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# Association between the thrombin generation potential and thrombin generation markers in PE patients

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NATF North American Thrombosis Forum

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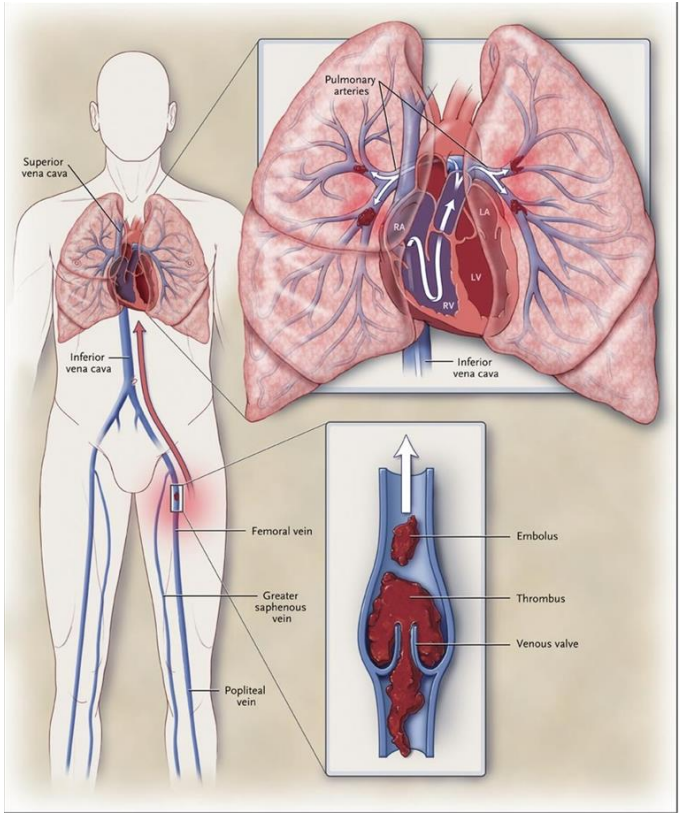
# Background

Pulmonary embolism is when an embolus travels to the lungs and causes a blockage in the pulmonary arteries

- Symptoms:
  - Shortness of breath
  - Chest pain
  - Coughing up blood
- Risk factors:
  - Active cancer
  - Post-surgical patients
  - Post-surgical immobility
  - Smoking
  - Obesity
  - Pregnancy
- Comorbidities:
  - Chronic heart disease
  - Chronic lung disease
  - Diabetes

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# Hypothesis

The hypothesis of this study is that despite the increase in thrombin generation biomarkers, the thrombin generation potential in PE may be reduced

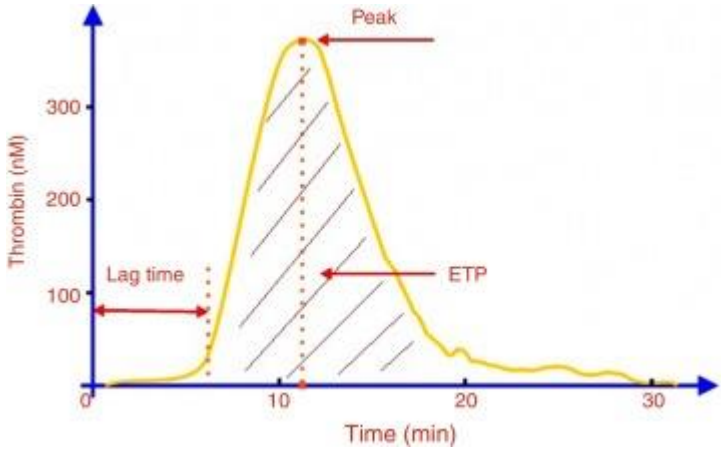
# Aim

The purpose of this study is to determine the thrombin generation potential in PE patients and its relevance to the thrombin generation markers, such as prothrombin fragment 1+2 (F1+2), thrombin antithrombin (TAT) and d-Dimer

# Thrombin Generation Potential

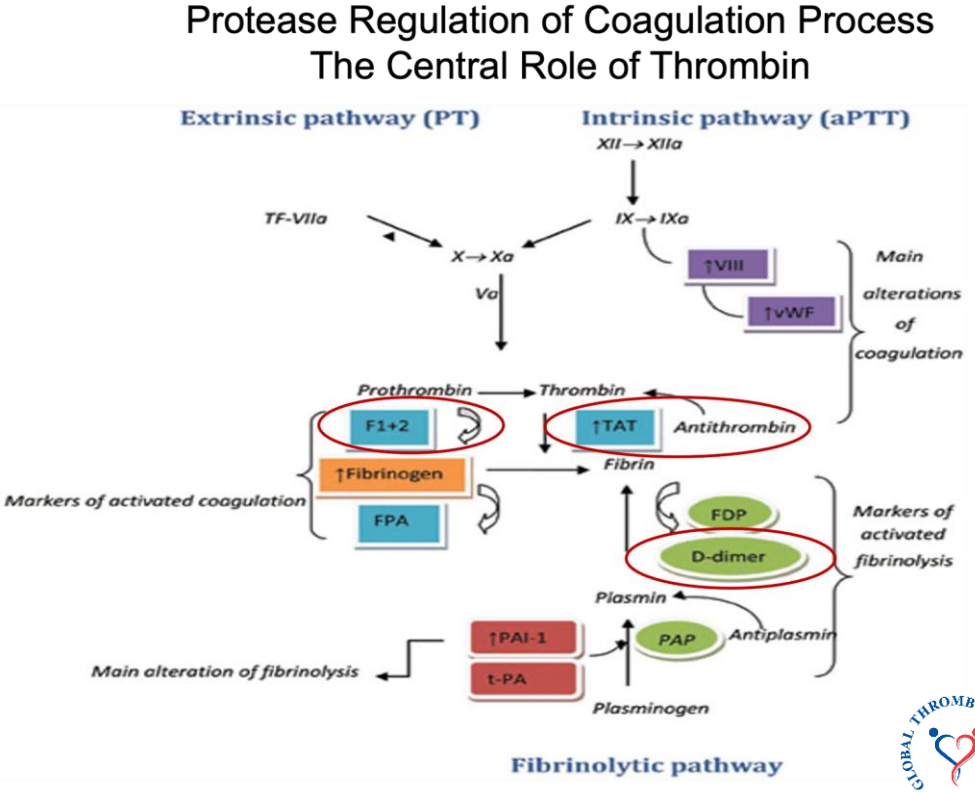
Thrombin generation tests can be used to identify coagulation and potential thrombosis

- Peak thrombin generation: highest amount of thrombin generated at a given time
- Endogenous thrombin potential (ETP): amount of thrombin that can be generated by the plasma after coagulation starts
- Lag time: amount of time it takes for the thrombin to be generated



# Thrombin Generation Biomarkers

- Prothrombin fragment F1+2:
  - Prothrombin → thrombin
  - Diagnoses thrombosis
  - Marker of thrombin generation & coagulation activation
- Thrombin antithrombin (TAT) complex:
  - Formed by binding AT to thrombin in a ratio of 1:1
  - Activation of coagulation
  - Associated with thrombosis
- D-dimer:
  - Fibrin degradation product, generates after a blood clot is degraded by fibrinolysis
  - Increased levels = coagulation problems



# Material and Method

**Sample Collection:**

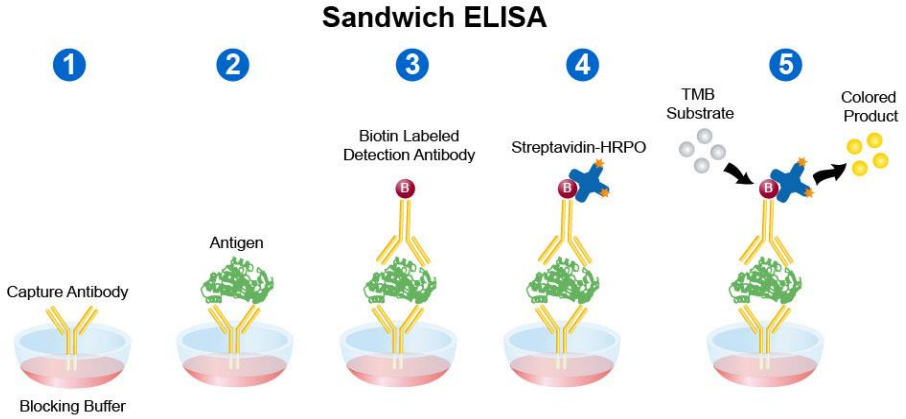
1. PE patient samples (n=150) within 24-72 hours (Loyola University Medical Center)
2. NHP (n=50) control from commercial vendor

**Biomarker Profiling:**

1. ELISA
    a. F1+2
    b. TAT
    c. D-Dimer
2. Fluorogenic Assay
    a.Thrombin Generation

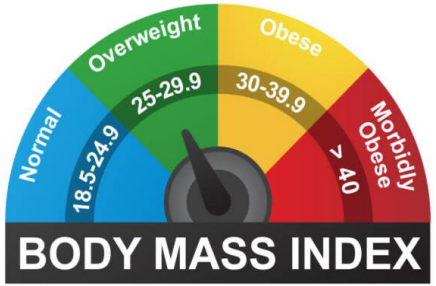
**Statistical Analysis:**

1. Mann-Whitney U Test
2. Spearman Correlation



# Demographics

48 %  52 %

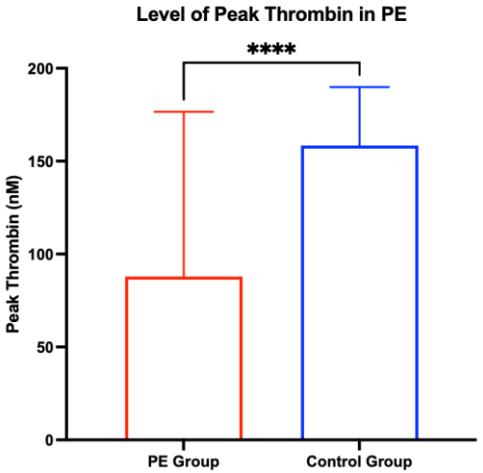


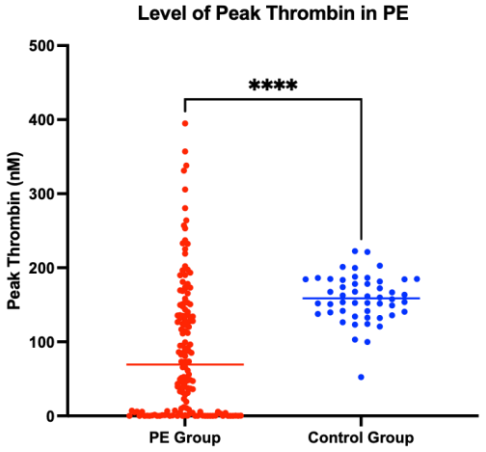

32.01

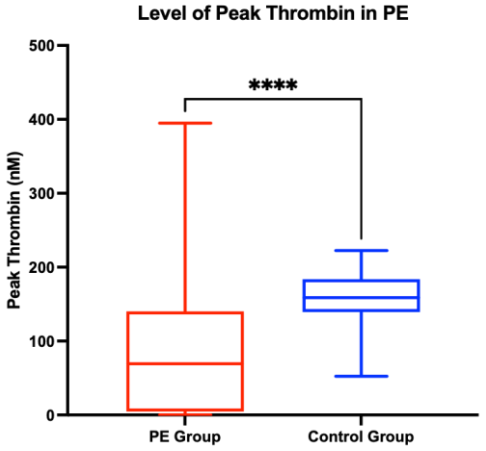

61.31

# Levels of Peak Thrombin

| C- Statistics | PE (n=150) | NHP (n=50) |
|---|---|---|
| Mean±SD | 87.94 ± 88.61 | 158.46 ± 31.46 |
| SEM | 7.24 | 4.45 |
| Median | 69.26 | 158.73 |
| Minimum | 0.00 | 52.35 |
| Maximum | 394.95 | 222.58 |
| Range | 394.95 | 170.23 |

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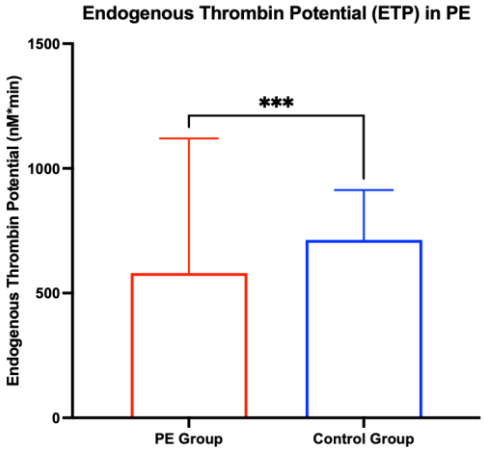
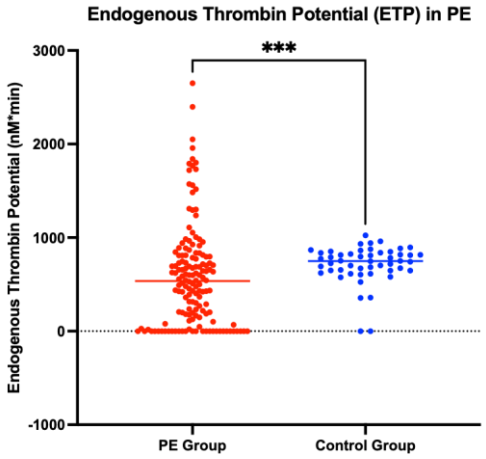
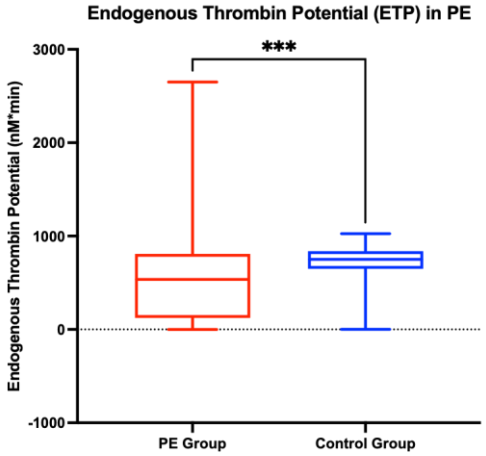
# Levels of Endogenous Thrombin Potential

| C- Statistics | PE (n=150) | NHP (n=50) |
|---|---|---|
| **Mean±SD** | 580.43 ± 539.54 | 713.37 ± 199.97 |
| **SEM** | 44.05 | 28.28 |
| **Median** | 535.51 | 748.88 |
| **Minimum** | -1.00 | -1.00 |
| **Maximum** | 2651.04 | 1023.32 |
| **Range** | 2652.04 | 1024.32 |

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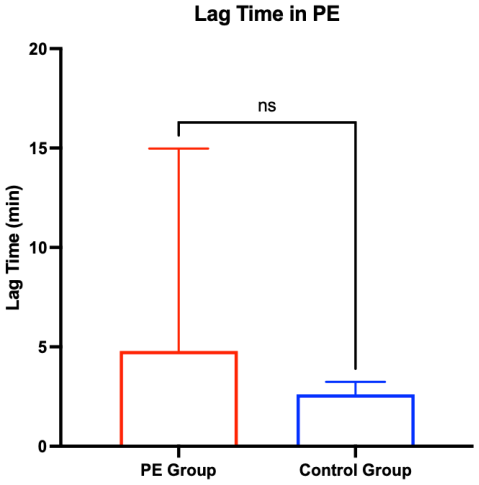
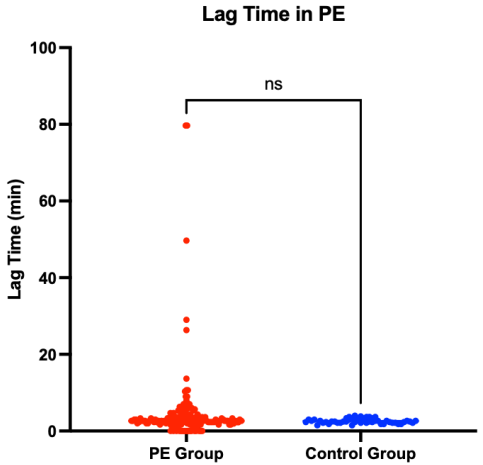
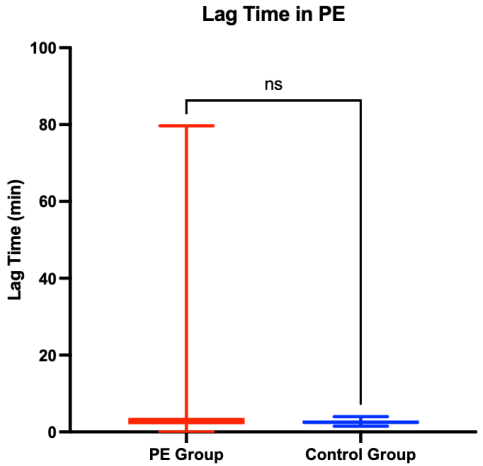
# Levels of Lag Time

| C- Statistics | PE (n=150) | NHP (n=50) |
|---|---|---|
| Mean±SD | 4.79 ± 10.18 | 2.61 ± 0.62 |
| SEM | 0.83 | 0.09 |
| Median | 2.67 | 2.48 |
| Minimum | 0.00 | 1.48 |
| Maximum | 79.67 | 4.00 |
| Range | 79.67 | 2.52 |

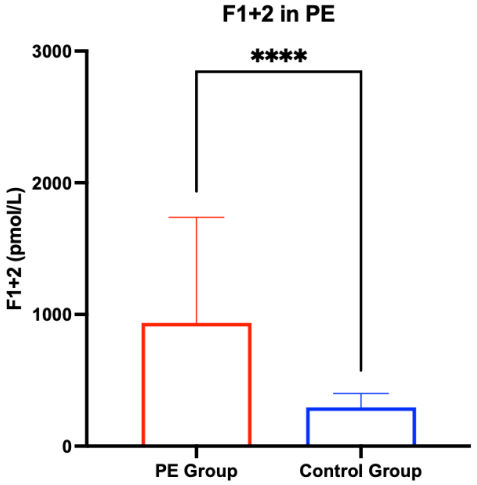


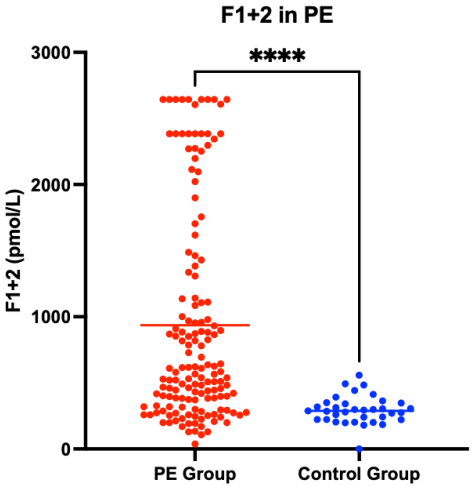



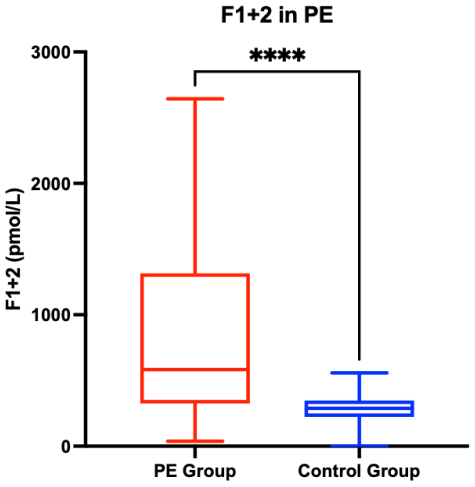



# Levels of F1+2

| C- Statistics | PE (n=150) | NHP (n=35) |
|---|---|---|
| Mean±SD | 936.36 ± 802.11 | 294.19 ± 104.99 |
| SEM | 65.49 | 17.75 |
| Median | 582.80 | 288 |
| Minimum | 37.88 | 0 |
| Maximum | 2642.80 | 558.27 |
| Range | 2604.92 | 558.27 |

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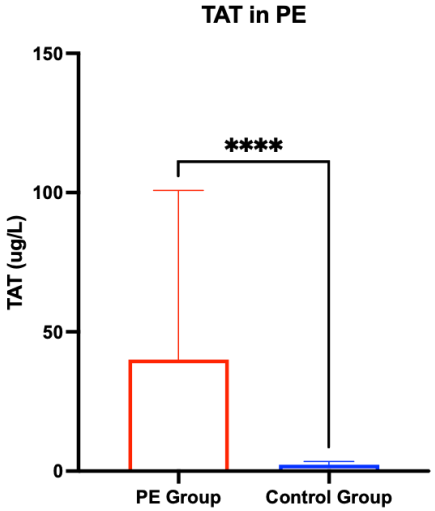
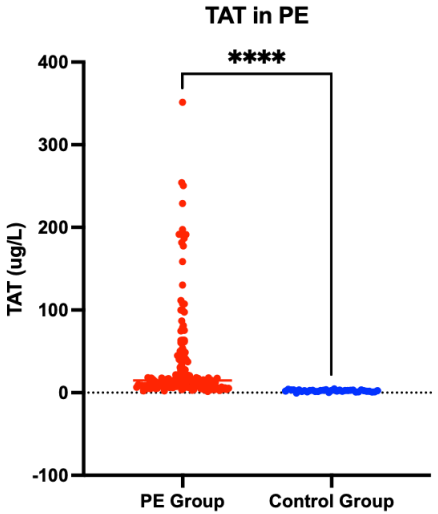
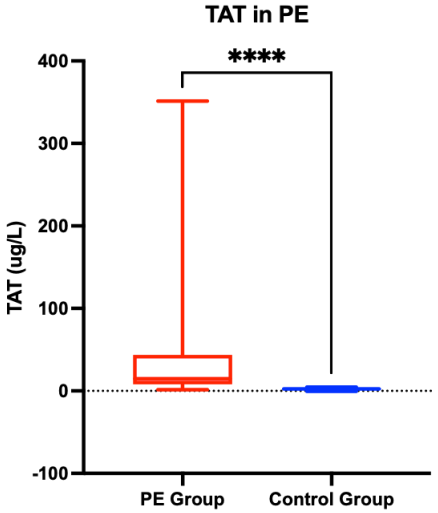
# Levels of TAT

| C- Statistics | PE (n=150) | NHP (n=35) |
|---|---|---|
| Mean±SD | 40.04 ± 60.76 | 2.29 ± 1.13 |
| SEM | 4.96 | 0.19 |
| Median | 14.71 | 2.40 |
| Minimum | 1.46 | -0.46 |
| Maximum | 351.35 | 4.67 |
| Range | 349.89 | 5.13 |

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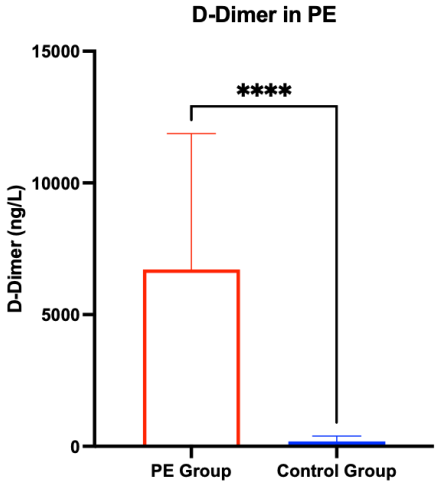
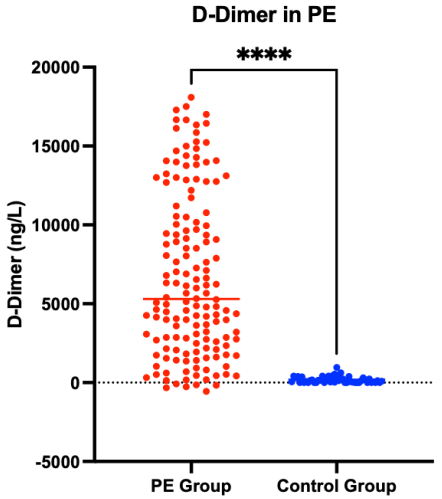
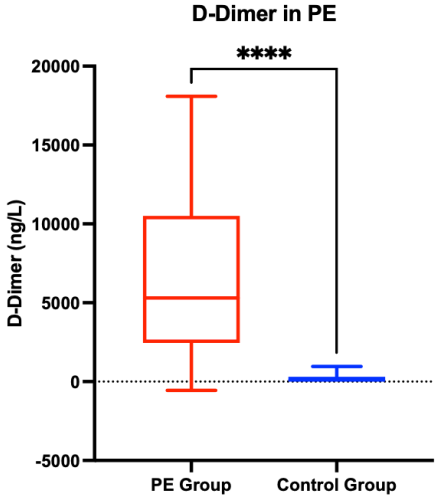
# Levels of D-Dimer

| C- Statistics | PE (n=150) | NHP (n=51) |
|---|---|---|
| **Mean±SD** | 6715.63 ± 5160.73 | 182.55 ± 205.77 |
| **SEM** | 421.37 | 28.81 |
| **Median** | 5302.33 | 121.71 |
| **Minimum** | -556.25 | 0 |
| **Maximum** | 18092.72 | 960.85 |
| **Range** | 18648.97 | 960.85 |

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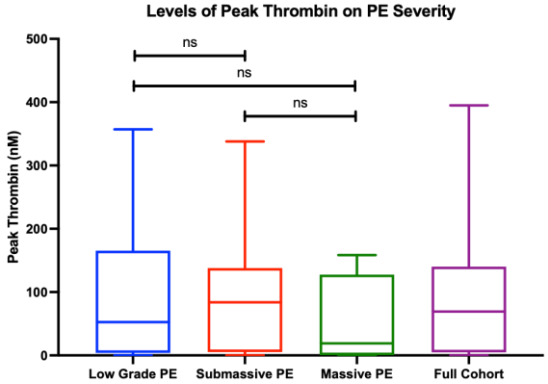
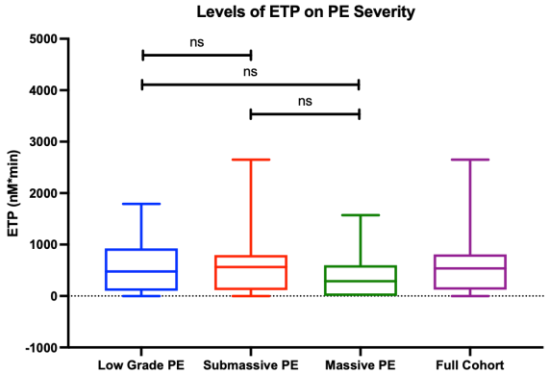
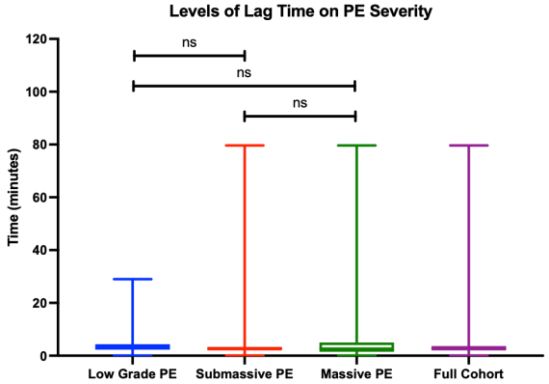
# Levels of Thrombin Generation Parameters and Biomarkers in PE Cohort on the Basis of PE Severity

| Biomarker | | PE Cohort (n=150) | Low Grade PE (n=47) | Sub-Massive PE (n=82) | Massive PE (n=9) |
|---|---|---|---|---|---|
| Thrombin Generation Potential | Peak Thrombin (nM) | 87.94 ± 88.61 | 84.32 ± 90.60 | 90.76 ± 87.25 | 54.17 ± 65.19 |
| | ETP (nM*min) | 580.43 ± 539.54 | 591.87 ± 541.03 | 588.83 ± 549.24 | 399.68 ± 504.84 |
| | Lag Time (min) | 4.79 ± 10.18 | 4.22 ± 5.46 | 4.62 ± 10.13 | 11.26 ± 25.71 |
| Thrombin Generation Markers | F 1+2 (pmol/L) | 936.36 ± 802.11 | 676.05 ± 565.38 | 1091.46 ± 896.15 | 1280.51 ± 775.51 |
| | TAT (ug/L) | 40.04 ± 60.76 | 28.07 ± 45.22 | 43.45 ± 59.45 | 75.12 ± 108.31 |
| | D-Dimer (ng/L) | 6715.63 ± 5160.73 | 4858.40 ± 4459.09 | 7290.82 ± 5262.51 | 14079.61 ± 4191.49 |

# Levels of Thrombin Generation Parameters in PE Cohort on the Basis of PE Severity

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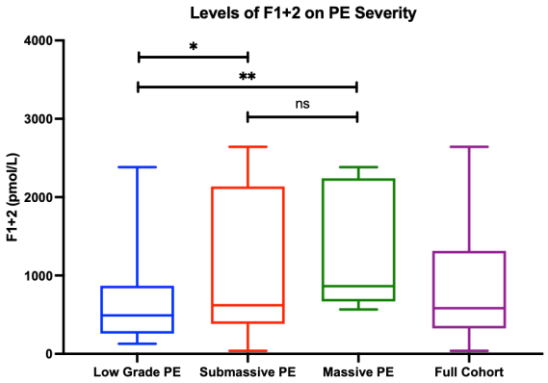
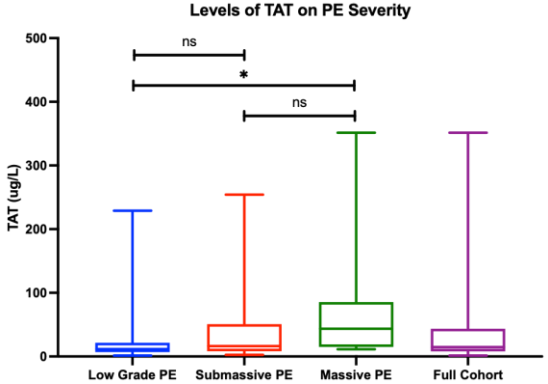
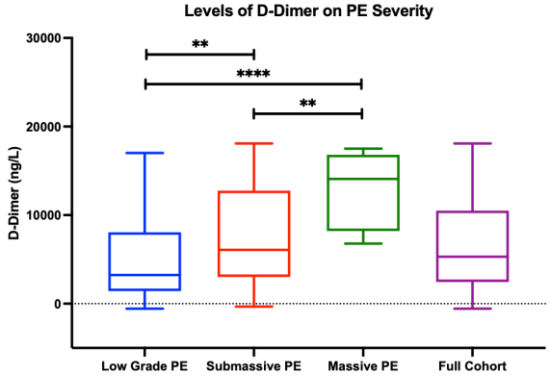
# Levels of Thrombin Generation Biomarkers in PE Cohort on the Basis of PE Severity

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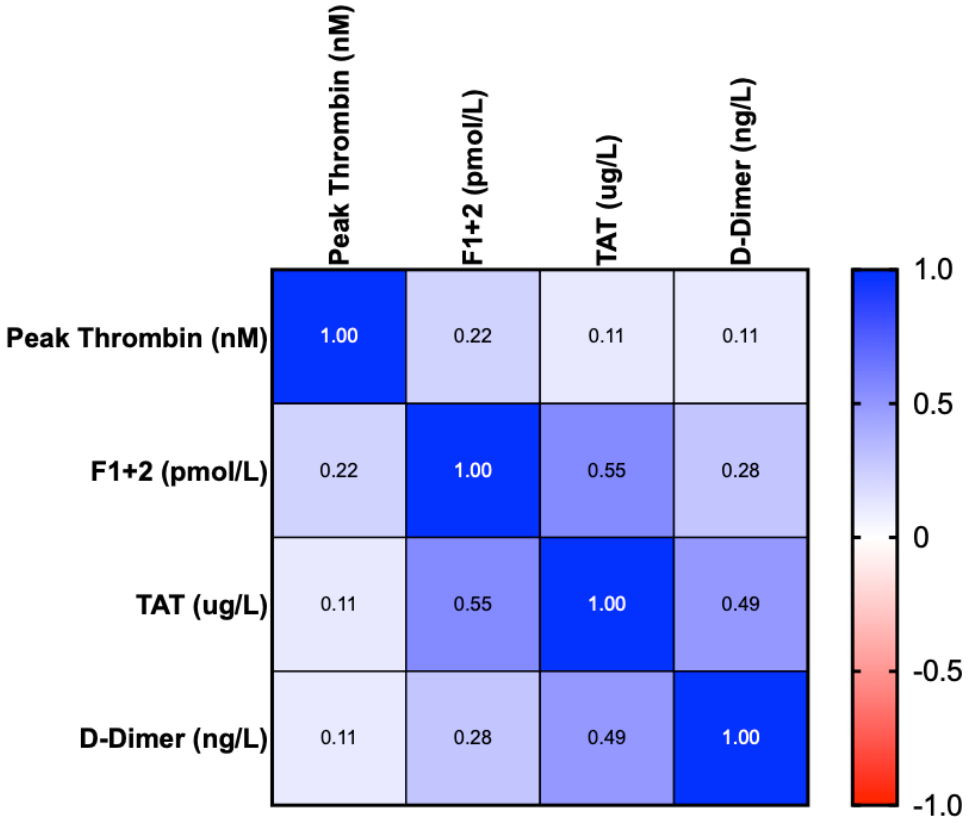
# Correlation Analysis



# Summary

| Variables | | PE Group | | Control Group | | P value |
|---|---|---|---|---|---|---|
| | | Mean ± SD | Range | Mean ± SD | Range | |
| Thrombin Generation Potential | Peak Thrombin (nM) | 87.94 ± 88.61 | 394.95 | 158.46 ± 31.46 | 170.23 | <0.0001 |
| | Endogenous Thrombin Potential, ETP (nM*min) | 580.43 ± 539.54 | 2652.04 | 713.37 ± 199.97 | 1024.32 | 0.0004 |
| | Lag Time (min) | 4.79 ± 10.18 | 79.67 | 2.61 ± 0.62 | 2.52 | 0.1649 (ns) |
| Thrombin Generation Markers | F1+2 (pmol/L) | 936.36 ± 802.11 | 2604.92 | 294.19 ± 104.99 | 558.27 | <0.0001 |
| | TAT (µg/L) | 40.04 ± 60.76 | 349.89 | 2.29 ± 1.13 | 5.13 | <0.0001 |
| | D-Dimer (ng/L) | 6715.63 ± 5160.73 | 18648.97 | 182.55 ± 205.77 | 960.85 | <0.0001 |

# Conclusion

1. Thrombin generation potential:
    a. ↓Peak thrombin and ETP = consumption of the coagulation factors
    b. ↑Lag time is due to the delay in the formation of thrombin
2. Thrombin generation biomarkers (F1+2, TAT and D-dimer):
    a. Continuous activation of the coagulation process in the PE patients
3. Correlation analysis:
    a. Peak thrombin is +ve correlated with F1+2
    b. F1+2 is +ve correlated with TAT and D-dimer
    c. TAT is +ve correlated with D-dimer as well
4. PE Severity:
    a. Biomarker levels increase as severity increases
    b. No difference between Low Grade PE and Submassive PE in parameters, but levels change in Massive PE

# Future Plans

1. Submit an abstract for the 2023 Experimental Biology Meeting
2. Possibly travel to Loyola for St. Alberts Day in October

# Acknowledgments

I would like to thank my mentor Dr. Siddiqui, as well as Dr. Laddu, Dr. Fareed, and the rest of the GTF and Hemostasis & Thrombosis Research Laboratory for their support and this opportunity.

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