THE ROLE OF BIOMARKERS IN THE DIAGNOSIS OF ACUTE AORTIC DISSECTION

Leanne Fava and Elisa Farrugia Pace
Tutors: Dr. Kurt Apap, Prof. Alex Borg

LIST OF ABBREVIATIONS

AD - Aortic Dissection
AAD - Acute Aortic Dissections
IRAD - International Registry of Aortic Dissections
CT Scan - Contrast-enhanced computed tomography
MRI - Magnetic resonance imaging
DSA - digital subtraction angiography.
MiRNA - MicroRNA

INTRODUCTION

GENERAL INTRODUCTION

Aortic dissection (AD), is characterised by the spontaneous development of false lumen in the innermost layer of the aorta and can present at various sites, with the ascending aorta being the most frequent location for its presentation. (1,2) Aortic dissections have a pathophysiologic sequence that includes aortic wall inflammation, apoptosis of vascular smooth muscle cells, aortic media degeneration due to inflammatory cell infiltration in the aortic media, elastin disruption, and vessel dissection. (1),(3) Consequently, immediate and accurate management and intervention are required, as mortality rates after the rupture exceed 80%.(2),(3)

Acute aortic dissection presents a great diagnostic challenge, as its typical signs and symptoms are nonspecific. The most common symptom includes acute onset of tearing chest, as well as pain in the back or abdomen, often described as 'sharp'. (1,2) Hypertension is a significant factor. Imaging tests are used to make a diagnosis, including transesophageal echocardiography, CT angiography, MRI, and contrast aortography, with recent studies showing that on a posterior-anterior chest x-ray, a widened mediastinum may also be a clinical presentation of aortic dissection. (1)

ANATOMICAL CONSIDERATIONS

The majority of Aortic Syndromes are caused by AD, with intramural haematoma and penetrating atherosclerotic ulcers accounting for the remainder. (4) Whilst acute AD and intramural haematoma have similar diagnostic and treatment challenges, the presence of an 'entry tear,' which is defined as a rupture in the intimal layer of the media, can distinguish the two pathologies. (1,3) If an aortic dissection is identified within 14 days after the commencement of the rupture, it is categorised as acute, and if it is diagnosed beyond two weeks, it is classified as chronic.

Dissections could interact with the true aortic lumen via intimal rupture at a distal site, permitting systemic blood flow to be maintained. Serious consequences include aortic valvular dilation and regurgitation, as well as heart failure. Another risk factor is a fatal aortic rupture through the adventitia into the pericardium, right atrium, or left pleural space. Dissection variants are thought to be precursors to classic aortic dissection, which include an intramural hematoma that separates the intima and media without a clear intimal tear or flap, an intimal tear and bulge without the presence of a haematoma or false lumen, as well as dissection, as well as a hematoma caused by atherosclerotic plaque ulceration.

The classification of AD is classified by the DeBakey system or the Stanford System. The DeBakey classification is of three types; Type I refers to lesions involving both the ascending aorta and descending aorta, Type II depicts lesions solely in the ascending aorta and Type III for the descending aorta.(3) With reference to the Stanford classification, Type A concerns dissections within the ascending aorta, regardless of anatomical entry location. Stanford Type A, Type I and II need immediate surgical intervention, reducing mortality rates by 20% within 30 days of onset. (1) Stanford Type B depicts lesions occurring in the descending aorta, and such distal AD may be managed medically, lowering mortality rates by 10% within the same time frame if no complications are present.(1,4)

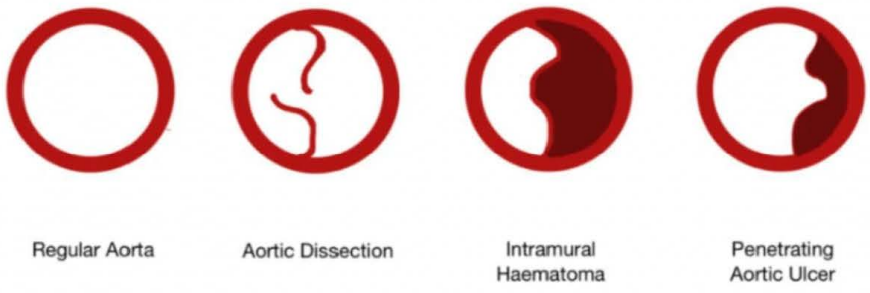

Figure 1: Simplistic diagram highlighting the changes in aortic lumen in different Acute Aortic Syndromes

DIAGNOSIS AND MANAGEMENT

At present, physicians consider biomarkers an attractive alternative diagnostic tool. Acute AD is depicted by the remodelling of the damaged aortic media via secondary thrombosis and inflammatory reactions. (4) Accordingly, various studies have focussed on biomarkers relating to such thrombotic and inflammatory reactions at the lesion site, as well as injury to the smooth muscle tissue and elastic laminae of the aortic media and interna. (1)

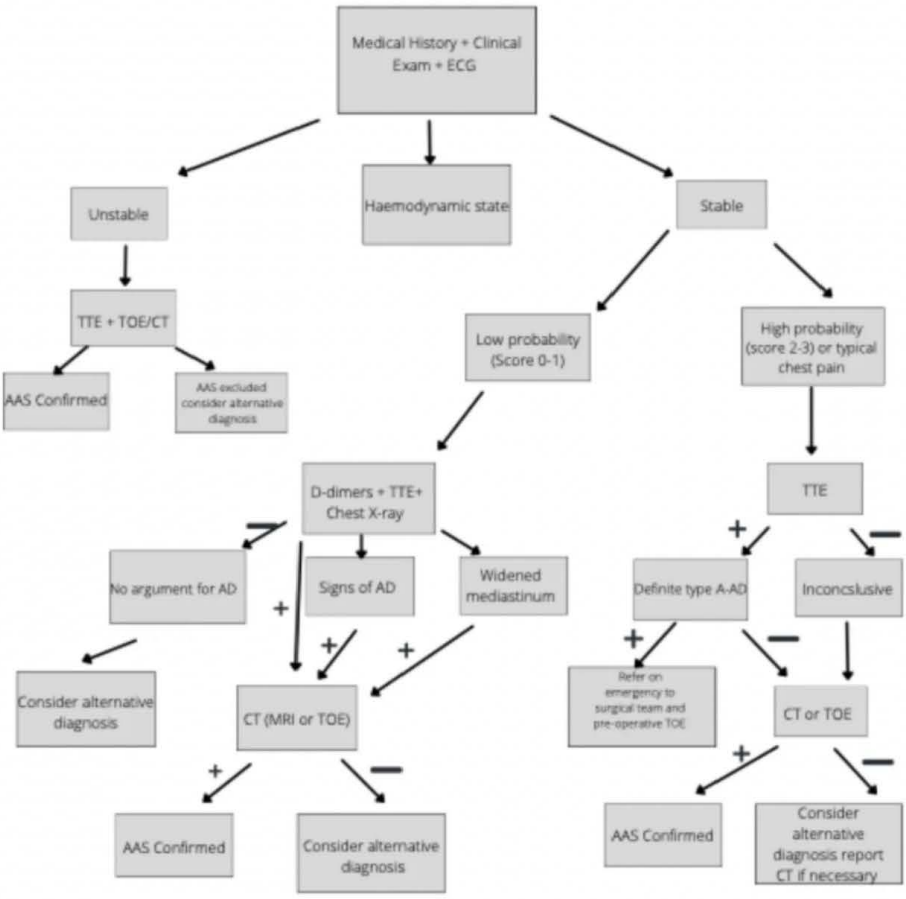

Figure 2: Diagnostic Algorithm of Potential D-dimer Patients, adapted from Wilcox G. Nursing patients with acute aortic dissection in emergency departments. Emerg Nurse. 2019 May 7;27(3):32–41.

Initial studies on biomarkers for AD show that circulating smooth muscle proteins are potential candidates for biomarkers, seeing as they are predominantly found in the aortic medial layer. Smooth muscle myosin heavy chains were investigated, and they showed significant rises in acute AD patients, with levels being 20-fold higher. While biomarkers had good diagnostic accuracy, they were shown to be high in the first 6 hours after onset, resulting in a short diagnostic window (5).

D-DIMER BIOMARKER

PATHOLOGICAL PROCESS OF D-DIMER

D-dimer is currently an available biomarker for AD. This molecule is comprised of two cross-linked fibrinogen D fragments. Although D-dimer is formed during fibrinolysis, it is a marker of thrombin activity and fibrin turnover, which reflects both haemostasis and fibrinolysis. D-dimer has an approximated 8-hour half-life and becomes detectable in the blood approximately 2 hours after index thrombus formation. D-dimer however, is not a specific marker for coagulation activity, limiting its utility. This is due to the close relationship between coagulation and inflammation, as well as the involvement of several factors in the coagulation cascade in each of these systems. Increased levels may be observed in conditions where fibrin is formed and later degraded, such as recent surgery, pregnancy, trauma events, heart disease as well as infection. (6,7)

The IRAD study on AD found that a cut-off level of 500ng/ml could be applied to both pulmonary embolism and AD within a day of symptom onset. The condition is linked to a rapid rise in D-dimer, with the first 6 hours following onset being the most significant. As a result, dissection during the first 6 hours can be safely ruled out. As a result, routine use of D-dimer helps risk-stratify people with query acute AD (5).

D-DIMER FORMATION FOLLOWING VESSEL TRAUMA

When blood clots are either being produced or broken down due to a damaged blood vessel, as what occurs in Aortic Dissection, a fibrin mesh and platelets form at the site of injury. This protective process against haemorrhage starts half an hour after trauma has occurred, and the vessel constricts due to spasms. (8) Collagen is exposed to the blood and cytokines and inflammatory markers are released via the extracellular matrix. The interactivity between tyrosine kinase receptors, glycoprotein receptors and G-coupled receptor proteins and von Willebrand Factor mediates the adhesion of platelets. (9)

During the propagation phase of coagulation, the prothrombinase complex FXa+FVa generates a large amount of thrombin on the activated platelet surface. Thrombin cleaves fibrinogen to fibrin, thus forming a stabilising fibrin network with FXIII. There are three major steps required for D-dimer formation, starting off when a polymerization site on fibrin is exposed, simultaneously when thrombin is cleaved. (8) This encourages the binding of fibrinogen or its monomer fibrin, in order to form thick fibrils. The role of plasmin is the proteolytic degradation of fibrin, and it remains fluid until thrombin cleaves 25% to 30% of plasma fibrinogen, allowing fibrin to polymerize while facilitating thrombin activation of plasma factor XIII. (7) Tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA) is required for plasminogen to plasmin conversion.

Plasminogen activator inhibitor (PAI)-1, which is a serine protease inhibitor, is an essential fibrinolytic system inhibitor that forms complexes with tPA and uPA quickly. PAI-1, an acute-phase protein, has high variability amongst individuals. Thrombin activatable fibrinolysis inhibitor (TAFI) is activated by thrombin and plasmin and is responsible for fibrinolysis. Thrombin remains bound to fibrin, and as more fibrin

molecules clump, plasma factor XIII bound to fibrinogen is activated. Until a fibrin gel is observed, a complex between soluble fibrin polymers, thrombin, and plasma factor XIII facilitates the formation of factor XIIIa. The second step of forming this biomarker is for Factor XIIIa forms intermolecular isopeptide links between lysine and glutamine residues in the soluble protofibrils and the insoluble fibrin gel to covalently cross-linked fibrin monomers. (9)

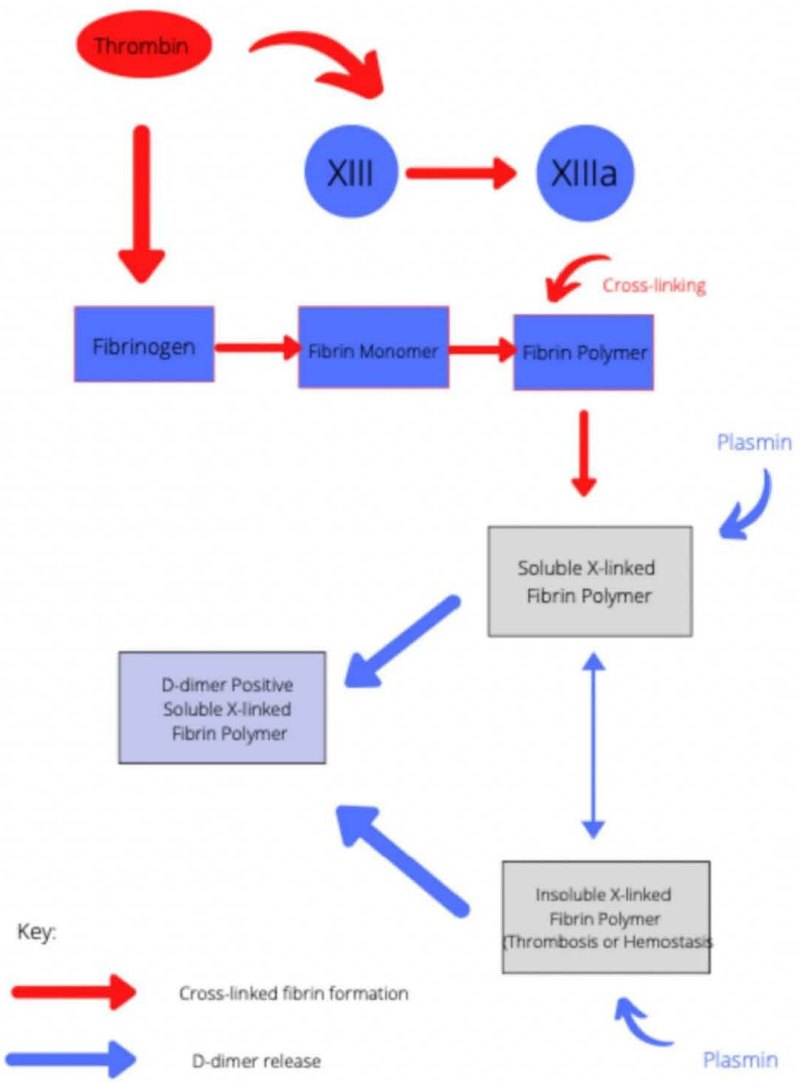

Figure 3: The Dynamic Formation of D-dimer, shown in three steps. Adapted from Adam SS, Key NS, Greenberg CS. D-dimer antigen: current concepts and future prospects. Blood. 2009 Mar 26;113(13):2878–87.

Until the action of plasmin releases the D-dimer antigen from crosslinked fibrin, it is undetectable. Plasmin formed on the fibrin surface by plasminogen activation cleaves fibrin at specific sites in the final step of D-dimer formation. Fibrin degradation products come in a wide range of molecular weights, including the D-dimer and fragment E complex terminal degradation products of cross-linked fibrin. (10)

DIAGNOSIS OF ACUTE AORTIC DISSECTION USING D-DIMER BIOMARKER

The function of biomarkers is to assess the risk and severity of the disease. This is measured by the sensitivity and specificity of a biomarker in relation to cardiac disease. (11) Sensitivity refers to the ability to correctly detect the disease in patients while specificity is related to the ability to identify patients that do not have the disease. Such screening tests are not diagnostic, however, they are used as identification means for the individual to know if they have a certain condition. (12)

D-dimer correlates positively with the extent of the dissection, as a key finding found is that the degree of aortic dissection and the condition of the false lumen were both reflected in the patients' admission D-dimer concentration. (13) The concentration of D-dimer was higher in patients with dissection that extended below the diaphragm level than in patients with dissection that did not extend to this extent. Low D-dimer concentrations are seen to be correlated with a tear that is either limited in the ascending aorta, or a thrombosed false lumen in the descending aorta. (14)

Thus far, studies state that there is no established acceptable failure rate for ruling out Acute Aortic Syndromes. This leads to high rates of AAS being misdiagnosed, reaching over 40%. D-dimer as a biomarker has several advantageous roles for Acute Aortic Dissection, due to such a test being less invasive, cost-effective and readily available in comparison to transesophageal echocardiography and MRI. Currently, D-Dimer is functionally used to test for short term outcomes of acute AD patients, with long-term outcomes yet to be explored, due to the fact that D-Dimer as a stand-alone test is inaccurate, due to the low specificity yet high sensitivity. In order for biomarkers to be widely used as a diagnostic test for dissection of the aorta, the D-Dimer biomarker must be paired with a biomarker with high specificity.

Studies and meta-analysis reviewed analysed patients with were diagnosed with acute AD, using the ADD-RS classification, of which patients with an Acute Aortic Dissection-Risk Score above or equal to 1 had a sensitivity of 95% (95% Confidence Index, 91.5-97.4) and specificity of 26.4% (95% CI, 24.3–28.7) when diagnosing Acute Aortic Dissection. It was found that the difference in concentration of D-d was significantly high, varying greatly from patient groups and is dependent on the extent of dissection, due to degradation fibrin fibres of an extensive false lumen would increase the D-dimer levels (15). Various meta-analyses conclude that 0.5ug/mL D-dimer cut off levels have a high sensitivity yet a relatively low specificity (95%-98% and 40%-60% respectively). Thus, this biomarker is termed sensitive yet non-specific (15,16).

A key finding found in this study is that the degree of aortic dissection and the condition of the false lumen were both reflected in the patients' admission D-dimer concentration. [25] The concentration of D-dimer was higher in patients with dissection that extended below the diaphragm level than in patients with dissection that did not extend to this extent. Similarly, the concentration of D-dimer was higher in patients with partially thrombosed or patented false lumen than in patients with fully thrombosed FL (16).

Moreover, low D-dimer concentrations are seen to be correlated with a tear that is either limited in the ascending aorta, or a thrombosed false lumen in the descending aorta. In addition, it was further reported that negative D-dimer results in patients correlated with a shorter dissection rate, yet it was inconclusive whether this related to a good prognosis rate (14).

Studies have confirmed that this biomarker has a high sensitivity for Acute Aortic Dissection diagnosis, due to the false lumen thrombosis results in tissue factor release activates the fibrin dissolution system, as well as the coagulation reaction endogenously. as mentioned previously. This study, like the previous studies mentioned in this systematic review, also confirmed that D-dimer levels are significantly more abundant in acute AD positive groups when compared to non-ADD clusters and control patients, further confirming its high diagnostic sensitivity but low specificity (17).

LIMITATIONS OF D-DIMER

The present studies discussed posed various limitations. There is no established acceptable failure rate for ruling out Acute Aortic Syndromes. This leads to high rates of misdiagnosis, reaching over 40%. In addition, it is often difficult with one cross-sectional CT image to assess the highest aortic width of the surgical site. These parameters alone will not appear to be sufficient in such situations to evaluate the long-term prognosis (18)

There is a limitation of the specific epitope on the D-dimer fragment that can be found for the monoclonal antibody used in a D-dimer assay, but greater than 20 different monoclonal antibodies are used in 30 different D-dimer tests (15). D-dimer as a biomarker is helpful, yet specificity and sensitivity of the test are sub-par, hence combined detection by using multiple biomarkers would improve diagnosis of Acute Aortic Dissection, especially when there is a lack of imaging equipment available (17).

Furthermore, a possible area for future research and literature would be Acute Aortic Dissection in patients of the age of 18 or younger. The majority of the present literature focus solely on patients above the age of 18, thus it is inconclusive whether D-dimer is a suitable and sensitive biomarker for sudden traumatic aortic injury in younger individuals.

MIRNA: STRUCTURE AND FUNCTION

MiRNA is small pieces of non-coding strands of RNA which are about 22 nucleotides long. The synthesis of miRNA is done through 2 different pathways which are the canonical pathway and the non-canonical pathway. The former pathway would involve pri-MiRNA synthesis from the genes, then this pri-MiRNA would be converted into pre-MiRNA through ribonucleotide III (Drosha) and DGCR8 (RNA binding protein DiGeorge Syndrome Critical Region 8). The pre-MiRNA is then transported to the cytoplasm through the exportin 5/RanGTP complex, where the terminal loop would be removed by Dicer forming the mature MiRNA duplex, this would then be loaded onto the argonaute proteins (AGO). On the other hand, the non-canonical pathways are grouped into the Drosha/DGCR8 independent and Dicer independent processes (19).

MiRISC (minimal RNA induced silencing complex) would interact with MRE (MiRNA response elements) which are found on the mRNA. When the mRNA and MRE fully match there would be AGO2 mediated splicing of the mRNA. When the miRNA binds to the 5'UTR on the mRNA it would suppress gene expression and when binding to the promoter region on the mRNA it would promote transcription (19).

The miRNAs have been linked to several cardiovascular diseases such as hypertension, heart failure, ischemic heart disease and left ventricular hypertrophy. MiRNA plays an important part in these diseases mentioned because it contributes to cardiac remodelling, therefore fibrosis and hypertrophy of myocytes can lead to the activation of this remodelling process (20).

EMERGING USES OF MIRNA IN AORTIC DISSECTION DIAGNOSIS

Recently miRNAs are also being considered to be used as a biomarker for the detection of aortic dissection or even detect a predisposing risk of people who are at risk of developing an aortic dissection such as people with hypertension. Apart from having the aforementioned benefit it also has a higher specificity than biomarkers that are currently being used such as D-dimer. The use of this biomarker would limit the error by which people are misdiagnosed which currently stands at around 25-50% due to offering different miRNAs which helps diagnosis of diseases such as myocardial infarction as opposed to aortic dissection (21).

Different miRNAs would have different biological pathways in which they play a role. The miRNA-15a is upregulated in diseases such as heart failure, myocardial ischemia and myocardial reperfusion injury (22). This miRNA has a specificity of 100% and therefore this can be used to track the progress of aortic dissection and also screen people which are prone to have the disease (23). MiRNA-23a is significantly important in cardiac development, myogenesis and hypertrophy, this would increase in cases of aortic dissection (23,24). This same miRNA has increased sensitivity and therefore can differentiate chest pain due to an aortic dissection or without an aortic dissection. Furthermore, it can also be used to monitor any suspected acute aortic dissections.

POSSIBILITY OF USING A COMBINATION OF MIRNAS FOR THE DIAGNOSIS

Studies also investigated the possibility of using the 4miRNAs which are miR-25, miR-29a, miR-155 and miR-26b and using them as a set to diagnose an acute aortic dissection. When combining these different types of miRNAs are tested together this would lead to a specificity of 100%, whilst the currently used biomarker D-dimer has a specificity between 96-60%. MiR-15a has the highest specificity (100%) and therefore this could be used as a prognostic marker, therefore this could be used to track the progress of an acute aortic dissection. Furthermore, this biomarker could be used to diagnose patients which have a subacute aortic dissection. MiR-23a has the highest sensitivity (91.9%), therefore this could be used to differentiate between chest pain which might not be the cause of aortic dissection and monitor an acute aortic dissection (20,23).

The use of these accurate MiRNA serum tests would be less invasive than transesophageal echocardiography which is currently used for the diagnosis of an aortic dissection. Furthermore, the use of serum tests would be less costly to conduct than CT scans which are used for the confirmation of an aortic dissection. Even Though, MiRNAs are very promising in what they offer to the field of medicine there still needs to be further tests done to assess the viability of these miRNAs. Further studies need to be deployed to find the best combination of miRNAs that can be used in tandem (19,20).

MIRNAS COMPARED TO D-DIMER (CURRENTLY USED BIOMARKER)

MiRNAs have a longer time window than D-dimer, therefore an aortic dissection could be diagnosed before treatment and surgery are too late for an option. Furthermore, serum-based tests would be less invasive than transesophageal echocardiography or CT scans which are more costly to conduct. However, despite all these advantages further experimentation needs to be carried out to test the viability and prognosis of aortic dissections as only limited research was done till now (19,20).

CONCLUSION

The importance of developing specific diagnostic biomarkers or rapid testing systems is to expedite the process of diagnosing and treating acute AD patients as soon as possible. Similarly, the development of new treatments or medical interventions with the goal of halting the growth of aneurysms and lowering the risks of acute AD results in a better prognosis for patients diagnosed with AA. The creation of such potential therapeutic agents or interventions does, in fact, provide longevity. Indeed, acute and chronic aortic conditions rely primarily on imaging modalities, and the likelihood that imaging will continue to be the primary tool in assessing such patients is high. Many studies, however, agree that studies of serum biomarkers to establish and monitor aortic diseases could be combined with current imaging tools to improve and advance diagnostic precision and patient management overall.

(1) Morello F, Piler P, Novak M, Kruzliak P. Biomarkers for diagnosis and prognosticstratification of aortic dissection: challenges and perspectives. Biomarkers in medicine 2014;8(7):931-941.

(2) Toghill BJ, Saratzis A, Bown MJ. Abdominal aortic aneurysm—an independent disease to atherosclerosis? Cardiovascular Pathology 2017;27:71-75.

(3) Mussa FF, Horton JD, Moridzadeh R, Nicholson J, Trimarchi S, Eagle KA. Acute aortic dissection and intramural hematoma: a systematic review. JAMA 2016;316(7):754-763.

(4) Cui J, Jing Z, Zhuang S, Qi S, Li L, Zhou J, et al. D-dimer as a biomarker for acute aortic dissection: a systematic review and meta-analysis. Medicine 2015;94(4).

(5) Suzuki T, Distante A, Eagle K. Biomarker-assisted diagnosis of acute aortic dissection: how far we have come and what to expect. Curr Opin Cardiol 2010;25(6):541-545.

(6) Giannitsis E, Mair J, Christersson C, Siegbahn A, Huber K, Jaffe AS, et al. How to use D-dimer in acute cardiovascular care. European Heart Journal: Acute Cardiovascular Care 2017;6(1):69-80.

(7) D-dimer understand the test.

(8) Adam SS, Key NS, Greenberg CS. D-dimer antigen: current concepts and future prospects. Blood, The Journal of the American Society of Hematology 2009;113(13):2878-2887.

(9) LaPelusa A, Dave HD. Physiology, Hemostasis. 2019.

(10) Tsai TT, Trimarchi S, Nienaber CA. Acute aortic dissection: perspectives from the International Registry of Acute Aortic Dissection (IRAD). European journal of vascular and endovascular surgery 2009;37(2):149-159.

(11) Ray P, Manach YL, Riou B, Houle TT, Warner DS. Statistical evaluation of a biomarker. The Journal of the American Society of Anesthesiologists 2010;112(4):1023-1040.

(12) Evaluating Screening Tests.

(13) Nazerian P, Mueller C, Soeiro AdM, Leidel BA, Salvadeo SAT, Giachino F, et al. Diagnostic accuracy of the aortic dissection detection risk score plus D-dimer for acute aortic syndromes: the ADvISED Prospective Multicenter Study. Circulation 2018;137(3):250-258.

(14) Nitta K, Imamura H, Kashima Y, Kamijo H, Ichikawa M, Okada M, et al. Impact of a negative D-dimer result on the initial assessment of acute aortic dissection. Int J Cardiol 2018;258:232-236.

(15) Itagaki R, Kimura N, Mieno M, Hori D, Itoh S, Akiyoshi K, et al. Characteristics and Treatment Outcomes of Acute Type AAortic Dissection With Elevated D-Dimer Concentration. Journal of the American Heart Association 2018;7(14):e009144.

(16) Nazerian P, Mueller C, Soeiro AdM, Leidel BA, Salvadeo SAT, Giachino F, et al. Diagnostic accuracy of the aortic dissection detection risk score plus D-dimer for acute aortic syndromes: the ADvISED Prospective Multicenter Study. Circulation 2018;137(3):250-258.

(17) Xiao Z, Xue Y, Yao C, Gu G, Zhang Y, Zhang J, et al. Acute aortic dissection biomarkers identified using isobaric tags for relative and absolute quantitation. BioMed research international 2016;2016.

(18) Mori K, Tamune H, Tanaka H, Nakamura M. Admission values of D-dimer and C-reactive protein (CRP) predict the long-term outcomes in acute aortic dissection. Internal Medicine 2016;55(14):1837-1843.

(19) O'Brien J, Hayder H, Zayed Y, Peng C. Overview of microRNA biogenesis, mechanisms of actions, and circulation. Frontiers in endocrinology 2018;9:402.

(20) Xu Z, Wang Q, Pan J, Sheng X, Hou D, Chong H, et al. Characterization of serum miRNAs as molecular biomarkers for acute Stanford type A aortic dissection diagnosis. Scientific reports 2017;7(1):1-11.

(21) Romaine SP, Tomaszewski M, Condorelli G, Samani NJ. MicroRNAs in cardiovascular disease: an introduction for clinicians. Heart 2015;101(12):921-928.

(22) Liu L, Liang Z, Lv Z, Liu X, Bai J, Chen J, et al. MicroRNA-15a/b are up-regulated in response to myocardial ischemia/reperfusion injury. Journal of geriatric cardiology: JGC 2012;9(1):28.

(23) Dong J, Bao J, Feng R, Zhao Z, Lu Q, Wang G, et al. Circulating microRNAs: a novel potential biomarker for diagnosing acute aortic dissection. Scientific Reports 2017;7(1):1-11.

(24) Wang S, He W, Wang C. MiR-23a Regulates the vasculogenesis of coronary artery disease by targeting epidermal growth factor receptor. Cardiovascular therapeutics 2016;34(4):199-208.