

MOLECULAR AND CELLULAR PATHOGENESIS OF ENDOTHELIAL LINING IN ATRIAL FIBRILLATION

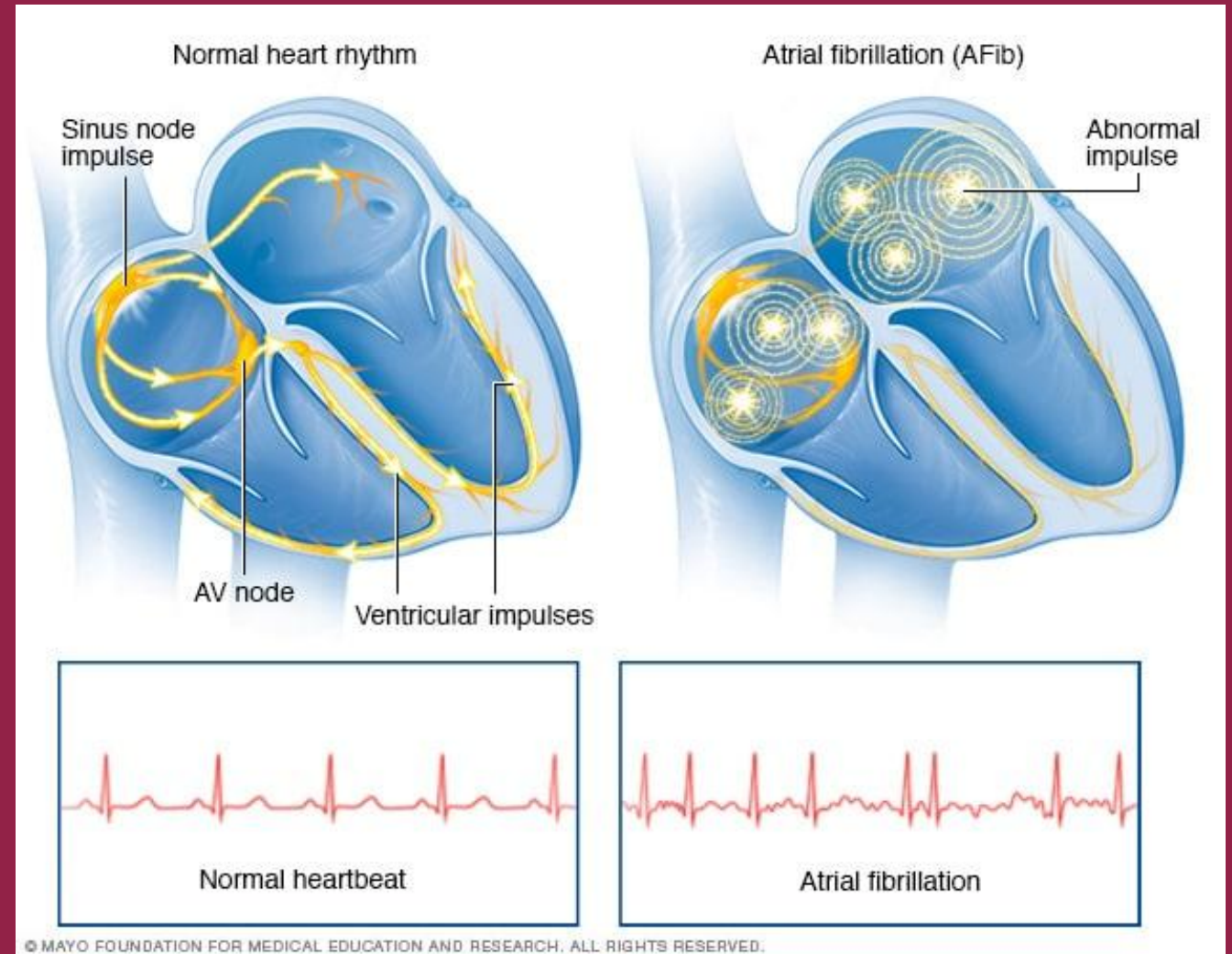


High School Scholars Day 2021

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BACKGROUND OF ATRIAL FIBRILLATION

- Atrial Fibrillation (AF) is a cardiac arrhythmia caused by a lack of coordination between the heart's upper and lower chambers. The miscoordination is caused by abnormal and chaotic impulses in the heart.
 - Can be caused by damage to heart's electrical system
 - May reduce the heart's efficiency at pumping blood
 - Can cause blood to pool in chambers
 - Blood flow to the rest of the body may become compromised
- Affects between 2.7-6.1 million in the United States today
 - Expected to rise to 12.1 million by 2050
- Symptoms include chest pain, fatigue, weakness, or shortness of breath



AIM/PURPOSE

- To establish the baseline blood samples for von Willebrand Factor (vWF), D-Dimer, and Interleukin-6 (IL-6) as they relate to Atrial Fibrillation
- To analyze the correlation between biomarkers of inflammation and thrombosis
- To assess the variance of vWF, D-Dimer, and IL-6 Levels between patients with AF compared with a control population

MATERIALS AND METHODS

- Blood Samples were Collected from Patients with confirmed diagnosis of AF
- The Normal Human Plasma (NHP) were Obtained from a Commercially Available Source (George King Biomedical Center)
- The Samples were Centrifuged to Make Platelet Poor Plasma
 - Stored in a frozen state at -80 degrees Celsius
- All Samples Were Then Analyzed for Certain Biomarkers Using Available Sandwich ELISA methods
- The Concentrations of the Specific Biomarkers Were Then Statistically Analyzed By Using Excel, GraphPad Prism, and IBM SPSS

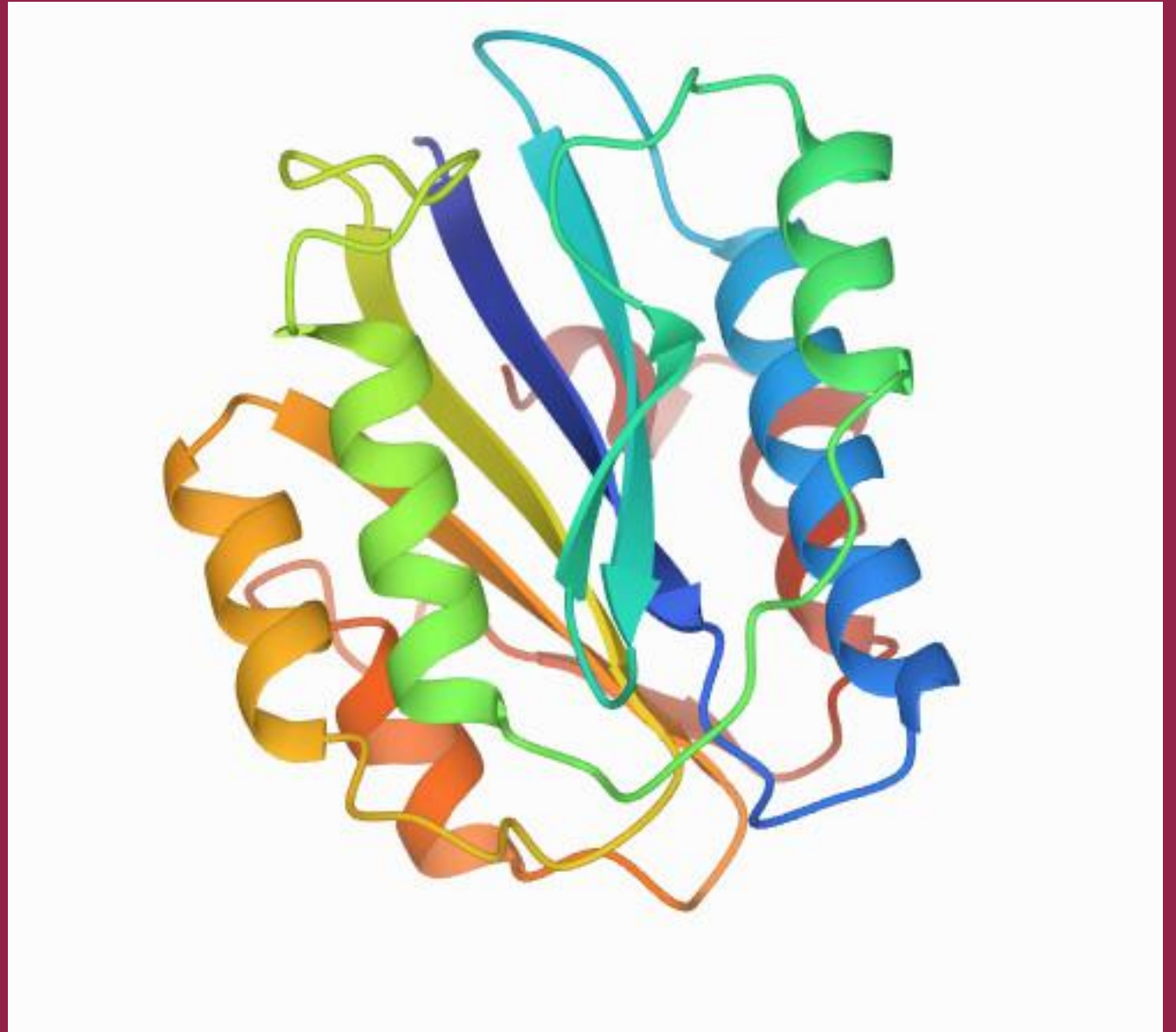
HYPOTHESIS

- Biomarkers of inflammation and thrombosis are present at higher levels in AF patients when compared to an NHP group.
- There will be a positive correlation between the biomarkers of inflammation and thrombosis

VON WILLEBRAND FACTOR (VWF) ANALYSIS

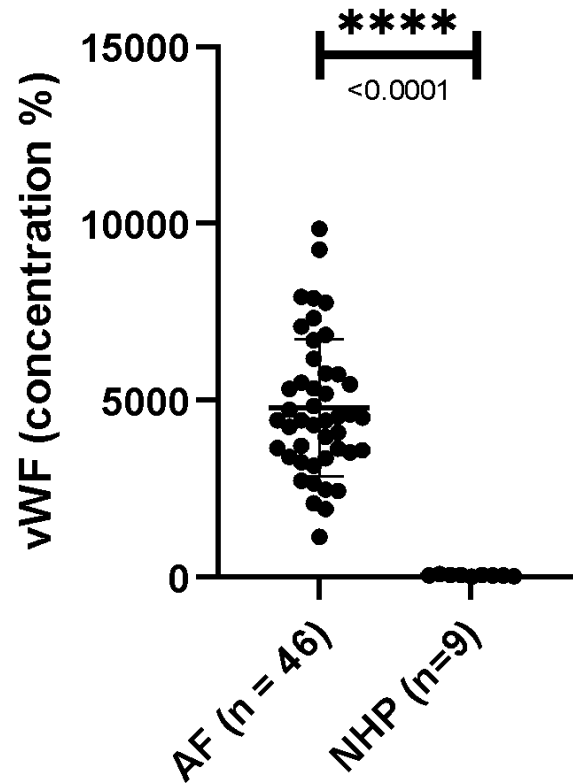
BACKGROUND OF VWF

- Structure
 - Composed of 2050-amino acid monomers
 - Rather large and classified as a multimeric plasma glycoprotein
- Function
 - Plays an essential role in blood clotting, as it binds to the walls of blood vessels which allows for the formation of the clot
 - Carries Factor 8 which is another protein that aids in blood clotting
- Location
 - Created in the endothelial cells and can be found in blood plasma
- Correlation to Atrial Fibrillation
 - vWF levels have been shown to correlate to endothelial damage, which is linked with Atrial Fibrillation



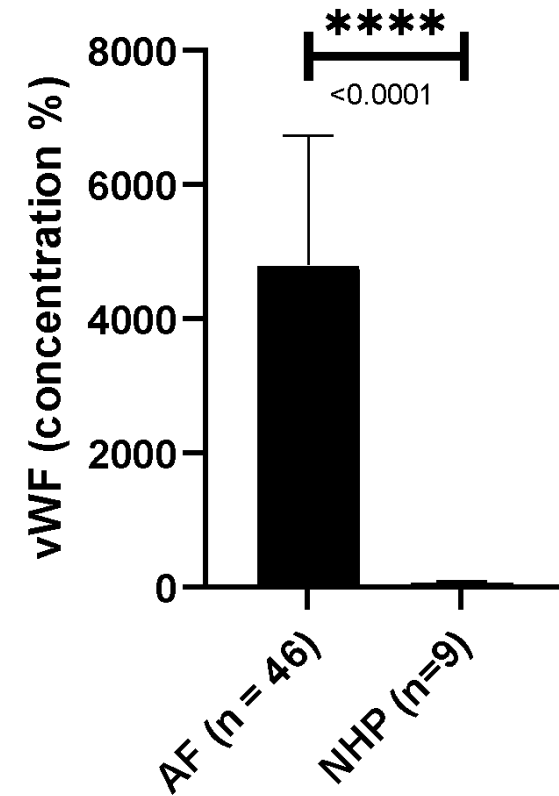
VWF RESULTS

von Willebrand Factor

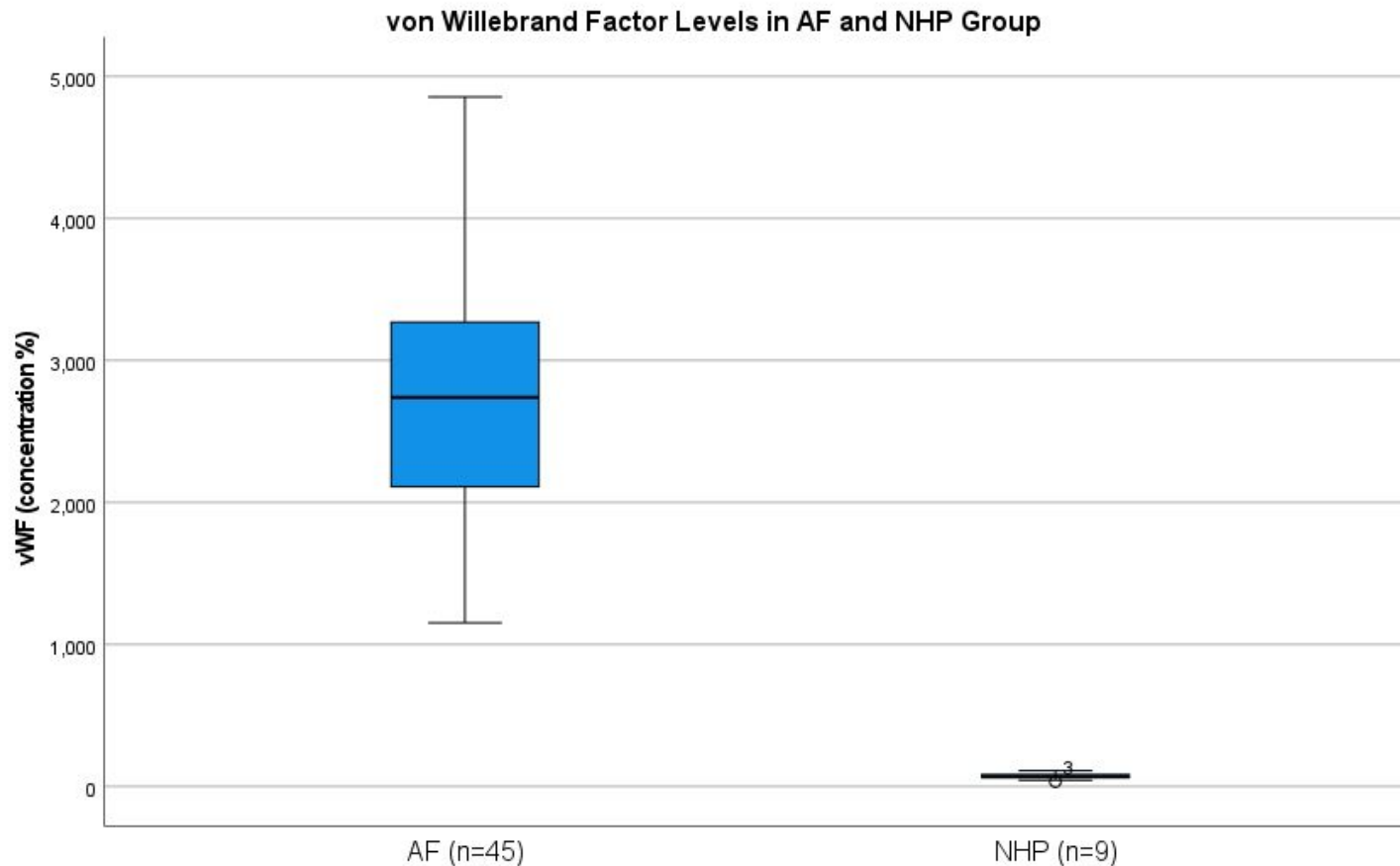


vWF Levels (concentration %)		
Sample		%
AF (n=45)	Average	4796.200
	SD	1919.586
	SEM	286.155
	Min	1153.000
	Max	9874.000
	Median	4456.000
	Mode	N/A
	IQR	2295.000
	25th Percentile	3479.000
	75th Percentile	5774.000
NHP (n=9)	Average	70.910
	SD	21.803
	SEM	7.268
	Min	33.960
	Max	111.500
	Median	67.640
	Mode	N/A
	IQR	29.690
25th Percentile	54.440	
75th Percentile	84.130	
% Change		6663.79

von Willebrand Factor



VWF QUARTILE ANALYSIS

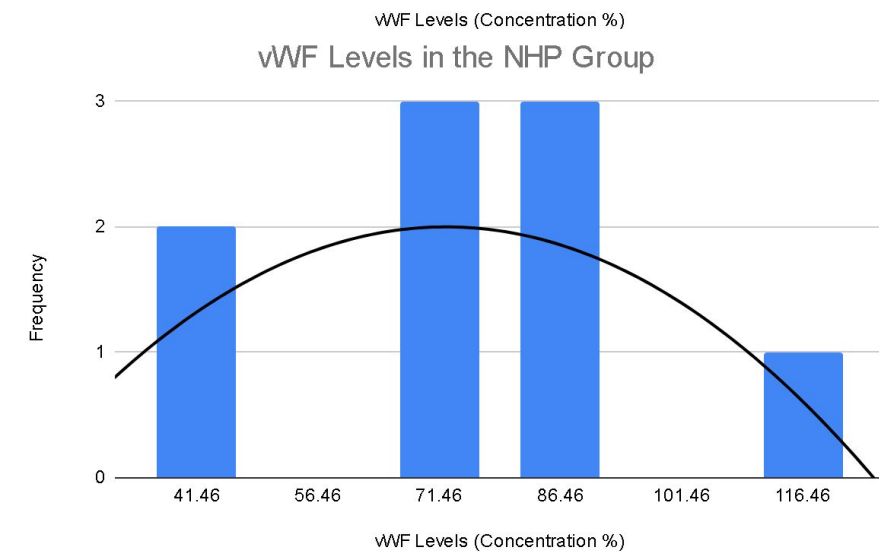
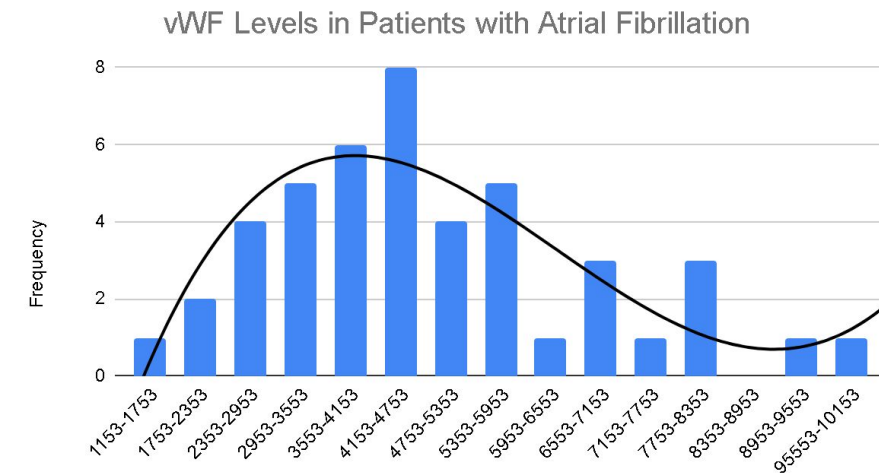


Sample		pg/mL
AF (n=45)	IQR	2295.000
	25th Percentile	3479.000
	50th Percentile	4456.000
	75th Percentile	5774.000

Sample		pg/mL
NHP (n=9)	IQR	29.690
	25th Percentile	54.440
	50th Percentile	67.640
	75th Percentile	84.130

VON WILLEBRAND FACTOR FREQUENCY DISTRIBUTION

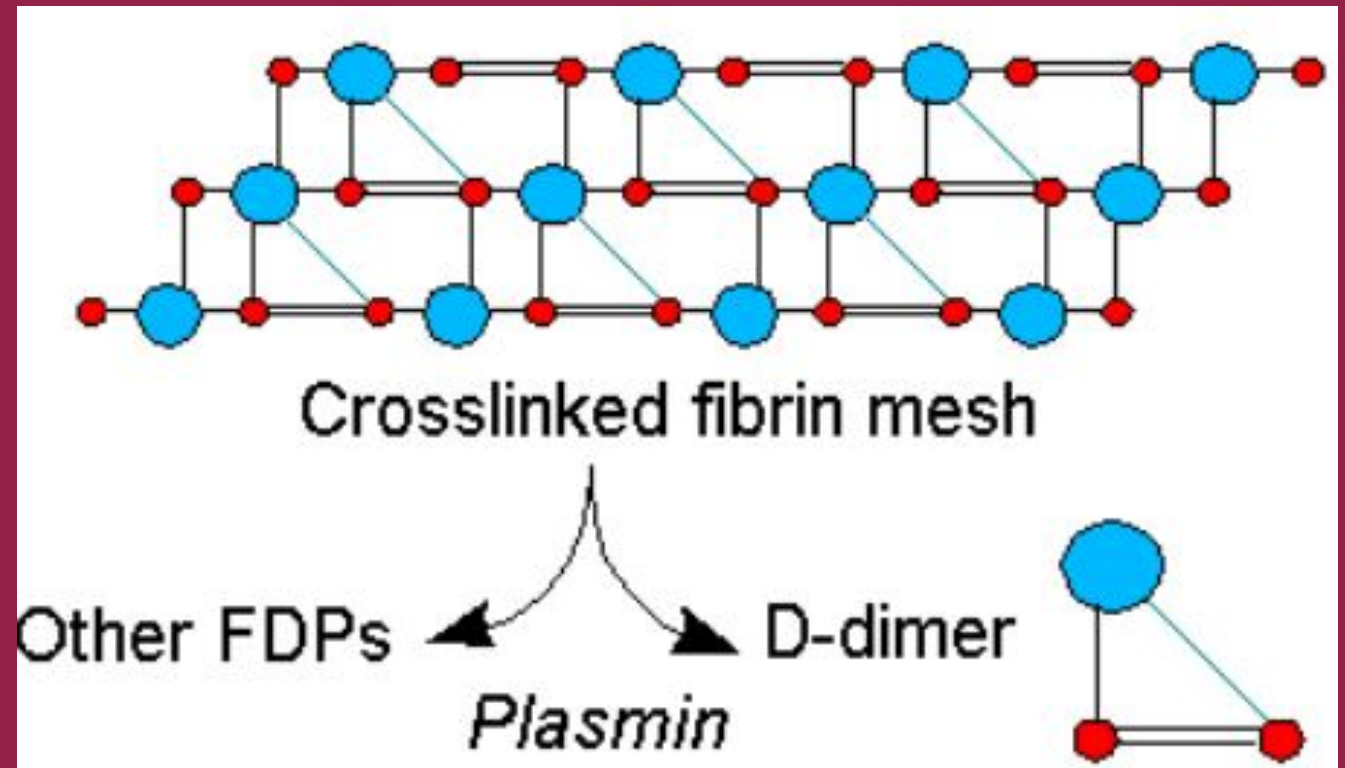
- AF Group
 - Slightly Right Skewed Data (Positive Skew)
 - Skewness: 0.378
- NHP Group
 - Symmetrical (Little to no Skew)
 - Skewness: 0.023
 - Data is Limited (n=9)



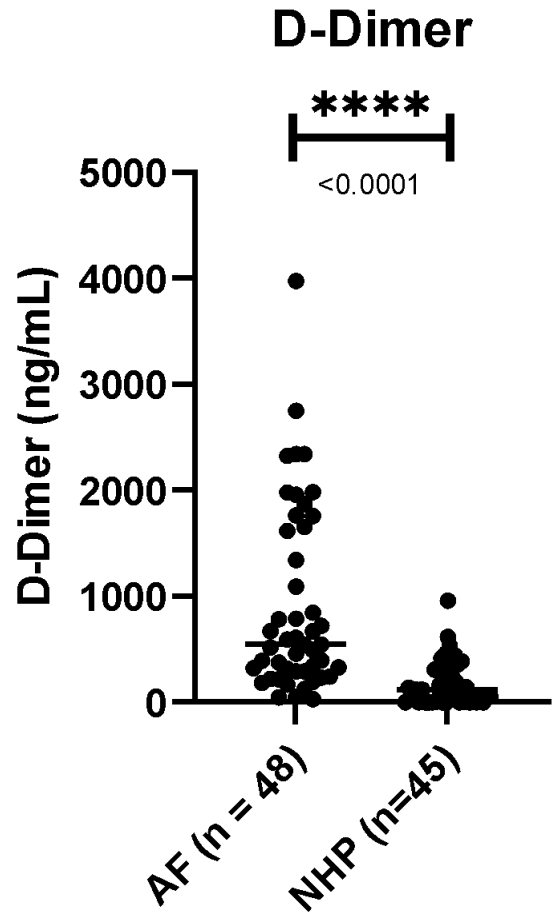
D-DIMER ANALYSIS

BACKGROUND OF D-DIMER

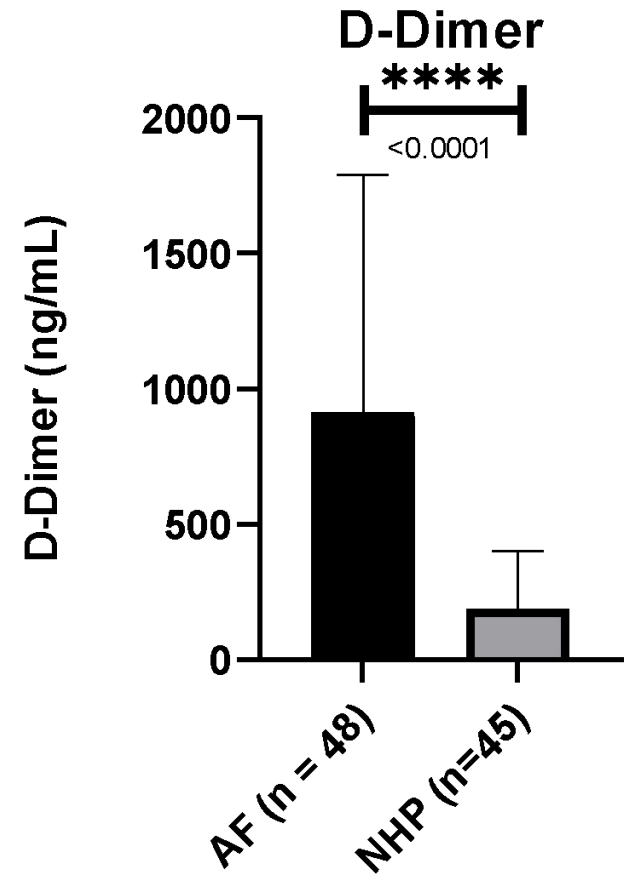
- Structure
 - Consists of α , β and γ peptide chains held together by disulfide bonds
- Production
 - Produced when fibrin is broken down
 - Fibrin is a major component of blood clots
 - High D-Dimer levels can indicate the presence of a blood clot (such as DVT or PE)
- Connection with Atrial Fibrillation
 - AF has been shown to be related to abnormal thrombogenesis which is known to increase levels of D-Dimer



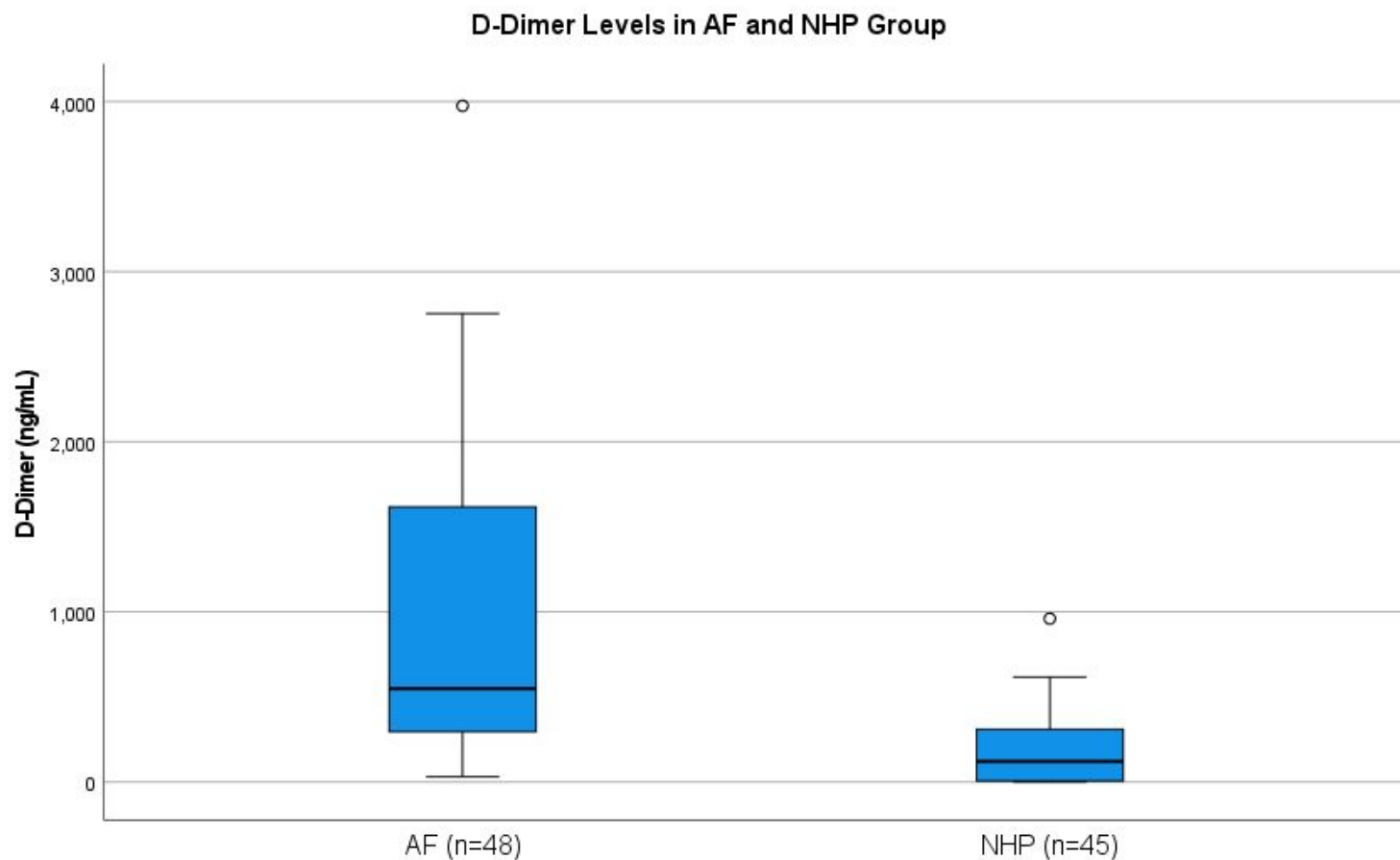
D-DIMER RESULTS



D-Dimer Levels (ng/mL)		
Sample	ng/mL	
AF (n=48)	Average	913.379
	SD	870.418
	SEM	125.634
	Median	546.250
	Mode	2345.100
	Min	31.900
	Max	3975.200
	IQR	1341.050
	25th Percentile	294.100
75th Percentile	1634.150	
NHP (n=45)	Average	187.726
	SD	210.118
	SEM	31.320
	Median	121.710
	Mode	0.000
	Min	0.000
	Max	960.850
	IQR	333.140
	25th Percentile	3.100
75th Percentile	336.240	
Percent Change (%)		386.549



D-DIMER QUARTILE ANALYSIS



Sample		ng/mL
AF(n=48)	IQR	1341.050
	25th Percentile	294.100
	50th Percentile	546.250
	75th Percentile	1634.150

Sample		ng/mL
NHP(n=45)	IQR	333.140
	25th Percentile	3.100
	50th Percentile	121.710
	75th Percentile	336.240

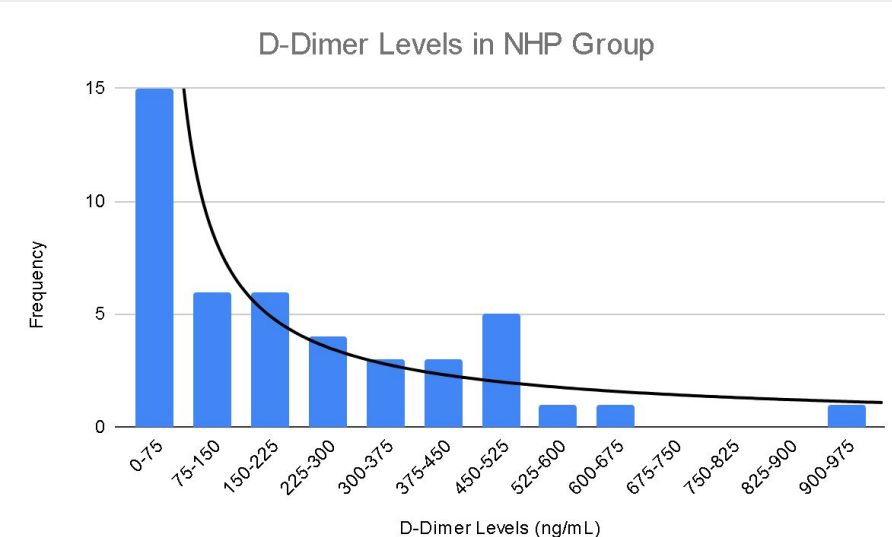
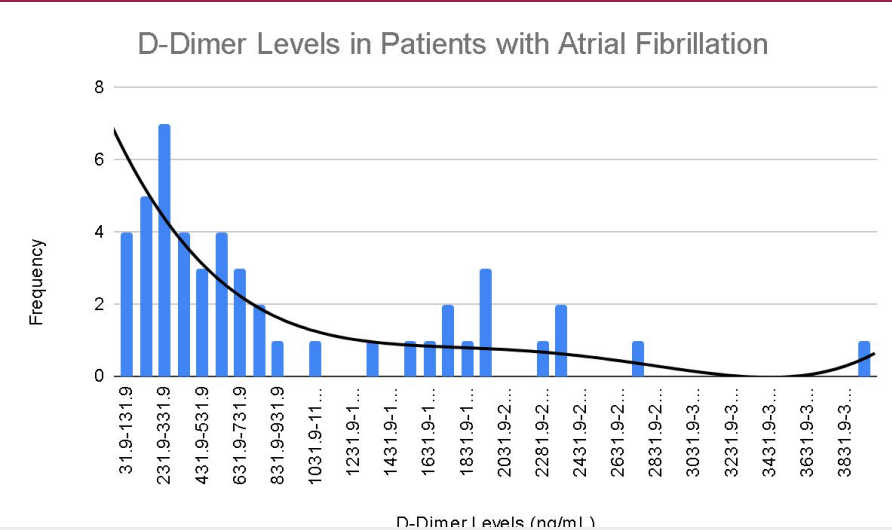
		NHP			
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	.00	11	22.9	24.4	24.4
	6.20	1	2.1	2.2	26.7
	6.98	1	2.1	2.2	28.9
	25.97	1	2.1	2.2	31.1
	29.84	1	2.1	2.2	33.3
	44.96	1	2.1	2.2	35.6
	49.61	1	2.1	2.2	37.8
	56.98	1	2.1	2.2	40.0
	57.75	1	2.1	2.2	42.2
	74.03	1	2.1	2.2	44.4
	94.19	1	2.1	2.2	46.7
	121.71	2	4.2	4.4	51.1
	130.62	1	2.1	2.2	53.3
	139.15	1	2.1	2.2	55.6
	143.41	1	2.1	2.2	57.8
	165.89	1	2.1	2.2	60.0
	188.37	1	2.1	2.2	62.2
	190.70	1	2.1	2.2	64.4
	229.46	1	2.1	2.2	66.7
	239.92	1	2.1	2.2	68.9
	265.50	1	2.1	2.2	71.1
	308.91	1	2.1	2.2	73.3
	310.08	1	2.1	2.2	75.6
	362.40	1	2.1	2.2	77.8
	386.43	1	2.1	2.2	80.0
	389.15	1	2.1	2.2	82.2
	419.38	1	2.1	2.2	84.4
	420.54	1	2.1	2.2	86.7
	442.25	1	2.1	2.2	88.9
	446.90	1	2.1	2.2	91.1
	466.67	1	2.1	2.2	93.3
	533.72	1	2.1	2.2	95.6
	617.44	1	2.1	2.2	97.8
	960.85	1	2.1	2.2	100.0
	Total	45	93.8	100.0	
Missing	System	3	6.3		
Total		48	100.0		

		AF			
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	31.90	1	2.1	2.1	2.1
	46.30	1	2.1	2.1	4.2
	54.90	1	2.1	2.1	6.3
	126.30	1	2.1	2.1	8.3
	170.30	1	2.1	2.1	10.4
	187.20	1	2.1	2.1	12.5
	194.40	1	2.1	2.1	14.6
	219.00	1	2.1	2.1	16.7
	223.60	1	2.1	2.1	18.8
	234.10	1	2.1	2.1	20.8
	245.10	1	2.1	2.1	22.9
	293.60	1	2.1	2.1	25.0
	295.60	1	2.1	2.1	27.1
	320.50	1	2.1	2.1	29.2
	321.40	1	2.1	2.1	31.3
	331.50	1	2.1	2.1	33.3
	345.20	1	2.1	2.1	35.4
	376.10	1	2.1	2.1	37.5
	395.30	1	2.1	2.1	39.6
	396.30	1	2.1	2.1	41.7
	459.20	1	2.1	2.1	43.8
	492.10	1	2.1	2.1	45.8
	519.40	1	2.1	2.1	47.9
	543.20	1	2.1	2.1	50.0
	549.30	1	2.1	2.1	52.1
	594.20	1	2.1	2.1	54.2
	609.20	1	2.1	2.1	56.3
	671.80	1	2.1	2.1	58.3
	672.10	1	2.1	2.1	60.4
	721.80	1	2.1	2.1	62.5
	785.40	1	2.1	2.1	64.6
	789.10	1	2.1	2.1	66.7
	845.90	1	2.1	2.1	68.8
	1093.10	1	2.1	2.1	70.8
	1344.20	1	2.1	2.1	72.9
	1617.10	1	2.1	2.1	75.0
	1654.20	1	2.1	2.1	77.1
	1762.10	1	2.1	2.1	79.2
	1763.80	1	2.1	2.1	81.3
	1872.10	1	2.1	2.1	83.3
	1959.30	1	2.1	2.1	85.4
	1982.10	1	2.1	2.1	87.5
	1987.30	1	2.1	2.1	89.6
	2326.40	1	2.1	2.1	91.7
	2345.10	2	4.2	4.2	95.8
	2753.80	1	2.1	2.1	97.9
	3975.20	1	2.1	2.1	100.0
Total		48	100.0	100.0	

FREQUENCY TABLES

D-DIMER FREQUENCY DISTRIBUTION

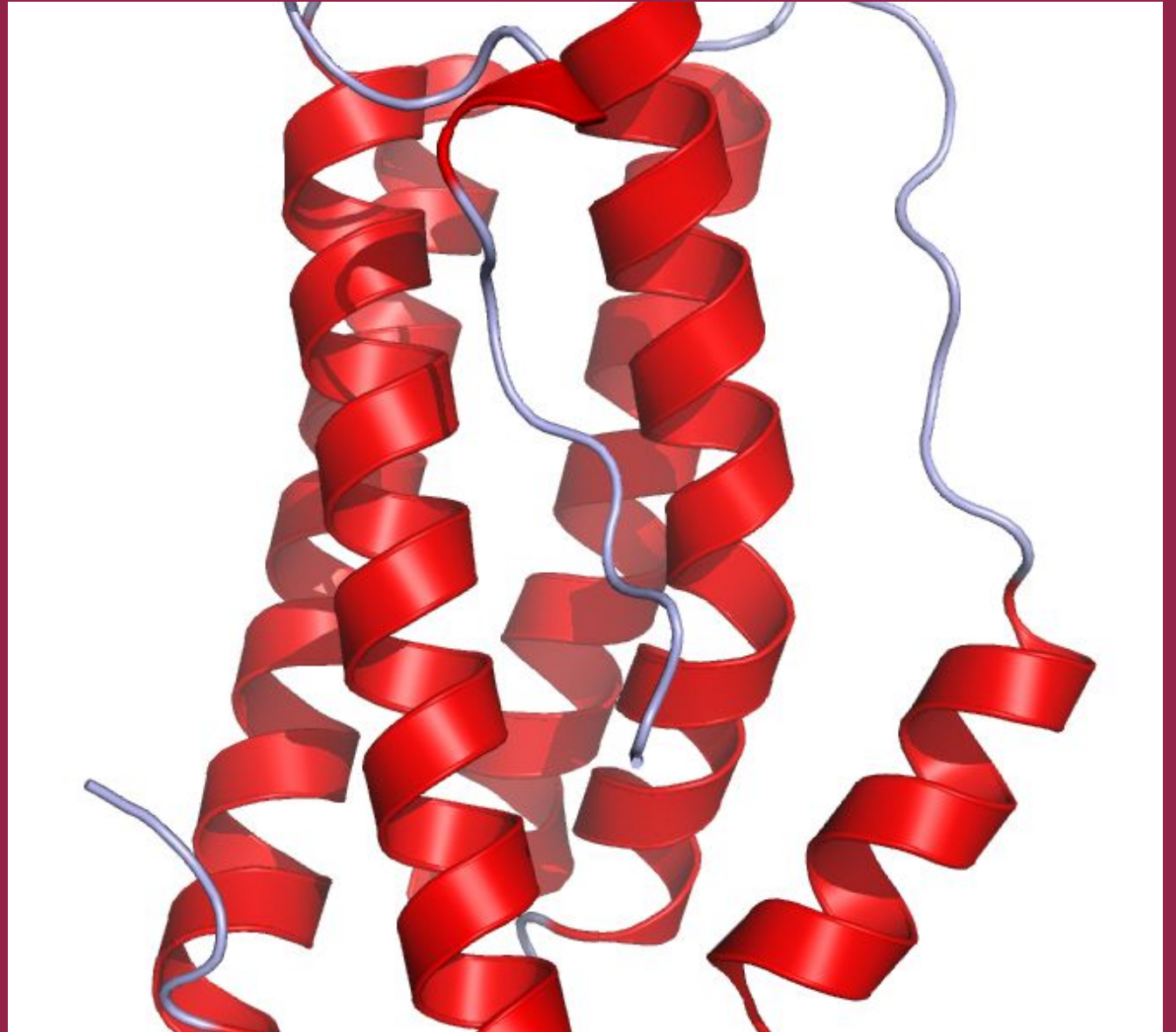
- AF Group
 - Right Skewed Data (Positive Skew)
 - Skewness: 1.441
- NHP
 - Right Skewed Data (Positive Skew)
 - Skewness: 1.445
 - L-Shaped Distribution



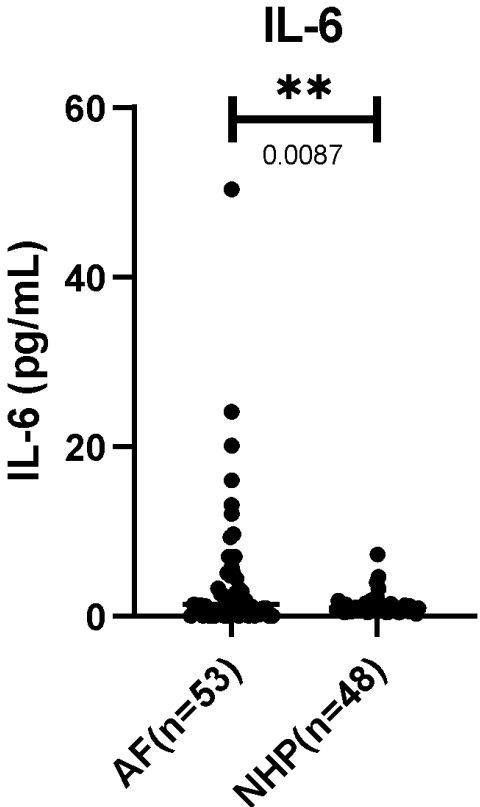
INTERLEUKIN-6 (IL-6) ANALYSIS

BACKGROUND OF INTERLEUKIN - 6

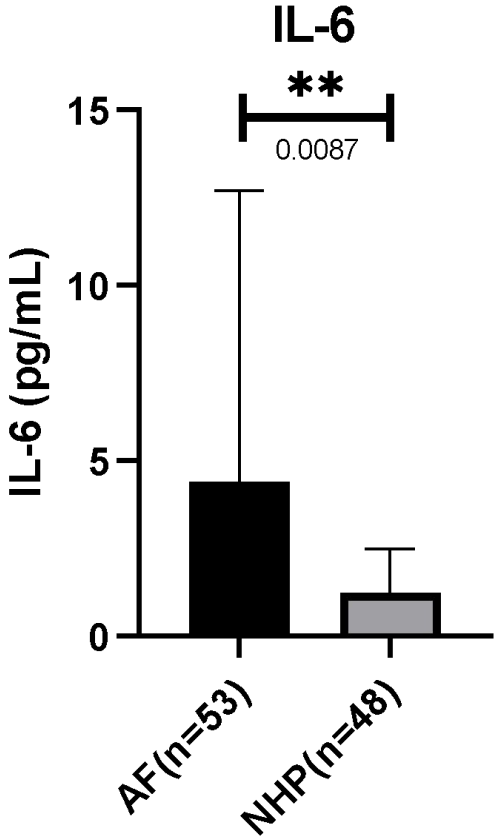
- Structure
 - Type of protein which is classified as a cytokine.
 - Composed of 4 helix bundles as well as a mini helix attached as seen in the image to the right
- Function
 - Primarily involved in regulating the body's immune response
 - Stimulates antibody production and the growth of B cells
- Location
 - Produced by macrophages and monocytes
 - Often found at the site of inflammation in the body
- Correlation to Atrial Fibrillation
 - Atrial Fibrillation is likely to cause inflammation in the cardiac tissue which can lead to increased levels of IL-6



IL-6 RESULTS

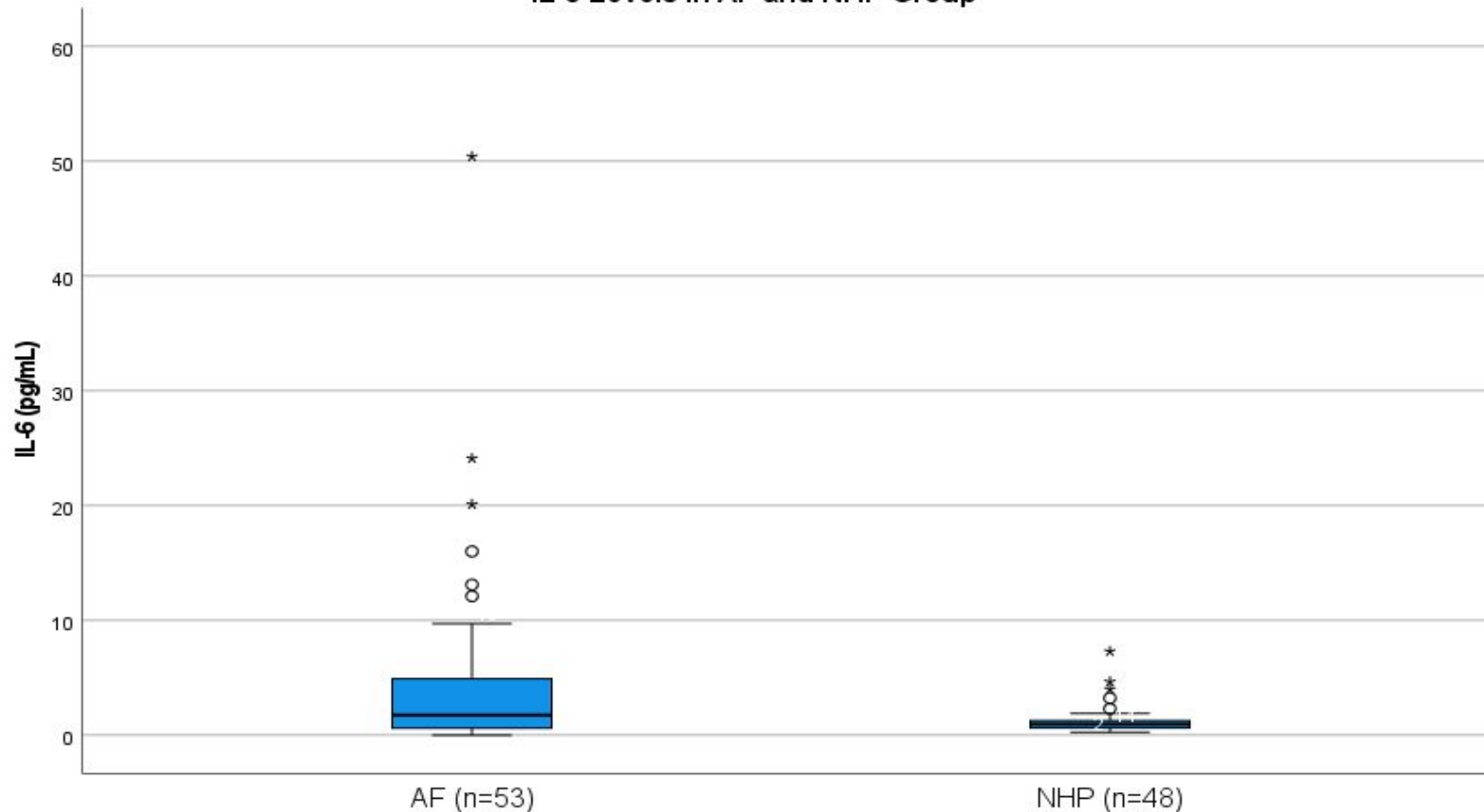


IL-6 Levels (pg/mL)		
Sample	pg/mL	
AF (n=53)	Average	4.409
	SD	8.188
	SEM	1.125
	Median	1.420
	Mode	0.000
	Min	0.000
	Max	50.400
	IQR	4.550
	25th Percentile	0.350
75th Percentile	4.900	
NHP (n=48)	Average	1.249
	SD	1.224
	SEM	0.178
	Median	0.940
	Mode	1.420
	Min	0.250
	Max	7.290
	IQR	0.662
	25th Percentile	0.613
75th Percentile	1.275	
% Change		253.002



IL-6 QUARTILE ANALYSIS

IL-6 Levels in AF and NHP Group



Sample		pg/mL
AF (n=53)	IQR	4.550
	25th Percentile	0.350
	50th Percentile	1.420
	75th Percentile	4.900

Sample		pg/mL
NHP (n=48)	IQR	0.662
	25th Percentile	0.613
	50th Percentile	0.940
	75th Percentile	1.275

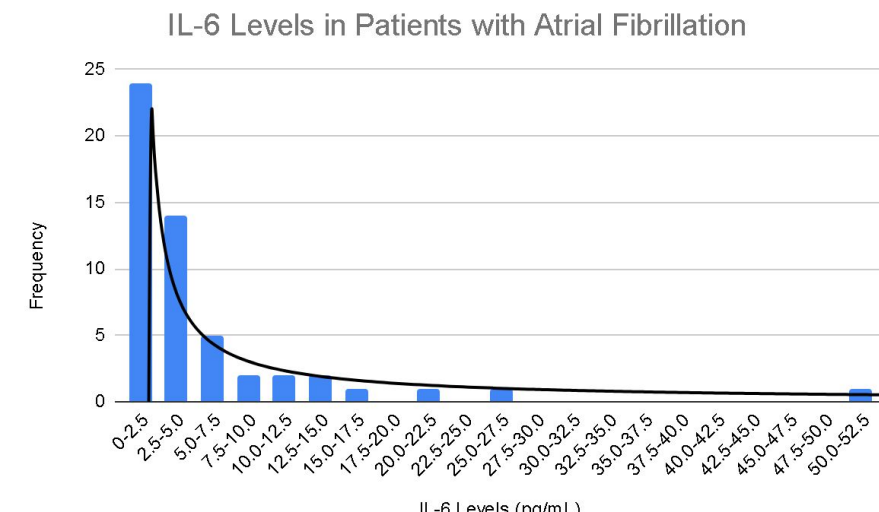
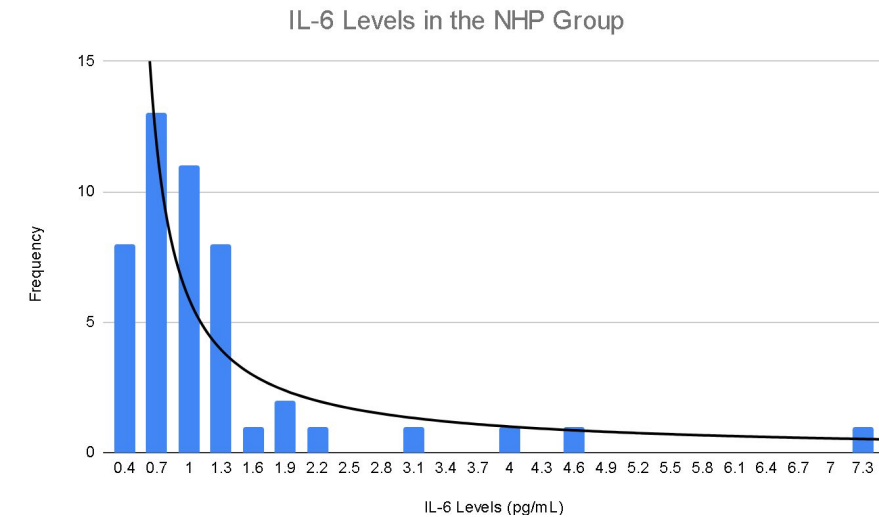
NHP				
	Frequency	Percent	Valid Percent	Cumulative Percent
Valid	.25	1	1.9	2.1
	.40	1	1.9	2.1
	.42	1	1.9	2.1
	.43	1	1.9	2.1
	.44	1	1.9	2.1
	.52	1	1.9	2.1
	.53	1	1.9	2.1
	.54	1	1.9	2.1
	.55	1	1.9	2.1
	.56	1	1.9	2.1
	.60	1	1.9	2.1
	.61	1	1.9	2.1
	.62	1	1.9	2.1
	.63	1	1.9	2.1
	.65	1	1.9	2.1
	.67	1	1.9	2.1
	.69	1	1.9	2.1
	.71	1	1.9	2.1
	.74	1	1.9	2.1
	.79	1	1.9	2.1
	.82	1	1.9	2.1
	.86	1	1.9	2.1
	.93	1	1.9	2.1
	.94	2	3.8	4.2
	.97	1	1.9	2.1
	.98	1	1.9	2.1
	.99	1	1.9	2.1
	1.02	1	1.9	2.1
	1.04	2	3.8	4.2
	1.09	1	1.9	2.1
	1.17	1	1.9	2.1
	1.18	1	1.9	2.1
	1.25	1	1.9	2.1
	1.26	1	1.9	2.1
	1.28	1	1.9	2.1
	1.36	1	1.9	2.1
	1.42	2	3.8	4.2
	1.52	1	1.9	2.1
	1.80	1	1.9	2.1
	1.88	1	1.9	2.1
	2.29	1	1.9	2.1
	3.22	1	1.9	2.1
	3.98	1	1.9	2.1
	4.64	1	1.9	2.1
	7.29	1	1.9	2.1
	Total	48	90.6	100.0
Missing	System	5	9.4	
Total		53	100.0	

AF				
	Frequency	Percent	Valid Percent	Cumulative Percent
Valid	.00	12	22.6	22.6
	.20	1	1.9	1.9
	.50	3	5.7	5.7
	.70	3	5.7	5.7
	.90	2	3.8	3.8
	1.20	3	5.7	5.7
	1.30	1	1.9	1.9
	1.40	2	3.8	3.8
	1.70	1	1.9	1.9
	1.80	2	3.8	3.8
	1.90	1	1.9	1.9
	2.20	1	1.9	1.9
	2.30	1	1.9	1.9
	2.60	1	1.9	1.9
	2.80	1	1.9	1.9
	2.90	1	1.9	1.9
	3.20	1	1.9	1.9
	3.30	1	1.9	1.9
	4.40	1	1.9	1.9
	4.80	1	1.9	1.9
	5.00	1	1.9	1.9
	5.10	1	1.9	1.9
	5.80	1	1.9	1.9
	7.00	2	3.8	3.8
	9.30	1	1.9	1.9
	9.70	1	1.9	1.9
	12.10	1	1.9	1.9
	13.10	1	1.9	1.9
	16.00	1	1.9	1.9
	20.10	1	1.9	1.9
	24.10	1	1.9	1.9
	50.40	1	1.9	1.9
Total		53	100.0	100.0

FREQUENCY TABLES

IL-6 FREQUENCY DISTRIBUTION

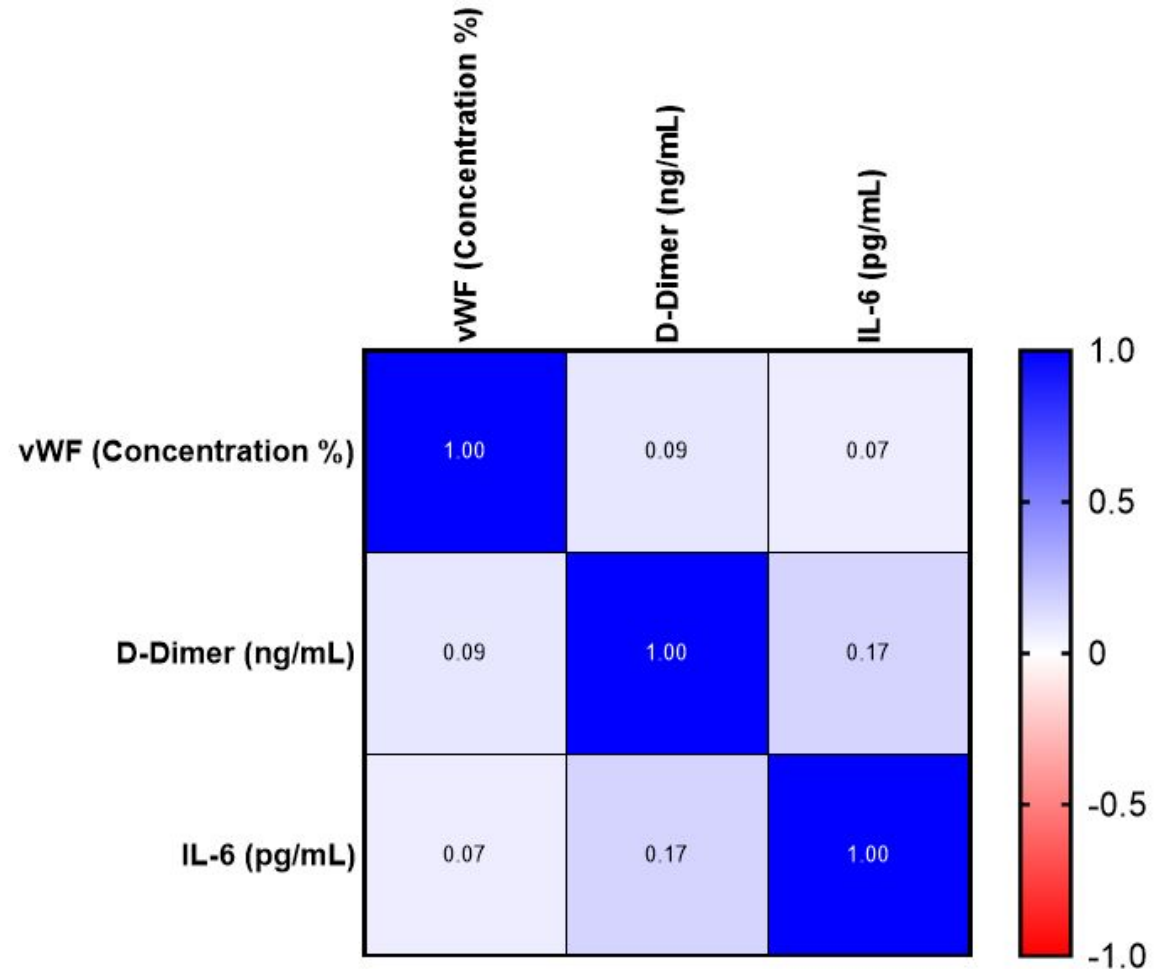
- AF Group
 - Right Skewed Data (Positive Skew)
 - Skewness: 3.792
 - L-Shaped Distribution
- NHP Group
 - Right Skewed Data (Positive Skew)
 - Skewness: 3.306
 - L-Shaped Distribution



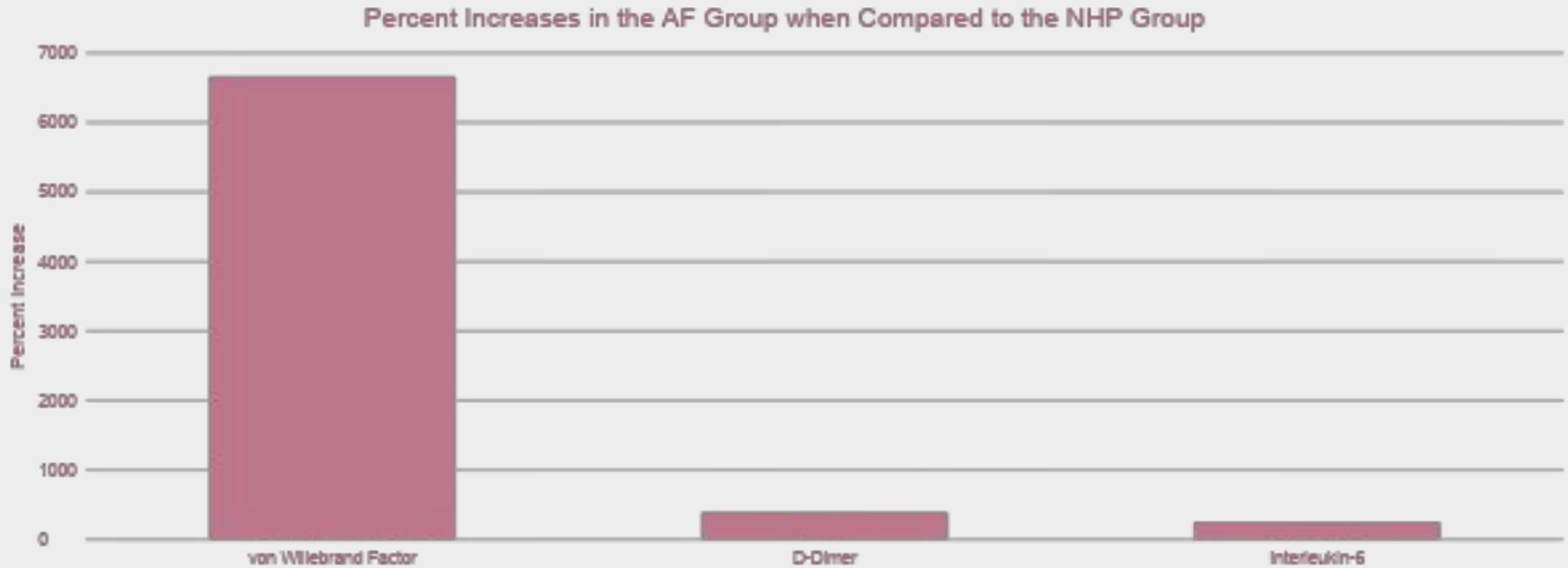
CROSS ANALYSIS

CORRELATION HEAT MAP

- Each box shows the r-value which represents how strong the correlation is between the respective biomarkers
- No substantial correlation between any of the three biomarkers (r-values above 0.7 are considered significant)



PERCENT INCREASES BETWEEN ALL BIOMARKERS



CONCLUSION

- The data supports the hypothesis that there will be an upregulation in biomarkers of inflammation and thrombosis (vWF, D-Dimer, IL-6) in AF patients.
 - vWF showed the greatest percent increase in AF patients when compared to the NHP group while IL-6 showed the lowest percent increase
- Unfortunately, there was no significant correlation between the biomarkers, so the second component of the hypothesis was not supported.
- Further investigation is needed as our data was limited by the modest sample size of the AF group

NEXT STEPS

- Submit abstract to FASEB
- Possibly visit Loyola University in October
- Further investigate the relation between the inflammatory and thrombotic biomarkers to see if there is any evidence of correlation among available literature online

THANK YOU FOR
YOUR TIME

