development is a candidate gene association using identified common variants. Examples of candidate genes with SNPs associated with AAA include those involved with inflammation (e.g., angiotensin-converting enzyme, interleukin-10, chemokine receptor 5), matrix remodeling (e.g., metalloproteinase-9, tissue inhibitor of metalloproteinase-1) and thrombosis (e.g., plasminogen activator inhibitor-1, nitric oxide synthase). However, the great majority of these associations have failed to be replicated in the literature. The reasons for failure of these studies to date likely stem primarily from the very low pre-test probability that any given single gene variant contributes to the susceptibility of a complex trait. Other reasons include the use of underpowered sample sizes, multiple testing, phenotypic heterogeneity, poor phenotype characterization, selection bias, population stratification, and incomplete knowledge of the complete set of allelic variants in the region of a candidate gene.

Importantly, most studies do not have satisfactory control groups (e.g., either poor age and gender matching or inadequate aortic imaging). Finally, the approach does not reflect the likely interaction between environment and genes in the development of AAA.

Biomarkers and Vascular Disease

The future development of effective therapeutics and their implementation for the treatment of human disease depends on the development of protein markers that can be accurately and reproducibly quantified in convenient clinical samples such as peripheral blood. Such biomarkers allow for early detection of disease and the opportunity for intervention and cessation or retardation of the disease process before fatal or life-threatening complications arise. Biomarkers serving as disease surrogates are critical for all stages of novel therapeutic development.

There is considerable experience with biomarkers in cardiovascular disease (15). It is now generally accepted that atherosclerosis is primarily an inflammatory process, and this hypothesis is supported by studies showing that inflammatory markers have some utility in predicting and monitoring vascular disease status. While C-reactive protein (CRP) has received the most attention in this regard, a number of other inflammatory markers have also shown correlations to vascular disease status or complications. Fibrinogen and soluble adhesion receptors have some correlation with the presence of risk factors, disease status, and risk for events (15). The correlation of these markers has been demonstrated in large cohorts of patients compared to controls, but their utility on an individual patient basis is still controversial. This is most likely due to the lack of specificity of general inflammatory markers for atherosclerotic disease. Indeed, the majority of CRP and fibrinogen is not derived from the vasculature and may signal inflammation in any organ. It is also possible that due to heterogeneity among the population at risk, a single marker cannot provide sufficient information for accurate prediction of disease. For similar reasons, the general markers of inflammation such as CRP and erythrocyte sedimentation rate (ESR) have been long abandoned as specific diagnostic markers in other inflammatory diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA).

The question remains as to which proteins can accurately detect disease as well as diagnose severity. In the case of AAA, biomarkers would ideally also allow for prediction of disease progression as well as the response to therapy. Molecular biological techniques using both human and animal model AAA tissue have posed several mechanisms of AAA development and identified a variety of candidate species that include inflammatory cytokines/chemokines (IL-1β, IL-6, IL-8, IL-10, IFNγ, CCL5, platelet-derived growth factor-A chain, transforming growth factor β (TGFβ), TNFα), serine proteases (plasmin, tPA, uPA), cysteine proteases (cathepsins S, K, D, L, H), metalloproteinases (MMP-1, MMP-2, MMP-3, MMP-9, MMP-12, MMP-13, MMP-14, TIMP-1, TIMP-2), adhesion molecules (ICAM1, CD11a/CD18), and oxidoreductases (heme-oxygenase 1, NADH-ubiquinone oxidoreductase 1β subcomplex 7, 12-lipoxygenase, inducible nitric oxide synthase) which may play roles in the pathogenesis of AAA disease (2).

Due to the key role of inflammation in AAA pathophysiology, several attempts have been made to associate markers of inflammation with AAA. CRP is associated with aneurysm size, but not expansion. Serum levels of inflammatory cytokines, like IL-1β, IL-6, IL-8, and TNFα, correlate with the presence of AAA while IFNγ has been tied to the rate of aneurysm expansion (2). As discussed earlier, there is an overlap in the risk factors, pathophysiology, and pathology between atherosclerosis and AAAs. Moreover, inflammatory markers could also reflect other concomitant pathological processes such as arthritis, poor dental hygiene, or infection. Therefore, the specificity of these individual markers for AAA disease remains questionable. However, while the correlation of these markers has been demonstrated in large cohorts of patients compared to controls, their utility on an individual patient basis is still controversial.

Some of the most compelling evidence for a candidate serological marker of AAA disease is the surrounding metalloproteinases, such as MMP-2 and MMP-9. MMP-9 is made by macrophages while MMP-2 is synthesized by smooth muscle cells and fibroblasts within the AAA lesion. Based on animal data, both MMP- 2 and MMP-9 appear to be the major players in

AAA pathogenesis. Mice deficient in either MMP develop smaller AAAs in various predictive models (7). Looking at a variety of human populations, investigators have demonstrated that elevated plasma MMP-9 levels are seen in patients with AAA. Furthermore, some have shown that levels of circulating MMP-9 are higher in patients with AAA than other types of atherosclerotic aortic disease. There have been two studies evaluating the response of plasma MMP-9 levels to open surgical repair and/or endovascular stent grafting. These studies have demonstrated that serum MMP-9 levels diminish the following successful repair, while patients who develop a perigraft endoleak have levels that remain elevated. Although this data looks promising, there are reports of patients with AAA that have lower or normal MMP-9 levels. Furthermore, efforts to associate MMP-9 levels with aortic diameter and disease progression have failed. Others have evaluated MMP-1 and MMP-2 without success. Thus, while available information points to a clear path for the development of clinically useful biomarkers, individual markers are unlikely to be found that can elucidate AAA activity with high fidelity across diverse patient populations. Accordingly, we must think in terms of panels of biomarkers, with the best chance for success being with proteins that are actually involved in the pathophysiology of the disease process. In addition, continued development of appropriate statistical analysis strategies that combine multiple protein measurements with known clinical variables and potentially novel biomarkers (e.g., imaging and genetic data) will be necessary.

In summary, while AAAs are recognized as a significant disease with high morbidity and mortality, identification of small aneurysms are dependent mostly upon incidental ultrasound evaluation. Furthermore, there remains no good way to predict which small lesions will grow to critical size lesions and at what pace. Protein profiles identified in the serum would appear to be a convenient monitoring tool that has the ability to be both highly sensitive and specific for AAAs. Not only would surveying these proteins in serum be informative as to the state of AAA development, the alteration in levels may be used to prognosticate AAA expansion as well as be indicative of responsiveness to clinical interventions. Characterizing such diagnostic protein profiles, combined with known risk factors as well as emerging genetic data, would undoubtedly contribute to the long-term objective of applying effective non-surgical therapies to the treatment of small AAA disease.

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POTREBA ZA NOVIM BIOMARKERIMA KOD ANEURIZME ABODMINALNE AORTE

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Sažetak

Aneurizma abdominalne aorte (AAA) je kompleksno oboljenje koje se definiše kao dilatacija infrarenalne aorte i često je praćena aterosklerozom, inflamacijom i trombozom. Važan je uzrok morbiditeta i mortaliteta u Sjedinjenim Američkim Državama kao i širom sveta. Bol u leđima, abdominalni bol i pulsativna abdominalna masa prisutne su kod izvesnog broja bolesnika, dok identifikacija malih aneurizmi predstavlja najčešće slučajan nalaz na ultrazvučnom pregledu. Takođe, za sada ne možemo sa sigurnošću predvideti kada će i koja manja aneurizma dostići kritičnu veličinu lezije i kojom brzinom, te zbog toga, značajan broj bolesnika prolazi kroz dugi, stresan i nedovoljno izvestan period praćenja.

Bazične nauke i kliničke studije dale su doprinos u razjašnjenju patofizioloških mehanizama nastanka AAA. Međutim, mehanizmi koji dovode do progresije i rupture AAA još uvek nisu dovoljno razjašnjeni. Zato, važan klinički izaziov u terapiji oboljenja predstavlja nedostatak: 1) efektivne neoperative terapije, koja bi imala ulogu da prevenira progresiju u ranim stadijumima oboljenja i 2) efektivne mogućnosti monitoringa aktivnosti oboljenja i terapijskog tretmana.

Serumski biomarkeri pružaju značajne informacije u proceni rizika za nastanak nekoliko oboljenja, kao što su kancer i koronarna arterijska bolest. Poznato je da su brojni inflamatorni markeri u korelaciji sa vaskularnim oboljenjima i komplikacijama, uključujući AAA. Do danas, njihova klinička vrednost u proceni individulanog rizika bolesnika ostaje nepoznata. Verovatno je da će novi biomarkeri (cirkulatorni proteini, genetski polimorfizam i imidžing tehnike), zajedno sa poznatim kliničkim faktorima rizika, pomoći u ovom nastojanju.

Ključne reči: **aneurizma abdominalne aorte, ateroskleroza, biomarkeri**