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Is there a role for biomarkers in thoracic aortic aneurysm disease?

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Abstract Thoracic aortic aneurysm (TAA) represents a major cause of mortality and morbidity in Western countries. The natural history of TAA is indolent, with patients usually being asymptomatic until a catastrophic event such as rupture or dissection ensues. As such, early diagnosis is crucial and the search is ongoing for a biomarker that can indicate the presence of TAA with sufficient accuracy to act as a screening tool. To date, no such marker has been developed for the diagnosis of non-familial or 'sporadic' TAA. However, our increased understanding of the pathogenesis of both familial and sporadic TAA has suggested potential candidates for diagnostic biomarkers. Many markers/pathways have been shown to have differential activity levels or expression in the aortic tissue of TAA. However, priority is given to markers that have shown differential levels in blood plasma, as blood tests represent the easiest route for mass screening for TAA. This review aims to evaluate the efficacy of clinical tests already in use in diagnosing TAA, explore novel proposed biomarkers and identify key areas of future interest.

Keywords Biomarkers · Thoracic aorta · Aneurysm · Dissection

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Introduction

Thoracic aortic aneurysm (TAA) represents a significant cause of morbidity and mortality worldwide. A large national database study found an incidence of 16 and 9 per 100000 population in men and women, respectively [1]. TAA is a silent disease, being asymptomatic in the majority of cases. The aneurysmal aorta grows slowly until it reaches a pivotal point, at which it either dissects or ruptures. Such complications are often associated with catastrophic outcomes, making timely detection of patients at risk of developing a thoracic aneurysm imperative. Such detection can be achieved by the development of screening tools such as biomarkers and genetic components.

As far back as 1968, Wilson and Jugner identified 10 key principals for early disease detection relating to both the disease and the screening test [2]. They described the need for a recognisable early symptomatic phase in the disease process to identify potential patients for screening. It is the lack of such a stage in TAA that poses a challenge in terms of screening for the condition. Instead, such screening would need to target groups known to be at increased risk of the disease. Identifying and understanding predisposing risk factors requires an in-depth understanding of the natural history of the disease as well as its associated conditions. In recent years, conditions such as bicuspid aortic valve (BAV), intracranial aneurysm, and bovine aortic arch, as well as a strong family history of aortic disease, have all been shown to be associated with TAA and dissection [3]. Furthermore, they have been proven to increase predilection to development of aortic aneurysm. Nevertheless, a large percentage of newly identified TAAs have no such associations and isolated incidental findings revealed during imaging studies (echocardiography, computed tomography, MRI) performed for other indications. Screening a population of

asymptomatic patients with few identified predisposing factors requires a simple, inexpensive and safe diagnostic test. Given the relatively low incidence of TAA in the general population, the use of imaging as a screening tool would be likely to be prohibitively expensive and time consuming. Also, the use of CT would involve exposing the majority of those screened to unnecessary radiation. As such, there has been increasing interest in the use of blood biomarkers for the early diagnosis of TAA [4].

In addition to diagnosis of TAA, research has focused on the use of biomarkers to monitor the progression of aneurysm development, in particular detection of imminent rupture or dissection [5, 6]. There is a similar role for biomarkers in the post-operative period in terms of monitoring both disease progression in non-operated aortic segments and the development of graft-related complications [7]. In this review, we aim to demonstrate current evidence pertaining to blood-borne biomarkers of TAA, focusing on circulating proteins and predisposing genetic variants measured from leukocyte-derived nucleic acids. Particular emphasis is paid to studies that have performed validation of their chosen markers and that quote their diagnostic ability in terms of positive or negative predictive values (NPVs), sensitivity or specificity.

Generalized laboratory tests for the diagnosis of aortic aneurysm

Several well-established clinical blood tests have been investigated for their suitability to act as a biomarker for aortic aneurysm, including products of haemostasis, acute phase reactants and homocysteine [8]. The vast majority of such studies have been done in the setting of abdominal aortic aneurysm (AAA), due to the increased prevalence and disease burden that this condition represents in comparison with TAA. The role of these general markers will briefly be discussed before focusing on novel biomarkers more relevant to the thoracic aorta.

D-Dimer

D-Dimer is a fibrin-degradation product that results from the fibrinolysis of a thrombus. Its primary use in clinical practice is in the detection of venous thrombo-embolism (VTE), such as deep vein thrombosis or pulmonary embolism, where it has an extremely high NPV, but poor positive predictive value (PPV). This means that, when negative, D-Dimer is a useful test to exclude thrombosis. However, a positive test can be triggered by any source of thrombosis in addition to VTE such as trauma, post-surgery or other conditions such as cancer, infections or disseminated intravascular coagulation [9, 10]. Evidence for the use D-Dimer in the diagnosis of TAA is lacking with the majority of studies focusing on AAA or acute aortic dissection (AAD). A meta-analysis of 9862 patients concluded that D-Dimer, fibrinogen and thrombin–antithrombin complex III are increased in AAA [11].

Several studies have investigated the use of D-Dimer in the diagnosis of AAD. In a study of 87 AAD patients and 133 controls, Suzuki et al. evaluated the diagnostic performance of D-Dimer using a cut-off of < 500 ng/ml within the first 24 h of symptom onset [12]. At this threshold, D-Dimer has a sensitivity of 96.6%, a specificity of 46.6%, a PPV of 37.6 and a NPV of 97.6 against all controls. The specificity and PPV were increased by reducing the time of D-Dimer testing to 6 h from symptom onset. Similarly, Eggebrecht et al. found that D-Dimer was elevated in AAD to a similar degree as it is elevated in pulmonary embolism, although no values for sensitivity or specificity are quoted [13].

Although several studies have evaluated the role of D-Dimer in AAD, few have investigated its role in the diagnosis of TAA pre-dissection. One small study by Yuan et al. compared 9 patients with TAA against 20 patients with AAD and 6 patients with coronary artery disease (CAD) and found significant, and approximately equal, elevations in D-Dimer levels in TAA and AAD patients compared to CAD patients [14]. In addition to D-Dimer, other marketers of haemostasis have been implicated in AAA formation with tissue plasminogen activator (tPA) corresponding positively with aneurysm expansion rate [15]. Similar studies have not been applied to the thoracic aorta and, to date, no assay of tPA is available for clinical use.

C-reactive protein and leucocyte

Highly sensitive C-reactive protein (hs-CRP), an acute phase protein that is elevated in response to inflammation, was found to be elevated in 39 patients with AAA [16]. Furthermore, the level of CRP was found to correlate positively with aneurysm size. Raised hs-CRP was also found by Yuan et al. in the setting of TAA [14]. Leukocyte count has previously been found to be elevated in cases of thoracic aortic dissection [13] and has also been found to be associated with the degree of plaque thickness. Elkind et al. demonstrate increased leukocyte counts in patients with aortic arch plaque thickness of > 4 mm [17]. Such elevated levels of leukocytes both initiate atherosclerosis and contribute significantly to its progression. However, the subtype of white cell and mechanism by which atherosclerosis is induced has not yet been determined [18].

Plasma homocysteine

Elevated plasma homocysteine levels are a risk factor for peripheral atherosclerotic disease including that of the

thoracic aorta [19]. Whilst several previous studies into the association of plasma homocysteine levels and aortic aneurysm reported conflicting results, a 2014 meta-analysis of 6445 patients showed elevated levels to be associated with an odds ratio for developing AAA of 3.29 (95% CI 1.66–6.51) [20]. For TAA, evidence is limited to a handful of studies with small numbers of patients. Sbarouni et al. demonstrated hyperhomocysteinaemia in 31 patients with acute dissection [21] with Giusti et al. showing similar findings in patients with Marfan's syndrome [22].

The applicability of the established tests described above to TAA has rarely been studied. The majority of evidence that does exist pertains to patients presenting with AAD. As such, the role of these markers as a diagnostic test prior to this pre-terminal event has not been established. Even in the context of AAD, studies into the use of established existing blood tests as biomarkers often involve small numbers of patients with no analysis of their efficacy in terms of sensitivity and specificity. Despite this, it is recognised that the above tests demonstrate low specificity for aneurysmal disease. D-Dimer, CRP, leukocyte count and homocysteine are upregulated in many disease states other than aortic aneurysm or dissection. This invalidates their use as a screening tool for the diagnosis of TAA. As such, their potential in clinical practice seems limited to excluding disease in the presence of a negative result. However, even this role is controversial since no marker is likely to have a 100% NPV. The case described by Thota et al. of aortic dissection in the presence of a normal D-Dimer result highlights the importance of the overall clinical presentation and the assessment of the treating physician over biomarkers currently in use [23]. If a peripheral blood biomarker for TAA is to be clinically useful it must be able to demonstrate a greater PPV than those tests currently in clinical use.

Focused biomarkers relevant for TAA

Aortic wall components

The media of the aortic wall is comprised of smooth muscle cells, connective tissue fibres and extracellular matrix (ECM). It is the connective tissue fibres, collagen and elastin, within the aortic wall that impart its elastic properties and strength. Their disruption is thought to play an important role in the pathogenesis of aortic aneurysm [24]. As such, research has focused on detecting differences in the composition of such molecules in the aortic wall of TAA patients compared to healthy controls. In this review, we will focus on blood-borne markers of collagen and elastin metabolism as potential biomarkers. Analysis of aortic wall specimens by Toumpoullis et al. identified changes in specific fibrillar collagens that occur in TAA. Collagen V and XI showed upregulation at both mRNA and protein levels, whereas collagen I and III showed downregulated protein levels in TAA [25]. Further work by Black et al. used proteomics to identify eight proteins that were differentially expressed in the aortic tissue of normal controls and TAA or varying sizes. The genetic sequences of all of these proteins were then tested for preliminary diagnostic efficacy using RT-PCR analysis of whole blood, in an attempt to find a blood-borne biomarker for TAA. Among the proteins tested, four and a half LIM domains protein 1 (FHL1) was found to be a useful whole blood biomarker for TAA. When FHL-1 was combined with Collagen (I), (III), (V) and (XI) to form a five-biomarker panel, upregulation of any 3 of the 5 by more than 1.5-fold correctly predicted TAA in 30 of 41 TAA patients (sensitivity 0.79, specificity 1) [26].

Despite several studies demonstrating altered elastin architecture in TAA in both animals and humans, no studies have yet identified any circulating biomarkers of elastin degradation in the setting of TAA. Wilson et al. demonstrated that two products of elastin metabolism, serum elastin peptides (SEP) and plasma elastin alpha-1-antitrypsin complex, are associated with aortic wall distensibility together with serum propeptide of type III procollagen (PIIINP), a marker of increased collagen neosynthesis. Increased breakdown of elastin with increased SEP and plasma elastin alpha-1-antitrypsin complex was found to be associated with increased vessel distensibility whilst increased PIIINP was associated with reduced distensibility [27]. Furthermore, Lindholt et al. developed a test for monitoring disease progression in patients with small AAA (+3 cm), validated by serial scans for aneurysm size. They found that a predictive model based on initial aneurysm size, SEP level and PIIINP level was able to predict 9 out of 10 patients that would require operation for AAA within 5 years (sensitivity 91%, specificity 87%) [28]. To date none of these markers have been investigated in the setting of TAA, but they remain a potential avenue for future study.

Fibrillin plays an important role in the aortic wall by providing a scaffold for elastin through the formation of microfibrils and defects in the fibrillin-1 gene are responsible for aortic dilatation in Marfan syndrome [29, 30]. Marshall et al. described the use of fibrillin fragment concentrations in the circulation as a potential biomarker for both TAA and dissection [31]. Interestingly, this study also reports that fragment concentration was altered with the anatomical location of aneurysms and whether or not acute dissection had occurred. TAA was significantly more common than AAA in the highest compared with lowest quartile of fibrillin-1 concentration (OR = 2.9; 95% CI 1.6–5.0), as was acute dissection when compared to TAA (OR = 2.9; 95% CI 1.6–5.3). Despite the strengths of this study, it is only able to identify fibrillin fragments as a potential future biomarker and does

not evaluate its use in diagnosing TAA in a population or its role in monitoring progression.

In addition to the potential use of products of collagen and elastin degradation directly, the mediators of this degradation have also been studied as potential biomarkers of TAA. The degradation of elastin and collagen is regulated by matrix metalloproteinases (MMP) [32]. The balance between MMPs and their naturally occurring inhibitors, tissue inhibitors of metalloproteinases (TIMPs), regulate the degree of proteolysis within connective tissues [33]. There is evidence that certain MMPs are increased in the wall of TAA, specifically, MMP 1, 9, 12, and 14, with MMP2 also rose in the presence of a BAV [34]. Kouillis et al. also demonstrated increased expression of MMP 1 and 9 in the aortic wall of TAA compared to healthy controls, with MMP2 and MMP9 being increased further in aortic walls that had undergone dissection. Furthermore, a ratio of MMP9 to TIMP1 of > 1 was found to be associated with the development of TAA [35].

Of critical importance to the use of MMPs as a potential biomarker of TAA, differential plasma levels of MMPs in thoracic aortic disease have also been demonstrated. Elevated plasma levels of MMP 9 are found following aortic dissection with maximal levels occurring approximately 2 weeks after the event [36]. Plasma levels of MMPs and TIMPs have also been shown to distinguish TAAs associated with either bicuspid or tricuspid aortic valves. When compared to normal aorta, TAA associated with BAV demonstrated increased plasma levels of MMP-1, MMP-2, and MMP-7, and decreased MMP-8 and MMP-9. In contrast, TAA associated with TAV demonstrated significantly increased MMP-1 only together with decreased MMP-8 and MMP-9 levels [37]. In addition to a potential diagnostic role in cases of aortic dissection, MMPs may have a role in disease monitoring post-surgical correction. Experiments performed in AAA showed that levels of MMP 3 and MMP 9 return to near normal levels following endovascular exclusion of AAA which is thought to be due to reduced aneurysmal wall tension. Such a decrease in levels might act as a biomarker of successful aneurysm exclusion and aid in the monitoring of progression or recurrence. However, large-scale clinical studies of MMPs have not yet been performed and are needed before their use can be adopted for diagnostic or monitoring of aortic aneurysm or dissection.

The renin–angiotensin system in TAA

The renin–angiotensin system (RAS) has been suggested to play a critical role in the development of TAA [38]. This resulted from the finding that the Angiotensin 1 (AT1) receptor antagonist, Losartan, fully attenuated the aortic pathology generated by mutant fibrillin-1 in mouse models of Marfan syndrome [39]. Further evidence was provided by the fact that chronic infusion of Angiotensin II promotes the development of ascending aortic aneurysms in mice [40]. A meta-analysis of 14 studies by Huang et al. demonstrated a consistent finding that the angiotensin-converting-enzyme I/D polymorphism is associated with the formation of aortic aneurysm, with an OR of 1.59 in TAA and 2.43 in AAD [41]. Finally, Li et al. demonstrated that levels of plasma ACE and mRNA expression from aortic tissue for both ACE and ACE2 were markedly reduced in cases of AAD and TAA compared to healthy controls or patients with CAD [42]. Furthermore, levels were significantly reduced in AAD compared TAA. This study also demonstrated that the ACE/ACE2 gene expression ratio in aortic tissue was significantly higher in AAD than TAA ($p = 0.025$), leading the authors to conclude that the imbalance of these two markers was important in the progression of disease and that ACE2 was functioning as a protective mechanism against the development of dissection. Overall, there is strong evidence that RAS is important in the development of TAAs. However, the body of evidence to date is mostly derived from mouse models. What little evidence exists in humans is limited to pilot studies with small numbers of patients. Whilst these studies have had some success in demonstrating differential plasma concentrations of ACE and gene expression in TAA, larger studies are required to validate their clinical efficacy.

Genetic markers of TAA

Genetic testing of DNA extracted from peripheral blood leukocytes has been shown to correlate well with tissues from around the body. As such, peripheral blood can act as a surrogate tissue as it is both readily available and contains a large number of gene transcripts [43]. This creates the opportunity for the transcriptome of peripheral blood to act as a biomarker of TAA.

TAA demonstrates a strong heritable pattern with approximately 20% of patients reporting a familial history of aneurysm formation [44]. As such, there is a potential role for genetic testing in relatives of patients with a pre-existing diagnosis of TAA. Such cases of familial TAA have historically been classified as syndromic or non-syndromic based on whether or not the genetic variant is present with other features of a recognised clinical syndrome. Common syndromes associated with TAA include Marfan syndrome, Loeys–Dietz syndrome, Ehlers–Danlos syndrome, familial TAA and dissection (TAAD), and BAV. However, the distinction between syndromic and non-syndromic TAA has become increasingly blurred as it is common for a pathogenic variant in a gene to present with a range of phenotypes on a spectrum from syndromic to non-syndromic [45]. In their overview of hereditable thoracic aortic disease, Milewicz et al. identify 13 common loci associated with familial TAA including FBN-1 (Marfan syndrome), TGFBR1 and 2

(Loey's Dietz), MYH11 and ACTA2 [45–48]. The high frequency of ACTA2 mutations in 15–20% of cases of familial TAA suggests that diagnostic sequencing of this gene should be performed in TAA patients with a family history of aneurysmal disease [49].

In addition to familial TAA, attempts have been made to identify underlying genetic variants predisposing to sporadic TAA or dissection (STAAD). Three broad strategies have informed approaches to investigating genetic traits in STAAD. First, as technology has advanced, several studies have investigated differential expression of multiple genetic loci in TAA and controls using GWAS or microarrays to identify new genes involved in aneurysm formation [50–52]. Many of the genes identified in this manner have been found to be involved in common pathways already known to be important in aneurysm formation such as FBN-1, TGF-beta signalling or the contractile apparatus of the aortic wall [53, 54]. Second, genetic predisposition to STAAD has been studied by looking at the pathogenic pathways underlying the genetic causes of TAA in an attempt to find common causality. The study of the pathogenesis of Marfan's and Loeys–Dietz syndrome highlighted the importance of TGF-β signalling in the development of thoracic aortic disease. Aneurysm formation has subsequently been found to be associated with altered matrix sequestration of the TGF-β latent complex, leading to uncontrolled release of TGF-β and subsequent activation of the TGF-β pathway [55]. Despite this, few studies report on the use of TGF-β levels as a biomarker for TAA. Suzuki et al. report that circulating TGF-β levels are elevated in patients with AAD and suggest that TGF-β might be used as a biomarker in non-Marfan aortic disease [56]. The final approach to studying the genetic basis of STAAD has been to look for underlying genetic causes for changes that are known to occur in aneurysm formation such as the altered structured and composition of the aortic wall. For example, in a recent meta-analysis of 19 studies, Li et al. investigated associations between matrix mettaloproteinase family polymorphisms and the development of aortic aneurysmal disease [57]. The authors found positive associations with SNPs of MMP 2, 3, and 13 with AAA, and MMP2 and 8 with TAA. They conclude that these may have the potential to form novel biomarkers for the prediction of aortic aneurysm. However, to date none of these markers have been clinically validated in large independent populations.

Discussion

The development of a clinically useful biomarker for TAA is enticing in terms of preventing the catastrophic consequences of aortic dissection or rupture by early intervention. However, at present there remains a paucity of evidence for the efficacy of any single biomarker. The existing biomarkers in clinical practice are rendered ineffective by their poor PPV, meaning that they are unable to reliably discriminate between aneurysmal disease and a number of co-existing conditions such as vascular disease, inflammatory disease or thromboembolic disease.

Due to the strong genetic links to the formation of TAA, interest has focused on identifying underlying causal and associated genetic loci for aneurysm formation. Studies performed to date in both sporadic and familial TAA have identified multiple steps at which different pathogenic mechanisms can contribute to the formation of aneurysmal disease. Such studies have informed our broader understanding of the pathogenic mechanisms of TAA, often confirming changes seen at a microscopic or macroscopic level. However, no one genetic variant has been found to have a strong enough association to act as a clinically useful diagnostic biomarker. Attempts have been made to develop such a biomarker using a panel of the most commonly associated variants. Wang et al. used a microarray to screen for variants in 29098 genes differentially expressed between 36 TAA patients and 25 controls. RNA was derived from peripheral blood samples. A 41-gene classification model was developed for the diagnosis of TAA. When this model was applied to a validation set of 22 TAA patients and 11 controls it was able to predict TAA status with a sensitivity of 72% and a specificity of 90%. This represents an improvement over the more established clinical tests such as D-Dimer or hs-CRP in the diagnosis of TAA, particularly in terms of sensitivity, as the gene expression signature appeared unique for TAA and did not show any significant overlap with that of atherosclerosis, or CAD [58]. However, such 'genetic signatures' have not yet been adopted into clinical practice for two reasons. First, whilst such tests may be beneficial in identifying patients at increased risk of TAA formation, they are still not accurate enough to replace imaging studies as the gold standard diagnostic tests. Second, at present, performing such tests in the quantities needed to effectively screen for TAA is prohibitively expensive.

A further limitation of the use of genetic biomarkers of TAA lies in the potential disconnect between changes at the level of the genome and resulting changes in protein expression. The rapidly expanding field of epigenetics seeks to account for this discrepancy. Epigenetics refers to modifications in the genome that alter gene expression without changing the DNA sequence and include DNA methylation, histone modifications and non-coding RNA [59]. How epigenetic phenomena alter gene expression in the pathogenesis of TAA is the subject of ongoing investigation, and is beyond the scope of the current review. However, such modifications have the potential to add an additional level of complexity in the use of genetic biomarkers for TAA as changes seen at a genomic level may not correspond with changes in the encoded protein and vice versa.

Initial attempts to identify by-products of aneurysm development such as elastin degradation and fibrillin fragment complications have shown promise but have not yet been validated in larger studies [27, 31]. As such we have no way of determining their diagnostic capabilities. However, as a general principal, the use of a biomarker generated by the unique changes in the vessel architecture that occurs after the onset of aneurysm formation is highly attractive in terms of its specificity for diagnosing TAA. They also have the advantage of being able to monitor disease progression, something that genetic biomarkers are unable to do due to the fixed nature of the genomic code. The ideal biomarker for TAA would be produced in the presence of aortic dilatation prior to dissection or rupture. It would be easily detectable in the peripheral blood and its concentration would reflect the degree of aortic dilatation, allowing use for both diagnosis and monitoring of disease progression. To date, no such biomarker exists for TAA. It is our opinion that further research into blood-borne markers of altered aortic wall architecture holds the greatest potential for developing clinically applicable biomarkers for TAA. Such research is facilitated by the collating of large numbers of patient tissue samples (both aortic wall and peripheral blood) with corresponding imaging data. For rare conditions such as TAA, such biobanks can be created by the development of national or international registries with multicentre data collection and input, as has previously been shown in the setting of AAD [12].

In summary, to date no biomarkers exist that can accurately diagnose the presence of TAA prior to dissection or rupture. Despite the growing body of evidence into genetic associations of TAA, the multifactorial nature of aneurysm formation and the wide range of genes found to play a role in its pathogenesis means that no one genetic test is likely to prove clinically efficacious. Existing evidence suggests that biomarkers derived from disrupted aortic wall components represent the most promising avenue for further study.

Compliance with ethical standards

Conflict of interest The authors do not wish to make any disclosures.

References

1. Olsson C, Thelin S, Stahle E, Ekbom A, Granath F. Thoracic aortic aneurysm and dissection: increasing prevalence and improved outcomes reported in a nationwide population-based study of more than 14,000 cases from 1987 to 2002. Circulation. 2006;114:2611–8.
2. Wilson JMG, Jungner G. Principles and practice of screening for disease. Public health papers, No. 34. Geneva: World Health Organization; 1968. pp 26–7.
3. Ziganshin BA, Elefteriades JA. Guilt by association: a paradigm for detection of silent aortic disease. Ann Cardiothorac Surg. 2016;5:174 – 87.
4. Black KM, Masuzawa A, Hagberg RC, Khabbaz KR, Trovato ME, Rettagliati VM, et al. Preliminary biomarkers for identification of human ascending thoracic aortic aneurysm. J Am Heart Assoc. 2013;2:e000138.
5. Elefteriades JA, Farkas EA. Thoracic aortic aneurysm clinically pertinent controversies and uncertainties. J Am Coll Cardiol. 2010;55:841–57.
6. Ramanath VS, Oh JK, Sundt TM 3rd, Eagle KA. Acute aortic syndromes and thoracic aortic aneurysm. Mayo Clin Proc. 2009;84:465–81.
7. Trimarchi S, Sangiorgi G, Sang X, Rampoldi V, Suzuki T, Eagle KA, et al. In search of blood tests for thoracic aortic diseases. Ann Thorac Surg. 2010;90:1735–42.
8. Golledge J, Tsao PS, Dalman RL, Norman PE. Circulating markers of abdominal aortic aneurysm presence and progression. Circulation. 2008;118:2382–92.
9. Lippi G, Bonfanti L, Saccenti C, Cervellin G. Causes of elevated D-dimer in patients admitted to a large urban emergency department. Eur J Intern Med. 2014;25:45–8.
10. Goldhaber SZ, Simons GR, Elliott CG, Haire WD, Toltzis R, Blacklow SC, et al. Quantitative plasma D-dimer levels among patients undergoing pulmonary angiography for suspected pulmonary embolism. JAMA. 1993;270:2819–22.
11. Sidloff DA, Stather PW, Choke E, Bown MJ, Sayers RD. A systematic review and meta-analysis of the association between markers of hemostasis and abdominal aortic aneurysm presence and size. J Vasc Surg. 2014;59:528–35.
12. Suzuki T, Distante A, Zizza A, Trimarchi S, Villani M, Salerno Uriarte JA, et al. Diagnosis of acute aortic dissection by D-dimer: the international registry of acute aortic dissection substudy on biomarkers (IRAD-Bio) experience. Circulation. 2009;119:2702–7.
13. Eggebrecht H, Naber CK, Bruch C, Kroger K, von Birgelen C, Schmermund A, et al. Value of plasma fibrin D-dimers for detection of acute aortic dissection. J Am Coll Cardiol. 2004;44:804–9.
14. Yuan SM, Shi YH, Wang JJ, Lu FQ, Gao S. Elevated plasma D-dimer and hypersensitive C-reactive protein levels may indicate aortic disorders. Rev Bras Cir Cardiovasc. 2011;26:573 – 81.
15. Lindholt JS, Jorgensen B, Shi GP, Henneberg EW. Relationships between activators and inhibitors of plasminogen, and the progression of small abdominal aortic aneurysms. Eur J Vasc Endovasc Surg. 2003;25:546–51.
16. Vainas T, Lubbers T, Stassen FR, Herngreen SB, van Dieijen-Visser MP, Bruggeman CA, et al. Serum C-reactive protein level is associated with abdominal aortic aneurysm size and may be produced by aneurysmal tissue. Circulation. 2003;107:1103–5.
17. Elkind MS, Sciacca R, Boden-Albala B, Homma S, Di Tullio MR. Leukocyte count is associated with aortic arch plaque thickness. Stroke. 2002;33:2587–92.
18. Ross R. Atherosclerosis–an inflammatory disease. N Engl J Med. 1999;340:115–26.
19. Tribouilloy CM, Peltier M, Iannetta Peltier MC, Trojette F, Andrejak M, Lesbre JP. Plasma homocysteine and severity of thoracic aortic atherosclerosis. Chest. 2000;118:1685–9.
20. Cao H, Hu X, Zhang Q, Li J, Wang J, Shao Y, et al. Homocysteine level and risk of abdominal aortic aneurysm: a meta-analysis. PLoS One. 2014;9:e85831.
21. Sbarouni E, Georgiadou P, Analitis A, Chaidaroglou A, Marathias A, Degiannis D, et al. High homocysteine and low folate concentrations in acute aortic dissection. Int J Cardiol. 2013;168:463–6.
22. Giusti B, Porciani MC, Brunelli T, Evangelisti L, Fedi S, Gensini GF, et al. Phenotypic variability of cardiovascular manifestations in Marfan Syndrome. Possible role of

hyperhomocysteinemia and C677T MTHFR gene polymorphism. Eur Heart J. 2003;24:2038–45.
23. Thota D, Zanoni S, Mells C, Auten JD. Acute, proximal aortic dissection with negative D-Dimer assay and normal portable chest radiograph: a case report. Mil Med. 2015;180:e164–7.
24. Tsamis A, Krawiec JT, Vorp DA. Elastin and collagen fibre microstructure of the human aorta in ageing and disease: a review. J R Soc Interface. 2013;10:20121004.
25. Toumpoulis IK, Oxford JT, Cowan DB, Anagnostopoulos CE, Rokkas CK, Chamogeorgakis TP, et al. Differential expression of collagen type V and XI alpha-1 in human ascending thoracic aortic aneurysms. Ann Thorac Surg. 2009;88:506–13.
26. Arnaud L, Haroche J, Limal N, Toledano D, Gambotti L, Costedoat Chalumeau N, et al. Takayasu arteritis in France: a single-center retrospective study of 82 cases comparing white, North African, and black patients. Medicine (Baltimore). 2010;89:1–17.
27. Wilson KA, Lindholt JS, Hoskins PR, Heickendorff L, Vammen S, Bradbury AW. The relationship between abdominal aortic aneurysm distensibility and serum markers of elastin and collagen metabolism. Eur J Vasc Endovasc Surg. 2001;21:175–8.
28. Lindholt JS, Heickendorff L, Vammen S, Fasting H, Henneberg EW. Five-year results of elastin and collagen markers as predictive tools in the management of small abdominal aortic aneurysms. Eur J Vasc Endovasc Surg. 2001;21:235–40.
29. Ramirez F, Sakai LY. Biogenesis and function of fibrillin assemblies. Cell Tissue Res. 2010;339:71–82.
30. Ramachandra CJ, Mehta A, Guo KW, Wong P, Tan JL, Shim W. Molecular pathogenesis of Marfan syndrome. Int J Cardiol. 2015;187:585 – 91.
31. Marshall LM, Carlson EJ, O'Malley J, Snyder CK, Charbonneau NL, Hayflick SJ, et al. Thoracic aortic aneurysm frequency and dissection are associated with fibrillin-1 fragment concentrations in circulation. Circ Res. 2013;113:1159–68.
32. Vine N, Powell JT. Metalloproteinases in degenerative aortic disease. Clin Sci (Lond). 1991;81:233–9.
33. Dollery CM, McEwan JR, Henney AM. Matrix metalloproteinases and cardiovascular disease. Circ Res. 1995;77:863–8.
34. Rabkin SW. The role matrix metalloproteinases in the production of aortic aneurysm. Prog Mol Biol Transl Sci. 2017;147:239–65.
35. Koullias GJ, Ravichandran P, Korkolis DP, Rimm DL, Elefteriades JA. Increased tissue microarray matrix metalloproteinase expression favors proteolysis in thoracic aortic aneurysms and dissections. Ann Thorac Surg. 2004;78:2106–10. **(discussion 10–1)**.
36. Sangiorgi G, Trimarchi S, Mauriello A, Righini P, Bossone E, Suzuki T, et al. Plasma levels of metalloproteinases-9 and -2 in the acute and subacute phases of type A and type B aortic dissection. J Cardiovasc Med (Hagerstown). 2006;7:307–15.
37. Ikonomidis JS, Ivey CR, Wheeler JB, Akerman AW, Rice A, Patel RK, et al. Plasma biomarkers for distinguishing etiologic subtypes of thoracic aortic aneurysm disease. J Thorac Cardiovasc Surg. 2013;145:1326–33.
38. Lu H, Rateri DL, Cassis LA, Daugherty A. The role of the renin-angiotensin system in aortic aneurysmal diseases. Curr Hypertens Rep. 2008;10:99–106.
39. Habashi JP, Judge DP, Holm TM, Cohn RD, Loeys BL, Cooper TK, et al. Losartan, an AT1 antagonist, prevents aortic aneurysm in a mouse model of Marfan syndrome. Science. 2006;312:117–21.
40. Rateri DL, Davis FM, Balakrishnan A, Howatt DA, Moorleghen JJ, O'Connor WN, et al. Angiotensin II induces region-specific medial disruption during evolution of ascending aortic aneurysms. Am J Pathol. 2014;184:2586–95.
41. Huang LG, Liu DB, Wang HQ. Angiotensin-converting enzyme I/D polymorphism and aortic aneurysm risk: a meta-analysis. Interact Cardiovasc Thorac Surg. 2014;19:782–7.
42. Li Y, Hu J, Qian H, Gu J, Meng W, Zhang EY. Novel findings: Expression of angiotensin-converting enzyme and angiotensin-converting enzyme 2 in thoracic aortic dissection and aneurysm. J Renin Angiotensin Aldosterone Syst. 2015;16:1130–4.
43. Liew CC, Ma J, Tang HC, Zheng R, Dempsey AA. The peripheral blood transcriptome dynamically reflects system wide biology: a potential diagnostic tool. J Lab Clin Med. 2006;147:126–32.
44. Coady MA, Davies RR, Roberts M, Goldstein LJ, Rogalski MJ, Rizzo JA, et al. Familial patterns of thoracic aortic aneurysms. Arch Surg. 1999;134:361–7.
45. Milewicz DM, Regalado E. Heritable Thoracic Aortic Disease Overview. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Mefford HC et al, editors. GeneReviews(R). Seattle: University of Washington; 1993. **(GeneReviews is a registered trademark of the University of Washington, Seattle. All rights reserved)**.
46. Pannu H, Fadulu VT, Chang J, Lafont A, Hasham SN, Sparks E, et al. Mutations in transforming growth factor-beta receptor type II cause familial thoracic aortic aneurysms and dissections. Circulation. 2005;112:513–20.
47. Pannu H, Tran-Fadulu V, Papke CL, Scherer S, Liu Y, Presley C, et al. MYH11 mutations result in a distinct vascular pathology driven by insulin-like growth factor 1 and angiotensin II. Hum Mol Genet. 2007;16:2453–62.
48. Renard M, Callewaert B, Baetens M, Campens L, MacDermot K, Fryns JP, et al. Novel MYH11 and ACTA2 mutations reveal a role for enhanced TGFbeta signaling in FTAAD. Int J Cardiol. 2013;165:314–21.
49. Guo DC, Pannu H, Tran-Fadulu V, Papke CL, Yu RK, Avidan N, et al. Mutations in smooth muscle alpha-actin (ACTA2) lead to thoracic aortic aneurysms and dissections. Nat Genet. 2007;39:1488–93.
50. Kim JH, Na CY, Choi SY, Kim HW, Du Kim Y, Kwon JB, et al. Integration of gene-expression profiles and pathway analysis in ascending thoracic aortic aneurysms. Ann Vasc Surg. 2010;24:538–49.
51. Sakai H, Suzuki S, Mizuguchi T, Imoto K, Yamashita Y, Doi H, et al. Rapid detection of gene mutations responsible for non-syndromic aortic aneurysm and dissection using two different methods: resequencing microarray technology and next-generation sequencing. Hum Genet. 2012;131:591–9.
52. Absi TS, Sundt TM 3rd, Tung WS, Moon M, Lee JK, Damiano RR Jr, et al. Altered patterns of gene expression distinguishing ascending aortic aneurysms from abdominal aortic aneurysms: complementary DNA expression profiling in the molecular characterization of aortic disease. J Thorac Cardiovasc Surg. 2003;126:344–57 **(discission 57)**.
53. Isselbacher EM, Lino Cardenas CL, Lindsay ME. Hereditary influence in thoracic aortic aneurysm and dissection. Circulation. 2016;133:2516–28.
54. LeMaire SA, McDonald ML, Guo DC, Russell L, Miller CC 3rd, Johnson RJ, et al. Genome-wide association study identifies a susceptibility locus for thoracic aortic aneurysms and aortic dissections spanning FBN1 at 15q21.1. Nat Genet. 2011;43:996–1000.
55. Gillis E, Van Laer L, Loeys BL. Genetics of thoracic aortic aneurysm: at the crossroad of transforming growth factor-beta signaling and vascular smooth muscle cell contractility. Circ Res. 2013;113:327–40.
56. Suzuki T, Trimarchi S, Sawaki D, Grassi V, Costa E, Rampoldi V, et al. Circulating transforming growth factor-beta levels in acute aortic dissection. J Am Coll Cardiol. 2011;58:775.

57. Li T, Lv Z, Jing JJ, Yang J, Yuan Y. Matrix metalloproteinase family polymorphisms and the risk of aortic aneurysmal diseases: a systematic review and meta-analysis. Clin Genet. 2017. https://doi.org/10.1111/cge.13050.
58. Wang Y, Barbacioru CC, Shiffman D, Balasubramanian S, Iakoubova O, Tranquilli M, et al. Gene expression signature in peripheral blood detects thoracic aortic aneurysm. PLoS One. 2007;2:e1050.
59. Kim HW, Stansfield BK. Genetic and epigenetic regulation of aortic aneurysms. BioMed Res Int. 2017;2017:7268521.