Biological and Medical Mechanisms of Intracranial Aneurysms and Biomarker Development

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Abstract: An intracranial aneurysm is one of the most debilitating medical conditions. Post rupture - only 30% of its victims are able to return to their pre-rupture lifestyle. The other 70% either didn't survive or suffered enough damage that they will be disabled for life. IAs can be prevented, but only if they are detected before it's too late. The methods utilized to obtain information were collecting information from different scientific journals and published works. Through combining this research, information about the pathophysiology of IAs, their progression, biomarkers for the same, and treatments was found. From this research, clinicians can develop a biomarker panel to be tested regularly in individuals over a certain age range. Further research could elaborate on biomarkers found in blood, so these biomarkers could be derived from a simple and inexpensive blood test.

1. Introduction

Intracranial aneurysms (IAs), also known as cerebral aneurysms or brain aneurysms, are dilations of cranial artery walls. Their global occurrence is approximately 3.2%. IA rupture frequently leads to subarachnoid hemorrhages (SAHs), which have a fatality rate of 50%. Many methods of detecting IAs have already been identified, such as magnetic resonance imaging (MRIs), CT scans (computed tomographies), computed tomography angiographies (CTAs), lumbar punctures, and classical angiographies. However, since patients may be asymptomatic or their symptoms overlap with those of other conditions, IAs are not regularly tested for, and most discoveries are accidental. In a majority of the cases, IAs are not even identified until they have ruptured, when it is too late for treatment. Clearly, a reliable form of early detection is crucial to effective treatment and decreasing patient mortality rates. Biomarkers can prove to be reliable indicators of the presence of an IA, its progression, or in some cases, the probability of developing an IA. Identification of some biomarkers can even lead to novel preventative and treatment methods for IAs. This paper will explore IA pathophysiology, identify a variety of biomarkers for IAs (including those suitable for blood-based tests), then explore some possible non-surgical treatments for the same.

Shivika was born in Hayward, California in 2008. She is currently a freshman at American High School. Shivika is interested in Biology and more specifically genetics, and plans to study it for her undergraduate and master's degrees. She also has a passion for community service, volunteering, and outreach, and is part of a variety of initiatives that work on the same. She is the Director of Curriculum Development and Organization Partnerships at EJ Grassroots, an organization dedicated to environmental justice education and solutions.

2. Causes of IAs

This section discusses the causes of IAs. Specifically, it focuses on inherited risk factors, risk factors developed over time, and lifestyle choices/patterns that make one more susceptible to developing an IA. It also describes the symptoms of an unruptured IA, and what happens when an IA ruptures.

2.1 Causes of IAs

Intracranial aneurysms (IAs) are caused by a variety of different factors. Some include genetic diseases (e.g. - autosomal dominant polycystic kidney disease, Ehlers-Danlos Syndrome), trauma or head injury, infection, tumors, atherosclerosis, female sex, high blood pressure, and use of cigarettes and narcotics. Also, if someone has a first-degree relative who has had an aneurysm, the risk of developing an IA becomes almost as high as 10%. Symptoms of an unruptured IA include dilated pupils, headaches (which seem similar to migraines), pain near the eye, double vision, blurred vision, and sensations of numbness or weakness on a certain side of the face. Symptoms of unruptured IAs typically overlap with those of other conditions, so the possibility of an IA is overlooked. Once detected, aneurysms are treated either by surgical clipping or endovascular coiling, both of which are high-risk procedures. The problem arises, however, when IAs go undetected. Symptoms of ruptured IAs are far more severe than those of unruptured IAs. Extreme photophobia, thunderclap headaches, nausea, vomiting, stiffness in the neck, and loss of consciousness characterize a ruptured aneurysm. IA rupture can even lead to a fatal subarachnoid hemorrhage (SAH) - a condition characterized by its 50.3% mortality rate.

3. Pathophysiological Factors for Aneurysm Formation

This section discusses the pathophysiology of IAs, and biomarkers associated with the pathophysiological development of aneurysms.

3.1 Pathophysiology

Since treatment is invasive and expensive, there has been a shift toward understanding the pathophysiology of IAs in order to determine which ones must be treated first (based on rupture risk). IA occurrence may have to do with processes that occur during embryonic development. The embryonic neural crest cell population generates smooth muscle cells that line intracranial arteries. If neural crest cells malfunction or are not positioned correctly, all vessels from that common embryonic origin will most probably develop aneurysms. On a physiological scale, intracranial aneurysms are caused by damage to the endothelial cells lining the artery wall. This damage/weakening may be caused by defects and degeneration of the endothelium, extracellular matrix, hemodynamic stress, and inflammation.

Pathophysiological factors for endothelial damage include endothelial cell apoptosis, pro-inflammatory endothelial cells, and breakdown of the endothelial barrier (Jung, 2018). Cell apoptosis

and degeneration, breakdown of collagen, damage to smooth muscle cells, the extracellular matrix, and internal elastic lamina, may be caused by a variety of factors including pro-inflammatory cells, apoptosis, breakdown of collagen, and inflammation caused by cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), cyclooxygenase (COX), matrix metalloproteinases (MMPs), etc.

The extracellular matrix (ECM) is the non-cellular part of all tissues/organs. It is a network of proteins and carbohydrates that surrounds cells (in multicellular organisms), providing structure and regulating different cell processes. Damage to the extracellular matrix has been shown to lead to vascular wall degeneration, thus fueling the development of an aneurysm. Matrix metalloproteinases (MMPs) lead to extracellular matrix degeneration, damaging elastin and collagen (both of which are ECMs). MMPs are enzymes which are responsible for tissue remodeling. Without sufficient counterbalance through the presence of metalloproteinase inhibitors, MMPs can cause tissue degeneration instead. In blood vessels, if there are too many MMPs, the vessel wall will be damaged, which increases susceptibility to aneurysms. Furthermore, a copper deficiency during embryogenesis can lead to abnormal blood vessels. These abnormalities can result in IA development during adulthood. However, copper deficiencies do not arise only in embryogenesis - they may occur in infancy as well. Feeding an infant cow's milk or formula without sufficient copper can lead to the same vascular abnormalities that cause aneurysms.

Another factor that plays a part in development of IAs is hemodynamics (the way blood flows through blood vessels). One of the most important factors in hemodynamics and IA prevalence is high wall shear stress (WSS). High WSS is the force that flowing blood puts on the arterial walls, thus damaging the inner lining of the walls. High wall shear stress leads to endothelial damage, medial damage, and smooth muscle cell degeneration. It induces the release of MMP-2 and MMP-9, which further degrade the extracellular matrix. WSS is particularly high at arterial junctions and bifurcations, so these areas become aneurysm prone. Poor lifestyle choices, such as smoking, result in elevated wall shear stress. The nicotine present in cigarettes causes vasoconstriction (constriction of blood vessels), and increases blood volume and viscosity. The resultant high blood pressure can lead to formation of an aneurysm.

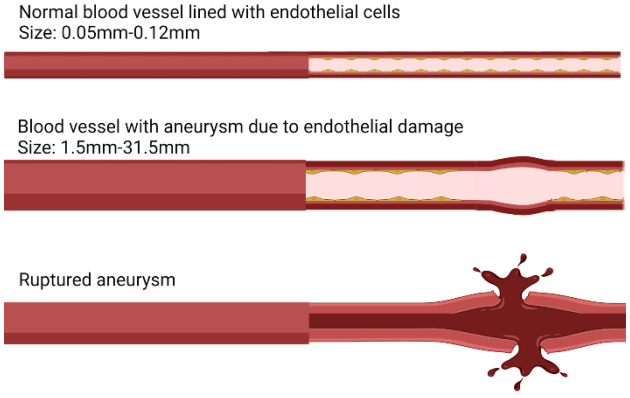

Figure 1. Blood Vessel Changes During Different Stages of IA Development. Figure 1 shows the progression of an aneurysm in an intracranial artery and the effects progression has on the same. The blood vessel is enlarged (from its normal size of 0.05-1.2mm to 1.5-31.5mm), and the vessel wall is damaged (as shown by the lack of endothelium surrounding the aneurysm area in the diagram of the second artery). This combined pressure and weakening leads to rupture of the aneurysm wall. Created and Copyrighted by Shivika Srivastava using Biorender.com.

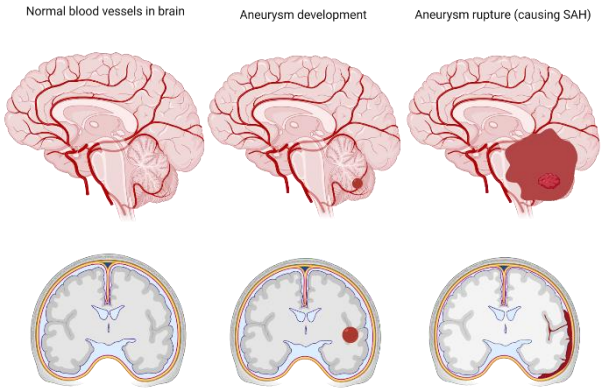

Figure 2. Changes in the Brain During Different Stages of IA Development. Figure 2 shows the progression of an aneurysm and its effect on the interior of the brain. Two perspectives are shown here - a hemisphere of the brain, and a coronary slice. Created and Copyrighted by Shivika Srivastava using Biorender.com.

4. Role of Biomarkers in IA Detection

This section discusses the definition and basics of biomarkers. Specifically, it explains why biomarkers are an effective technique in detecting IAs.

4.1 Role of Biomarkers in IA Detection

Currently, intracranial aneurysms are hard to diagnose, since they require expensive detection methods such as MRIs or CTAs. Furthermore, symptoms of unruptured IAs are very similar to those of the common everyday ailments. Most discoveries of IAs are made accidentally when screening for another disease, or when they rupture and it is too late for treatment. Hence, a reliable, quick, and simple way is required for IAs to be detected and then treated. Biomarkers are measurable characteristics that serve as indications of "normal biological processes, pathogenic processes or responses to an exposure or intervention" (Califf, 2018). Biomarkers allow for simple, effective, and easy-to-obtain characteristics to assess a patient's health. Biomarker technology (especially for IAs) is being studied rigorously, and research is being developed that show different biomarkers for these silent killers. Clearly, biomarkers would be the best way to detect IAs before they rupture.

5. Biomarkers for IAs

This section discusses a variety of biomarkers for IA detection. Specifically, it describes different molecules, genetic biomarkers, biomarkers for endothelial dysfunction, and oxidative stress.

5.1 Tumor Necrosis Factor-α

Tumor necrosis factor-α (TNF-α) has been proven to be an effective biomarker for IA detection. TNF-α "is a proinflammatory cytokine with an important role in the pathogenesis of several diseases. Its encoding gene is located in the short arm of chromosome 6 in the major histocompatibility complex class III region" (Torres et al., 2020). TNF-α has been found in high levels in the walls of aneurysmal blood vessels, which raises the possibility of TNFR1 (tumor necrosis factor receptor 1) being an effective biomarker to detect IAs. When testing for venous and arterial TNF-α levels using endovascular catheters in patients, Torres et al. showed that there was 455 pg/mL more TNFR1 in

aneurysm patients than in healthy patients (p=0.074). Patients with more than 1658 pg/mL of TNFR1 had a 12.03 odds ratio of having an aneurysm (a very high probability of having an IA). TNFR1/TNF-α is a reliable biomarker for the presence of an intracranial aneurysm.

5.2 Genetic Biomarkers

This section discusses genetic loci (gene locations on chromosomes) and genetic biomarkers which can be found in blood.

5.2.1 Genetic Loci

Many genetic loci (locations of genes on chromosomes) have been identified to encode for the presence of IAs. Studies have worked on identifying these loci (using linkage and candidate gene studies based on microsatellite markers and single-nucleotide polymorphisms [SNPs]) and determining their LODs (logarithm of the odds; an estimate of if two loci are near enough to be linked or related; LODs that are more than 3 are considered significant). These results have shown that loci for IAs depend on ethnicity. One study showed that locus 1p34.3-36.13 was significant for North Americans (LOD=4.2) and Dutch (LOD=3.18), locus 2p13 was significant for Dutch (LOD=3.55), locus 4q32 was significant for familial IAs (FIAs) (LOD=3.5), 5p12.2-14.3 was significant for French-Canadians (LOD=3.57), 7q11 was significant for Japanese sibling pairs (LOD=3.22), 8p22.2 was significant for Koreans (LOD=3.61), 11q24-25 was significant for North Americans (LOD=4.3), 12p12.3 was significant for familial IA studies (LOD=3.1), 13q14-21 was significant for French-Canadians (LOD=4.56), 14q23-21 was significant for North Americans (LOD=3.0), 17cen was significant for Japanese (LOD=3.0), 19q13 was significant for both Finnish sibling pairs (LOD=3.16) and Japanese (LOD=4.1), and Xp22 was significant for Dutch (LOD=4.54). The results are summarized in the table below.

Chromosome	Arm (p[2], q[3])	Area	Full locus	Ethnicity	Logarithm (LOD[1])
1	p	34.3-36.13	1p34.3-16.13	North American	4.2
				Dutch	3.18
2	p	13	2p13	Dutch	3.55
4	q	32	4q32	FIA[4]	3.5
5	p	15.2-14.3	5p15.2-14.3	French-Canadian	3.57
7	q	11	7q11	Japanese sibling pairs	3.22
8	p	22.2	8p22.2	Korean	3.61
11	q	24-25	11q24-25	North American	4.3
12	p	12.3	12p12.3	FIA[4]	3.1
13	q	14-21	13q14-21	French-Canadian	4.56
14	q	23-21	14q23-21	North American	3.0
17	cen[5]		17cen	Japanese	3.0
19	q	13	19q13	Finish sibling pairs	3.16
				Japanese	4.1
X	p	22	Xp22	Dutch	4.54

1 - Logarithm of the Odds (LODs) value greater than three is considered significant.
2 - p is petit, indicates short arm of chromosome
3 - q is queue, indicates long arm of chromosome
4 - FIA indicates familial intracranial aneurysm study
5 - cen indicates closeness to centromere of gene

Table 1. Genetic Loci Significantly Associated with the Presence of Intracranial Aneurysms. Table 1 shows different genetic loci associated with brain aneurysms, the loci's location on the chromosome, which ethnicity the loci are associated with, and the LOD that shows the probability of an aneurysm. Created and Copyrighted by Shivika Srivastava.

5.2.2 Blood-based Genetic Detection

One strong candidate for detection of IAs would be blood-based genetic markers. Research has found 18 genes obtained from whole blood transcriptomes (finding the complete set of RNA in unaltered/untreated blood of humans) that mark high probability of the presence of IAs. Poppenberg et al. used blood samples, RNA sequencing, and LASSO (least absolute shrinkage and selection operator) regression to determine the appropriate gene markers for IAs. These are ATF3 (p <0.001), CBWD6 (p=0.001), CCDC85B (p=0.001, CCR8 (p<0.001), CHMP4B (p=0.007), CLEC4F (p=0.002), CXCL10 (p<0.001), FN1 (p=0.06), MT2A (p<0.001). MZT2B (p=0.008), PCSK1N (p=0.018), PIM3 (p<0.001). SLC37A3 (p=0.032), ST6GALNAC1 (p<0.001), TCN2 (p<0.001), TIFAB (p=0.007), TNFRSF4 (p<0.001), and UFSP1 (p=0.003). As is evident, the p-values, which indicate the probability of an event occurring (while assuming the null hypothesis is true), are very low. This indicates a strong positive correlation between the gene marker and presence of IAs. The results are summarized in the table below.

Gene	P-value*
ATF3	<0.001
CBWD6	0.001
CCDC85B	0.001
CCR8	<0.001
CHMP4B	0.007
CLEC4F	0.002
CXCL10	<0.001
FN1	0.06
MT2A	<0.001
MZT2B	0.008
PCSK1N	0.018
PIM3	<0.001
SLC37A3	0.032
ST6GALNAC1	<0.001
TCN2	<0.001
TIFAB	0.007
TNFRSF4	<0.001
UFSP1	0.003

Table 2. Genes Strongly Correlated with the Presence of IAs. Table 2 shows 18 different genes correlated with IAs, and their significance. Lower p-values indicate higher significance. Created and Copyrighted by Shivika Srivastava.

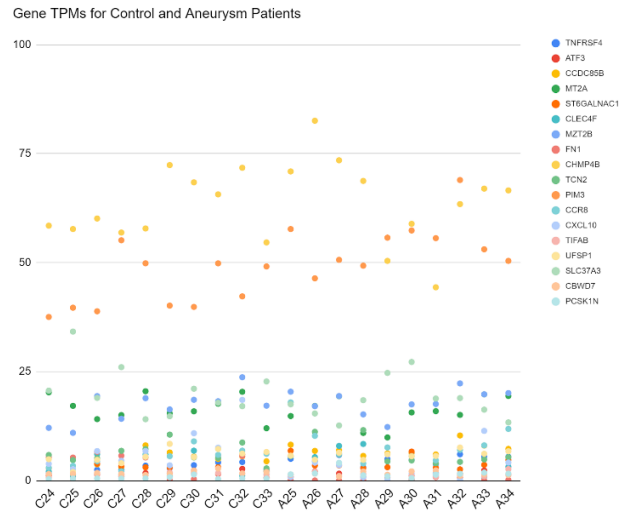

Figure 3. Gene Transcripts Per Million (TPMs) for Control Group and Patients with Aneurysms. As seen in figure 3, gene TPMs, which indicate levels of gene expression, are shown. "C" indicates patients in the control group, and "A" indicates patients in the aneurysm group. The numbers next to either letter are for patient identification. Created and Copyrighted by Shivika Srivastava.

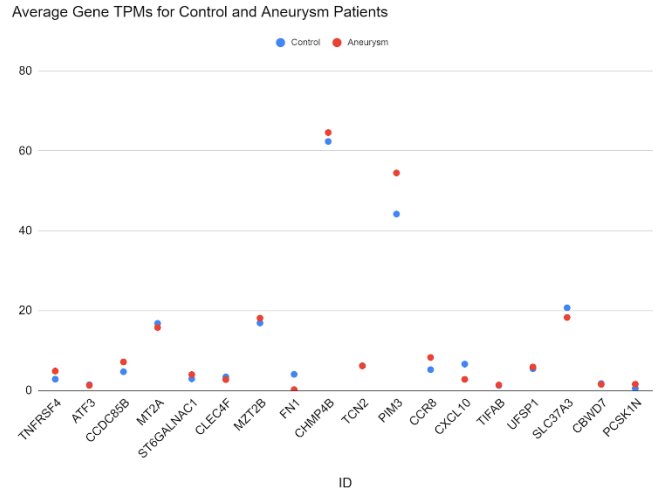

Figure 4. Average Gene Transcripts Per Million (TPMs) for Control and Aneurysm Patients. Figure 4 shows average gene TPMs of different genes in control patients and aneurysm patients. Relative gene TPMs for aneurysms and non-aneurysmal patients seemed to vary on the type of gene that was being expressed. Created and Copyrighted by Shivika Srivastava.

The genes mentioned above have also been shown to be related to inflammation, ECM degradation, release of MMPs and TNF-α, all of which are important factors in the pathogenesis of IAs. Hence, it can be concluded that these 18 genes are reliable biomarkers for detection of IAs.

The 18 genes above have been proven to have a correlation with IA pathogenesis on a physiological level. They have been proven to cause inflammation and arterial wall degradation. Of these eighteen, four genes (TNFRSF4, TIFAB, MT2A, PIM3) have been associated with NF-κB. NF-κB is a signaling pathway which causes inflammation in the arterial wall and has been shown to lead to development of IAs. Furthermore, NF-κB increases MMP-9, which has been proven to be an important factor in IA pathogenesis, and MCP-1, which brings macrophages to the arterial wall - which is another sign of an IA. TNFRSF4 is part of the TNF (tumor necrosis factor) receptor superfamily, and is involved in the activation of NF-κB (thus leading to arterial degradation). Some of these gene markers have been shown to lead to extracellular matrix degradation, which, as discussed above, is a key characteristic of intracranial aneurysms. CCR8 and CXCL10 relate to inflammatory response through the upregulation of M1 macrophages, which are proinflammatory and cause damage to the vessel wall.

5.3 Interleukin-6 (IL-6)

Another reliable indication of UIAs (unruptured intracranial aneurysms) is the interleukin-6 (IL-6) quotient. IL-6 is a proinflammatory cytokine. IL-6 can be found in both cerebrospinal fluid (CSF; the fluid that flows in the subarachnoid space, i.e. - in/around the brain and spinal cord) and in serum (the liquid portion of blood). The IL-6 quotient, which is the ratio of CSF IL-6 concentration to serum IL-6 concentration, can be used to determine presence of a UIA. Chrzanowski et al. used in vitro diagnostic immunoassays to determine these IL-6 levels. The paper describes that the median IL-6 quotient in patients with UIA (1.78) was greater than the median quotient in those without (the control group; 0.87). The results are shown in the graph below.

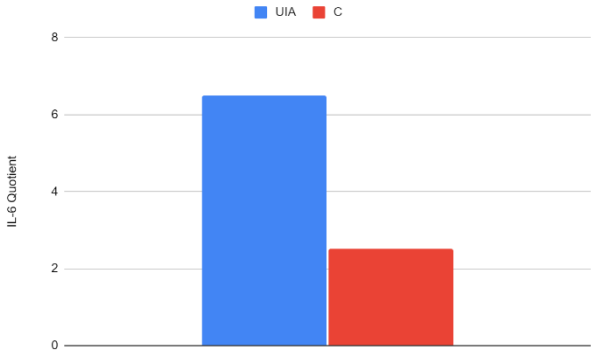

Figure 5. IL-6 Quotient Results in Patients with IAs and Patients in the Control Group. As seen in Figure 5, data was collected from a study that calculated the IL-6 quotient in patients with UIAs and those without. Created and Copyrighted by Shivika Srivastava.

The median IL-6 score for the UIA group is significantly higher than that of the control group, showing that patients with UIAs will have higher levels of the IL-6 quotient. Thus, IL-6 can serve as a potential biomarker for UIAs. Furthermore, the concentration of CSF IL-6 showed a strong positive correlation with the number of IAs. Lower CSF IL-6 concentrations were proven to correlate with multiple aneurysms (5.08 pg/mL for multiple UIAs vs. 4.14 pg/mL for a single UIA; p=0.0227). Additionally, lower CSF IL-6 concentrations and lower IL-6 quotients were shown to relate to larger sized aneurysms (≥5.3mm; CSF IL-6: 4.00 pg/mL for large size vs. 5.19 pg/mL for smaller size, p=0.0022; IL-6 quotient: 1.33 for large size vs. 2.51 for smaller size, p=0.0183). However, there was no significant correlation between either of these parameters and IA progression. The results are summarized in the following table.

Characteristic		CSF IL-6 concentration (pg/mL)	IL-6 quotient
Aneurysm Number	Multiple	5.08	No significant correlation
	Single	4.14	
Size of Aneurysm	≥5.3mm (Large)	4.00	1.33
	<5.3mm (Small)	5.19	2.51

Table 3. CSF IL-6 and IL-6 Quotient Corresponding with Different IA Characteristics. Table 3 summarizes the results of CSF IL-6 and IL-6 quotient measurement results. The results show that the number of aneurysms has no significant correlation with the number of aneurysms, although higher CSF IL-6 levels have been shown to correspond with a larger number of aneurysms. However, lower levels of CSF IL-6 and in the IL-6 quotient have been shown to correlate with a larger aneurysm size. Created and Copyrighted by Shivika Srivastava.

5.4 CRP as a biomarker for presence of fusiform aneurysms

Fusiform aneurysms (FAs) are the most challenging types of IAs to detect and treat, due to their morphology and location in the brain. They can be up to 1.5 times a healthy artery's size. FAs can also lead to SAH's, so it is crucial to identify these IAs as well. C-reactive protein (CRP) is a protein that has been shown to contribute to oxidative stress. Telles et al. studied 35 patients with FAs (and 70 patients without) using medical records from 2010-2019. Of those given FA cases, 13 were ruptured FAs, and 22 were unruptured. Serum CRP levels were measured in mg/dL as an indication of CRP levels. Patients with FAs were shown to have higher median levels of CRP than those without FAs (0.61 mg/dL versus 0.29 mg/dL), with a strong positive correlation between serum CRP levels and FA presence ($p<0.01$).

5.5 Neutrophil Extracellular Traps (NETs)

Neutrophil extracellular traps (NETs) are another reliable biomarker to determine presence of IAs. Reduction of NETs can even prevent rupture risk. Neutrophil extracellular traps are "networks of unraveled chromatin decorated with histones and antimicrobial… enzymes" (Korai et al., 2022). Previous research has proven the pathophysiological role of NETs in forming IAs, by damaging the extracellular matrix, causing tissue inflammation, and more. Higher levels of NETs have been found in patients with IAs, as Torres et al. found when taking blood samples and have been shown to increase rupture risk.

Inhibiting NET production/release may reduce IA rupture risk. NETs are formed by the enzyme peptidyl arginine deiminase 4 (PAD4). Different methods were used to reduce PAD4 in mouse models, in an effort to reduce rupture risk of an aneurysm. There was no difference in rate of aneurysm formation. However, global knockout (complete elimination) of PAD4 in mouse models was shown to decrease rupture risk from 82% to 25%. Granulocyte-specific knockout of PAD4 (temporarily switching off the PAD4 gene in granulocytes) reduced rupture risk from 87% to 36%. Another way NETs were reduced was using the pharmacological blockade of chlorine-amidine (Cl-amidine). Doing so would inhibit PAD4, once again reducing rupture risk from 93% to 36%. Lastly, NETs were resolved using the enzyme DNase, which controls inflammation and tissue damage. Resolution of an NET means the clearing out of NETs after they finish their work in the body. This method reduced rupture risk from 81% to 33%. The results of this experiment are summarized in the table below.

		Global Knockout of PAD4	Granulocyte-Specific Knockout of PAD4	Pharmacological blockade of NET formation using Cl-amidine	Resolution of NETs by DNase
Rate of aneurysm formation	Control	No difference			
	Experimental	No difference			
Rate of aneurysm rupture	Control	82%	87%	93%	81%
	Experimental	25%	36%	36%	33%

Table 5. Reduced IA Rupture Risk using Different Methods of Decreasing Number of NETs. Table 5 shows the reduced risk of IA rupture after different methods of mitigating NET formation were used (global knockout of PAD4, granulocyte-specific knockout of PAD4, pharmacological blockade of NET formation using Cl-amidine, and resolution of NETs by DNase, respectively). Overall, global knockout of PAD4 seemed to achieve the lowest rupture risk, and thus, it may be considered as a possible treatment method for UIAs in the future. Created and Copyrighted by Shivika Srivastava.

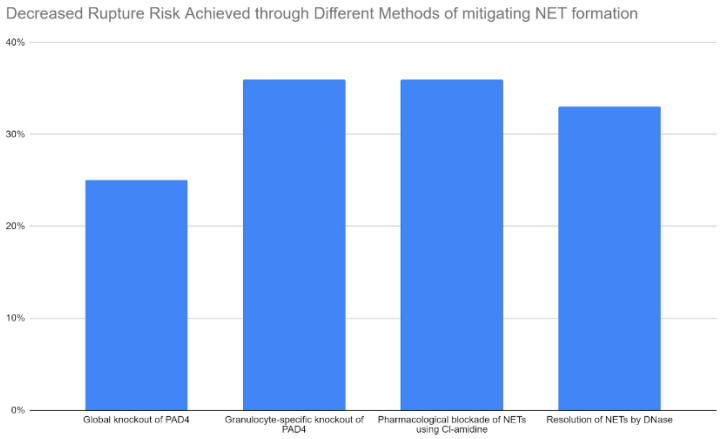

Figure 5. Decreased Rupture Risk Achieved Through Different Methods of Mitigating NET Formation. Figure 5 shows the IA rupture risk after NET migration methods were used. The most effective method was global knockout of PAD4, followed by resolution of NETs by DNase, and then granulocyte-specific knockout and pharmacological blockade, both of which achieved the same decreased risk. Created and Copyrighted by Shivika Srivastava.

Although these four methods did not reduce the rate of IA formation, they reduced the rate of rupture significantly. Future non-surgical treatment methods for IAs could include pharmacological blockade using Cl-amidine, resolution of NETs by DNase, and global and granulocyte-specific knockout of PAD4.

5.6 Endothelial Dysfunction

As discussed in the pathophysiology portion of this paper, endothelial defect/dysfunction is one of the main physiological causes leading to IA development and rupture. Endothelial functions include NO (nitric oxide; maintains structural integrity of vessel wall) production, vasodilation (expansion of blood vessels), inflammation and thrombolytic activity regulation, and general endothelial health maintenance. When endothelium doesn't function properly, these operations are not carried out properly. This results in weakening of the vessel wall (which is a risk factor for IAs). Thus, a biomarker for assessing endothelial dysfunction is crucial in detection of IAs.

MMP-9 has been shown to lead to inflammatory cells migrating into the arterial walls. It is also known that if MMPs do not have enough inhibitors, the ECM and artery wall will be damaged. Tissue inhibitors of matrix metalloproteinases (TIMPs) must be present in the correct ratio to balance out the inflammatory and damaging properties of MMPs (released by endothelial cells), as measured by the flow-dependent dilation and Laser Doppler. Thus, the MMP/TIMP ratio can be an effective biomarker for assessing endothelial dysfunction.

Furthermore, the angiopoietin-related protein 2 (ANGPTL2), has been shown to be a driving factor for cardiovascular diseases (CVDs; e.g. - atherosclerosis). ANGPTL2 levels were measured in aortic tissue (from apolipoprotein E). ANGPTL2 promotes NF-κB, a proinflammatory pathway. Increased levels of NF-κB have been shown to lead to increased risk of developing an IA.

In addition, endoglin (also known as CD105 or TGF-β receptor III) has been shown to lead to endothelial dysfunction and the

migration of inflammatory cells into blood vessels. Endoglin levels were reduced using atorvastatin treatment, which showed that a soluble form of endoglin could serve as a potential biomarker for CVDs. Both endothelial dysfunction and inflammatory cell migration (which are caused by high endoglin levels) can contribute to IA formation.

Homocysteine is an amino acid that increases risk of stroke. Serum homocysteine levels were measured to make this association. “Thus, Wald et al. [27] suggest that lowering homocysteine concentrations by 3 μmol/L from current levels would reduce the risk of… stroke by 24%” (Zhang, 2022). Research shows that homocysteine activity has led to damage of blood vessels (by release of angiotensin II) and endothelial dysfunction. This correlation has been proven in mouse models as well.

A new and emerging biomarker for vascular inflammation is circulating endothelial cell microparticles. Circulating endothelial cells are parts of endothelium that have detached from full endothelium. They are formed due to over-activation of endothelium or too much endothelial apoptosis (programmed cell death) or necrosis (unprogrammed/uncontrolled cell death). Endothelial microparticles are membrane-bound vesicles that are released from endothelial cells. Examples of endothelial microparticles are E-selectin and annexin-V+. Elevated levels of annexin-V+ (a protein involved in apoptosis) are a reliable indication of endothelial dysfunction correlated with vascular inflammation.

5.7 Oxidative Stress

Oxidative stress is a major factor in the formation of IAs. Oxidative stress is defined as “injury due to increased production and/or decreased removal of free radicals” (Starke et al., 2013). Free radicals are molecules that have unpaired electrons. The human body produces Reactive Oxygen Species (ROS), which, when produced in excess, results in oxidative stress. Oxidative stress has been shown to lead to vascular inflammation, endothelial injury, apoptosis, activation of MMPs, and recruitment of inflammatory cells - all of which consequently lead to formation of aneurysms. Free radicals augment hemodynamic stress as well. Starke et al. shows that many biomarkers which contribute to oxidative stress overlap with the biomarkers for intracranial aneurysms. Pathways of oxidative stress are biological, chemical, and biochemical processes that result in the excessive production of ROS, or more ROS than the body’s antioxidant system can counter. Different pathways have different biomarkers, which, as stated above, overlap with the biomarkers for IAs. Biomarkers for the atherosclerosis pathway include: NADPH, oxidized LDL, myeloperoxidase, COX, LIPOX, NOS, IL-1β, TNF-α, IL-6, MCP-1, and other molecules. Biomarkers for endothelial function, hemodynamic stress, and hypertension include: NADPH, NF-κB, COX, NOS, VCAM, IL-8, mPGEs-1, and angiotensin II. Biomarkers for vascular smooth muscle cell (SMC) inflammation and matrix remodeling modulation, as well as apoptotic cell death include: NADPH, NOS, TNF-α, NF-κB, KLF4, VCAM, MMP, and MCP. Lastly, the biomarkers for chronic inflammation, vascular wall remodeling, and apoptotic cell death include: NF-κB, IL-1β, IL-8, IL-12, VCAM, MMP, MCP-1, NOS, and NADPH. The results and overlap is summarized in the table below. Some overlapping biomarkers are beyond the scope of what has been discussed in this paper.

Pathway	Biomarker (Oxidative Stress)	Biomarker Overlap (with biomarkers for IAs)
Atherosclerosis/arterioscleros-is	NADPH Oxidized LDL Myeloperoxidase COX LIPOX NOS IL-1β TNF-α IL-6 MCP-1 Other molecules	COX TNF-α IL-6
Endothelial function, hemodynamic stress, hypertension	NADPH NF-κB COX NOS VCAM IL-8 mPGEs-1 Angiotensin II	NF-κB COX
Vascular SMC inflammation, matrix remodeling modulation; apoptotic cell death	NADPH NOS TNF-α NF-κB KLF4 VCAM MMP MCP	TNF-α NF-κB MMP
Chronic inflammation, vascular wall remodeling, apoptotic cell death	NF-κB IL-1β IL-8 IL-12 VCAM MMP MCP-1 NOS NADPH	NF-κB MMP

Table 6. Biomarkers for Oxidative Stress Based on Pathway, and Overlap with IA Biomarkers. Table 6 shows different pathways for oxidative stress, their biomarkers, and which of those biomarkers have been proven to lead to IAs. The overlap between biomarkers for oxidative stress and those for intracranial aneurysms may provide a reliable set of biomarkers for IAs (since oxidative stress is one factor that causes IAs). Created and Copyrighted by Shivika Srivastava.

6. Clinical Factors for Intracranial Aneurysm Detection

This section discusses a variety of factors that can aid in the quick detection of an intracranial aneurysm.

6.1 Clinical Factors for Intracranial Aneurysm Detection

There are many clinical factors that can help in detection of IAs. Symptoms of IAs include pain behind the eye, vision changes, double vision, dilated pupils, headaches (which seem similar to migraines), and numbness or weakness on a certain side of the face. Although these symptoms overlap with many other conditions, clinicians should also test for IAs, in case there is a more serious underlying issue. Tests that are currently in use to identify IAs include lumbar punctures, CT scans, MRIs, CTAs, and classical angiographies. However, less expensive diagnostic techniques revolve around biomarkers. As discussed in this paper, the biomarkers that can be used to detect IAs include: TNF-α, IL-6, COX, MMPs, wall shear stress, genetic loci (1p34.4-16.13, 2p13, 4q32, 5p15.2-14.3, 7q11, 8p22.2, 11q24-25, 12p12.3, 13q14-21, 14q23-21, 17cen, 19q13, and Xp22), blood-based gene markers (ATF3, CBWD6, CCDC85B, CCR8, CHMP4B, CLEC4F, CXCL10, FN1, MT2A, MZT2B, PCSK1N, PIM3, SLC37A3, ST6GALNAC1, TCN2, TIFAB, TNFRSF4, and UFSP1), NF-κB, NETs, MMP-9, ANGPTL2, endoglin, and homocysteine. These biomarkers, some of which may be derived

from the blood or from a tissue sample, are accurate, reliable, simple ways to detect IAs. All of the above discussed are factors clinicians can use to detect IAs.

7. Nonsurgical Treatments to Reduce Rupture Risk

This section discusses nonsurgical, low-risk treatments for IAs that will reduce their risk of rupture.

7.1 Nonsurgical Treatments to Reduce Rupture Risk

Along with NETs, there are many nonsurgical treatments that can be done to reduce aneurysm rupture risk. Curcumin is a phenol (chemical compound; carbolic acid) found in the common household spice, turmeric. Previous research has used forearm blood flow as a measure of possibility of IA development. 12 weeks of a curcumin supplement was given to patients "In healthy middle-aged and older adults, 12 weeks of curcumin supplementation improves resistance artery endothelial function by increasing vascular nitric oxide bioavailability and reducing oxidative stress, while also improving conduit artery endothelial function" (Santos-Parker et al., 2017). This same course of supplement led to better endothelial function and less forearm blood flow (FBF). Less FBF led to less pressure on the artery walls, coupled with better response to more or less blood flow by the arteries. The results are summarized in the table below.

	Forearm blood flow	Artery dilation/Endothelial Function
Curcumin	P=0.02	5.7±0.4 36% increase P=0.001
Placebo	No effect; P>0.6	4.4±0.4 No change P=0.1

Table 7. Relation between Curcumin Supplement and Forearm Blood Flow, and Artery Dilation/Endothelial Function. Table 7 explains the difference in forearm blood flow (FBF) and artery dilation/endothelial function in patients given a curcumin and a placebo supplement. The group given the curcumin supplement showed a high correlation with decreased FBF, and a 36% increase in artery dilation/endothelial function. Created and Copyrighted by Shivika Srivastava.

Endothelial dysfunction has been defined as a lack of bioavailability of NO, and inability to perform proper vasodilation. NO is formed by endothelial nitric oxide synthase (eNOS) from L-arginine, a semi-essential amino acid. However, NO synthesis can only be performed if there are enough B vitamins available (such as B6 and B12). NO has been shown to lead to many CVDs. L-arginine and B vitamins have been proven to reverse endothelial damage and vascular degradation, and thus, could be used as a potential nonsurgical treatment for unruptured IAs. Menzel et al. studied supplements given to patients that contained a combination of L-arginine and B vitamins (B6, folic acid, B12), and their correlation with vascular health. "The primary efficacy analysis revealed superiority of the nutritional intervention over placebo ($p = 0.0349$) in reducing the deterioration of endothelial function. While in the active group ΔΔRHI increased (0.371 ± 0.122), almost no change could be detected in the placebo group (0.031 ± 0.100), thus demonstrating a significant improvement in vascular function in the intervention group. Moreover, the intervention reduced BP and homocysteine levels" (Menzel et al., 2018). Clearly, this treatment of L-arginine and B vitamins was effective in reducing the risk of IA rupture (by reducing the magnitude of causes of IA formation). Apart from supplements, one of the best ways to reduce IAs is to make certain lifestyle changes. Weight loss, cardiovascular exercise, and dietary changes have been shown to reverse endothelial damage.

8. Post-rupture Recovery Process

This section discusses the process that takes place after an aneurysm ruptures, and further treatment that must be pursued.

8.1 Post-rupture Recovery Process

After an aneurysm has ruptured, it requires immediate medical attention. Aneurysm rupture can lead to a subarachnoid hemorrhage. Most patients with ruptured IAs are admitted to an Intensive Care Unit (ICU) for 10-14 days post rupture. Doctors and medical professionals may perform surgical treatments such as surgical clipping or endovascular coiling (to prevent blood flow to the aneurysm), artery bypass surgery (to block the aneurysm), and superficial temporal artery to middle cerebral artery bypass (STA-MCA) bypass. Patients with ruptured intracranial aneurysms may undergo many complications as well. For example, a patient may undergo hydrocephalus. Hydrocephalus is a condition where an excess of CSF (cerebrospinal fluid) is built up in the brain, increasing pressure. In order to treat hydrocephalus, doctors may insert a catheter, either a lumbar or ventricular catheter, to drain the excess fluid in the brain. Another condition that may develop after 5-10 days post-rupture, is vasospasm. Vasospasm is a condition where excess blood flow causes constriction and expansion of cranial blood vessels. Vasospasm can be treated by injection in the artery, which is done by a catheter. If ruptured aneurysms are not treated in time, the risk of rebleeding is extremely high.

Conclusion

Intracranial aneurysms, or IAs, are very common, and they frequently lead to fatal subarachnoid hemorrhages. Although many methods of detecting IAs exist and are in use, they are expensive. Moreover, symptoms of IAs overlap with those of many everyday conditions, so IAs are often overlooked. Thus, many different biomarkers for unruptured IAs were studied in detail and evaluated. This paper discussed pathophysiology of IAs, biomarkers (genetic, endothelial dysfunction biomarkers, oxidative stress, etc), clinical factors for detection, post-recovery process, and nonsurgical ways to reduce risk of IA rupture. Biomarkers, post-recovery processes, and rupture-risk reduction methods could greatly benefit clinicians in proceeding to effectively diagnose, then use a non-invasive, inexpensive method to treat, IAs. Future studies should focus on evaluating the efficacy of each biomarker, and figuring out which is the best one for IA detection. Future research may also include introducing a blood-based IA detection blood panel that may be involved in routine checkups.

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Keywords: Intracranial Aneurysm • Rupture • Subarachnoid Hemorrhage • Biomarker • Non-surgical Treatments

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