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Evaluation of the NAVI Model for Thrombogenicity Assessments: Correlation between *in vitro* hemocompatibility test results and *in vivo* thrombus scores

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SOT MDCPSS Webinar

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A Critical Evaluation of the NAVI Model for Thrombogenicity Evaluation of Cardiovascular Medical Devices: Correlation between *In Vitro* Hemocompatibility Test Results and *In Vivo* Thrombus Scores

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INTRODUCTION

According to ISO 10993-4: 2017 direct blood-contacting medical devices should be evaluated for thrombogenic potential, with the *in vivo* 4hr non-anticoagulated venous implant (NAVI) model as a standard test procedure used to assess acute thrombogenicity. However, limitations in test methodology such as inherent differences in individual animal thrombogenic reactivity, lack of statistical power, placement in venous anatomy, incubation period, and lack of anticoagulants may limit the NAVI model's predictiveness of the thrombogenic potential of a medical device under clinical-use conditions.

ISO 10993-4:2017 also indicates that the manufacturer may decide if *in vitro* tests are an appropriate alternative to *in vivo* testing. Common *in vitro* tests used to assess coagulation are: [partial thromboplastin time (PTT)], platelet counts (% loss), and complement (SC5b-9) activation. To determine if there is a correlation between *in vitro* test results and elevated *in vivo* thrombus scores, the results of *in vivo* thrombogenicity studies were compared to corresponding *in vitro* data (PTT, platelet counts and SC5b-9 complement activation) in the same devices. The data were obtained from several CRO's throughout 2006–2018. Therefore, test methodologies may vary slightly based on the study protocol used to perform the test.

Results from 15 NAVI studies showed thrombus scores were variable among individual animals (i.e. scores differed by 2 among three animals in half of the datasets). In this analysis, studies were divided into two groups: a thrombogenic group with scores of > 2 in at least 2 animals and a less thrombogenic group with scores of ≤ 2 in at least 2 animals. Although no association between platelet results (counts normalized to blood) and elevated thrombus scores was observed, PTT results (clotting time normalized to plasma) were on average 20% lower, and SC5b-9 levels were statistically significantly higher in the thrombogenic group. These results suggest reduced PTT and increased SC5b-9 may be associated with *in vivo* thrombogenicity in the NAVI model. Historically, platelet counts have been assessed using sodium citrate anticoagulation. Platelet counts utilizing heparinized human blood per ASTM F2888:2019 may improve the correlation. In conclusion, *in vitro* hemocompatibility tests, possibly combined with data from a dynamic *in vitro* flow loop may provide a feasible and appropriate alternative to *in vivo* thrombogenicity testing.

OBJECTIVE

To conduct a review of previous *in vivo* NAVI study results and determine if there is any correlation with *in vitro* hemocompatibility tests including PTT, platelet and leukocytes counts, and SC5b-9 complement activation.

BACKGROUND

Test	Standard	Purpose
SC5b-9 Complement Activation (CAA)	ISO10993-4: 2017	Complement activation has the potential to indicate whether a test article, when exposed to Normal Human Serum, can affect the hemolytic, platelet function, and thrombogenic potential of NHS.
Partial Thromboplastin Time (PTT)	ASTM F2382-18 ISO10993-4: 2017	Clotting time can indicate whether a test article, when exposed to human plasma, has the potential to activate the intrinsic clotting pathway.
Platelet & Leukocyte Counts (PLC)	ASTM F2888-19 ISO10993-4: 2017	Platelet counts can indicate whether a test article, when exposed to human whole blood, has the potential to cause thrombus formation.

The NAVI data set included a total of 17 studies performed between 2006 – 2018 on externally communicating devices or implant devices indicated for limited (<24 hour) or long-term (> 30 days) contact duration, respectively. Evaluations were conducted in duplicate or triplicate (2-3 animals) and thrombus formation was scored using an observation-based scoring system consistent with the NAVI/AVI model scoring scheme described by ISO 10993-4 (2017).

IN VIVO NAVI SCORING SCHEME

Thrombus formation score description	Score
No significant thrombosis (a very small clot is acceptable at insertion).	0
Minimal thrombosis, one location.	1
Minimal thrombosis, multiple locations.	2
Significant thrombosis, >1/4 to ≥ ½ the surface of the implant, vessel patent	3
Significant thrombosis, >½ the surface of the implant, vessel patent	4
Vessel completely occluded	5

Ref: ISO 10993-4:2017, Annex C, Table C.1.

METHODS

The following common parameters were used to determine the in-scope studies:

- The data set was included if hemocompatibility testing was performed on the test/control articles in both the NAVI study and the *In Vitro* assays.
- The selected *In Vivo* NAVI test/control article results were split into 2 categories: high and low.

  The high category was defined as *In Vivo* scores with 2 or more results > 2.

  The low category was defined as *In Vivo* scores with 2 or more results ≤ 2.
- Based on the *in vivo* score category for the selected test/control articles, the corresponding *in vitro* results were appropriately entered and evaluated.
- Results were analyzed using an unpaired t test with Welch's correction.

Partial Thromboplastin Time (PTT)

The following parameters were used to determine the in-scope PTT studies:

- Data obtained from four BSC studies and two WuXi AppTec studies were used to analyze the relationship between clotting time and thrombus formation.
- If two PTT studies were performed on the same test article, then the lower, more conservative value was used for evaluation.
- Normalized values were used for analyses, as the negative control clotting values varied between 157.3 – 368.8 seconds depending on laboratory or protocol used. The normalized values provides for a comparison of results across labs.

Complement Activation (CAA)

The following parameters were used to determine the in-scope CAA studies:

- Data obtained from fourteen BSC studies were used to analyze the relationship between complement activation and thrombus formation.
- If two CAA studies were performed on the same test article then the higher, more conservative, value was used for evaluation.
- C3a results were excluded from this assessment. ISO 10993-4 lists C3a testing as optional, as the SC5b-9 fragment is considered a more robust marker to represent complement activation.

Platelet and Leukocyte Count (PLC)

The following parameters were used to determine the in scope PLC studies:

- Data obtained from seven BSC studies were used to analyze the relationship between platelet counts and thrombus formation.
- Normalized values were used for analyses, as the platelet counts for the negative control (untreated blood) ranged between 120 – 283 X $10^3/\mu L$ depending on test protocol used. The normalized values provides for a comparison of results across labs.

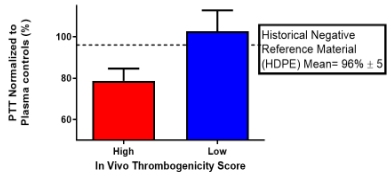
RESULTS

PTT

- Six PTT test results were determined to fall within the high category (*In Vivo* scores with 2 or more results > 2). The average normalized result for this category was 79% of the concurrent negative control.
- Three PTT test results were determined to fall within the low category (*In Vivo* scores with 2 or more results ≤ 2). The average normalized result for this category was 103% of the concurrent negative control.
- The test results in the high category were statistically significantly ($p < 0.05$) lower than the results in the low category Therefore, the results indicate a correlation between increased thrombus formation in the *in vivo* tests and a decreased clotting time in the PTT test.
- Although the sample size of the low category was small, the average normalized results were higher than the historical negative controls. In contrast, the average normalized result for the high category was 17% lower when compared to the historical negative reference material mean value.

PTT (% of negative plasma control)	
High	Low
78	92
87	112
69	104
77	
79	
82	

Partial Thromboplastin Time



CAA

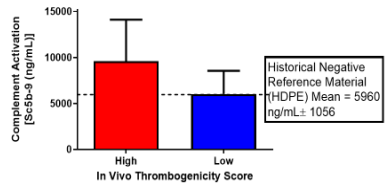
- Eleven CAA test results were determined to fall within the high category. The average result for this category was 9,594 ng/mL ± 4303.
- Seven CAA test results were determined to fall within the low category. The average normalized result for this category was 6,027 ng/mL ± 2520.
- The test results in the high category were statistically significantly ($p < 0.05$) higher than the results in the low category. Therefore, the results indicate that there is a correlation between increased thrombus formation in the *in vivo* tests and an increased SC5b-9 fragment activation in the CAA test.
- Test results for the low category were almost identical to historical negative controls.

CAA SC5b9 Concentration (ng/mL)	
High	Low
7821	6032
11264	8085
16969	5677
4684	10320
5017	3582
8007	3022
3520	5473
16381	
9264	
9451	
13157	

RESULTS

CAA Continued

Complement Activation (Sc5b-9)

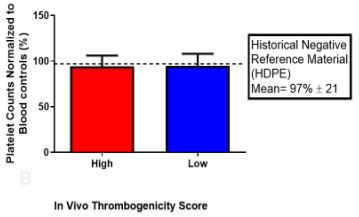


Platelet and Leukocyte Counts

- Six PLC test results were determined to fall within the high category. For the platelet counts, the average test article response in the high category was 94% of the negative control.
- Three PLC test results were determined to fall within the low category. For the platelet counts, the average test article response in the high category was 95% of the negative control.
- There was no statistical significance ($p > 0.05$) between the test article results in the high category compared to the results in the low category. Therefore, the results indicate that there is no correlation between increased thrombus formation in the *in vivo* tests and a decreased platelet counts in the PLC test.
- Test results for the low category were almost identical to historical negative controls.
- Leukocyte Counts were excluded from this assessment. According to ASTM F2888-19, leukocyte counts are less sensitive to evaluate thrombogenic potential and acceptance criteria has not yet been established.

PLC (% of negative blood control)	
High	Low
103	103
105	102
76	80
85	
103	
103	

Platelet Counts



SUMMARY

Our analysis on 17 NAVI studies completed on blood-contacting devices and the corresponding *in vitro* hemocompatibility results for the same devices show that for devices found to have high thrombogenic scores (scores of ≥3 in at least 2 animals):

- PTT results (clotting time normalized to plasma) were on average 20% lower
- SC5b-9 levels were statistically significantly higher
- No association between platelet counts and elevated thrombus scores was observed

DISCUSSION/ CONCLUSION

- The PTT assay is capable of differentiating materials with different thrombogenic potentials (Lu, Q., et. al, 2014). Our results with cardiovascular devices provide further evidence that reduced PTT may be associated with *in vivo* thrombogenicity.
- Overall, SC5b-9 levels were greater in the group with high *in vivo* thrombogenic responses than in the group with low *in vivo* thrombogenic responses. Our results with cardiovascular devices provide evidence that elevated SC5b-9 activation may be associated with *in vivo* thrombogenicity.
- The lack of correlation between platelet count and *in vivo* thrombus formation could be associated with the use of citrated blood in this dataset. Recent publications by Lu, *et al.*, 2015 and Schatz, *et al.*, 2019 have shown that the use of sodium citrate anticoagulation significantly inhibits platelet responsiveness and that minimal heparinization (final concentration 1 unit/ml) optimizes the response to commonly used control materials. Further evaluations utilizing heparinized human blood per ASTM F2888:2019 may improve this correlation.
- Since there is no absolute pass/fail criteria for a clinically acceptable level for PTT, CAA or P&L, it is important to include a marketed comparator device to assess biological relevance of the *in vitro* results. Reduced PTT (50-80% of negative control) was found in 22% (20/89) of the historical dataset. Additionally, the average historical mean PTT for positive controls varied from 31% to 47% between data generated by two different CROs.
- *In vitro* hemocompatibility tests such as PTT, CAA and P&L are conducted in static conditions and they are useful in evaluating hemocompatibility for changes in device materials. There are many challenges in performing a NAVI study and factors such as implant technique, device placement and individual animal differences in thrombotic potential lead to inconsistent thrombogenicity predictions. *In vitro* hemocompatibility tests, possibly combined with data from a dynamic *in vitro* flow loop may provide a feasible and appropriate alternative to using the *in vivo* NAVI model.

REFERENCES

ASTM (2019) Standard Practice for Platelet Leukocyte Count—An *In-Vitro* Measure for Hemocompatibility Assessment of Cardiovascular Materials. ASTM F2888-19. American Society for Testing and Materials.

ISO (2017) Biological evaluation of medical devices - Part 4: Selection of tests for interactions with blood. International Standards Organization, Geneva, Switzerland.

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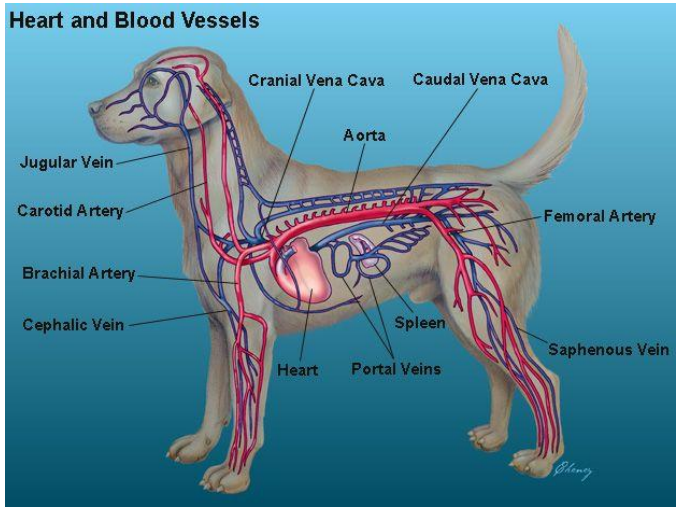
Lu, Q., Shamsie, M., Nehrer, J., and Malinauskas, R. (2014) Partial Thromboplastin Time and Prothrombin Time for the Hemocompatibility Evaluation of Biomaterials. Society for Biomaterials Annual Meeting.

Schatz, T., Howard, S., Porter, D., Grove, K., Smith, M. E., and Chen, Y. (2019) Improved Method for the ASTM Platelet and Leukocyte Assay: Use of Minimal Heparinization in a Screening Test for Hemocompatibility of Blood-Contacting Medical Devices. *Journal of Medical Devices* **13**, 011004.

BACKGROUND NAVI

- Direct blood-contacting devices should be evaluated for thrombogenic potential
- The non-anticoagulated venous implant (NAVI) model is a standard *in vivo* thrombogenicity test
- Method:
  - Absence of anticoagulants
  - Insert up to 15 cm of catheter-shaped device into veins of animals for 4 hours
  - Canine jugular vein
  - N = 2 to 3
  - Control: legally marketed comparator device



NAVI CAVEATS

Caveats in test methodology limit the NAVI model's predictiveness

- inherent differences in "thrombotic potentials" among individual animals
- lack of statistical power
- placement in venous anatomy
- incubation period
- lack of anticoagulants

Reference: ISO 10993-4:2017 Annex C, Table C.3

IN VITRO THROMBOSIS TEST METHODS

Test	Standard	Purpose
SC5b-9 Complement Activation (CAA)	ISO10993-4: 2017	Complement activation has the potential to indicate whether a test article, when exposed to Normal Human Serum, can affect the hemolytic, platelet function, and thrombogenic potential of NHS.
Partial Thromboplastin Time (PTT)	ASTM F2382-18 ISO10993-4: 2017	Clotting time can indicate whether a test article, when exposed to human plasma, has the potential to activate the intrinsic clotting pathway.
Platelet & Leukocyte Counts (PLC)	ASTM F2888-19 ISO10993-4: 2017	Platelet counts can indicate whether a test article, when exposed to human whole blood, has the potential to cause thrombus formation.

OBJECTIVE

In vivo thrombogenicity is influenced by device materials as well as geometry and surface characteristics

Can *in vitro* thrombosis assays predict *in vivo* thrombogenicity?

To conduct a review of previous NAVI studies and determine if there is any correlation between NAVI scores and *in vitro* thrombosis test results

NAVI SCORING SCHEME

Thrombus formation score description	Score
No significant thrombosis (a very small clot is acceptable at insertion).	0
Minimal thrombosis, one location.	1
Minimal thrombosis, multiple locations.	2
Significant thrombosis, >1/4 to ≤ ½ the surface of the implant, vessel patent	3
Significant thrombosis, >½ the surface of the implant, vessel patent	4
Vessel completely occluded	5

RESULTS FROM NAVI STUDIES

Study #	TA Animal #1	TA Animal #2	TA Animal #3	CA Animal #1	CA Animal #2	CA Animal #3
1	4	3	3	1	2	2
2	3	0	1	4	0	3
3	2	4	2	3	2	1
4	1	3	3	0	3	3
5	3	3	2	3	3	3
6	3	2	2	1	2	2
7	4	4	3	0	2	0
8	1	0	1	2	1	3
9	3	4	4	2	3	3
10	0	0	2	2	3	4

NAVI RESULTS Continued

Study #	TA Animal #1	TA Animal #2	TA Animal #3	CA Animal #1	CA Animal #2	CA Animal #3
11	4	4	4	4	4	4
12	3	1	4	3	2	4
13	3	4	4	4	4	3
14	2	0	2	2	0	3
15	1	1	2	0	0	1
16	2	3	2	2	3	2

NAVI scores of 3 and higher in 2 or more animals occurred in half of this dataset.

NAVI SCORES WERE VARIABLE

Study #	TA Animal #1	TA Animal #2	TA Animal #3	CA Animal #1	CA Animal #2	CA Animal #3
1	4	3	3	1	2	2
2	3	0	1	4	0	3
3	2	4	2	3	2	1
4	1	3	3	0	3	3
5	3	3	2	3	3	3
6	3	2	2	1	2	2
7	4	4	3	0	2	0
8	1	0	1	2	1	3
9	3	4	4	2	3	3
10	0	0	2	2	3	4

NAVI RESULTS WERE OFTEN INCONSISTENT

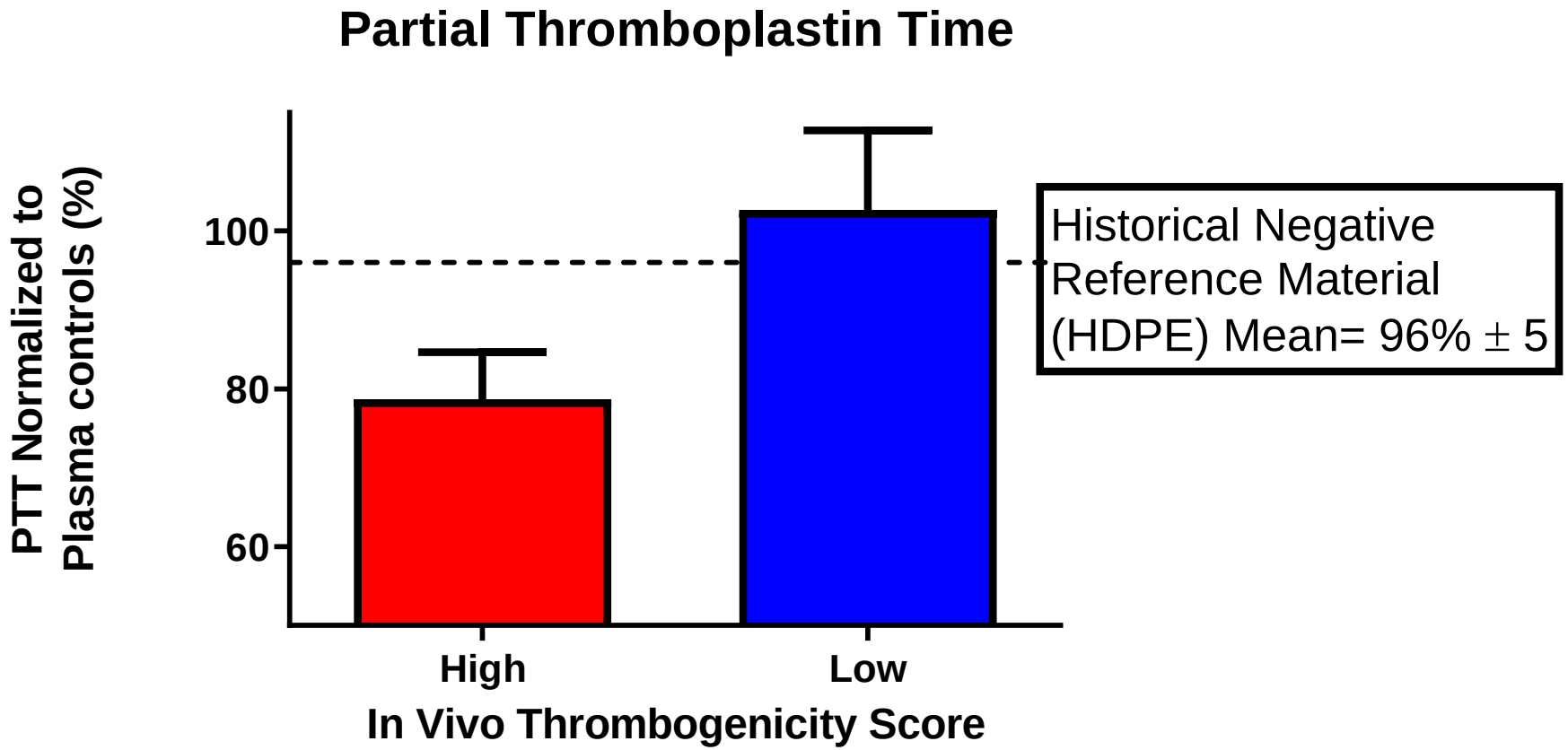
Study #	TA Animal #1	TA Animal #2	TA Animal #3	CA Animal #1	CA Animal #2	CA Animal #3
11	4	4	4	4	4	4
12	3	1	4	3	2	4
13	3	4	4	4	4	3
14	2	0	2	2	0	3
15	1	1	2	0	0	1
16	2	3	2	2	3	2

Scores differed by 2 among three animals in almost half of the dataset (14/32 = 44%).

ANALYSIS METHOD

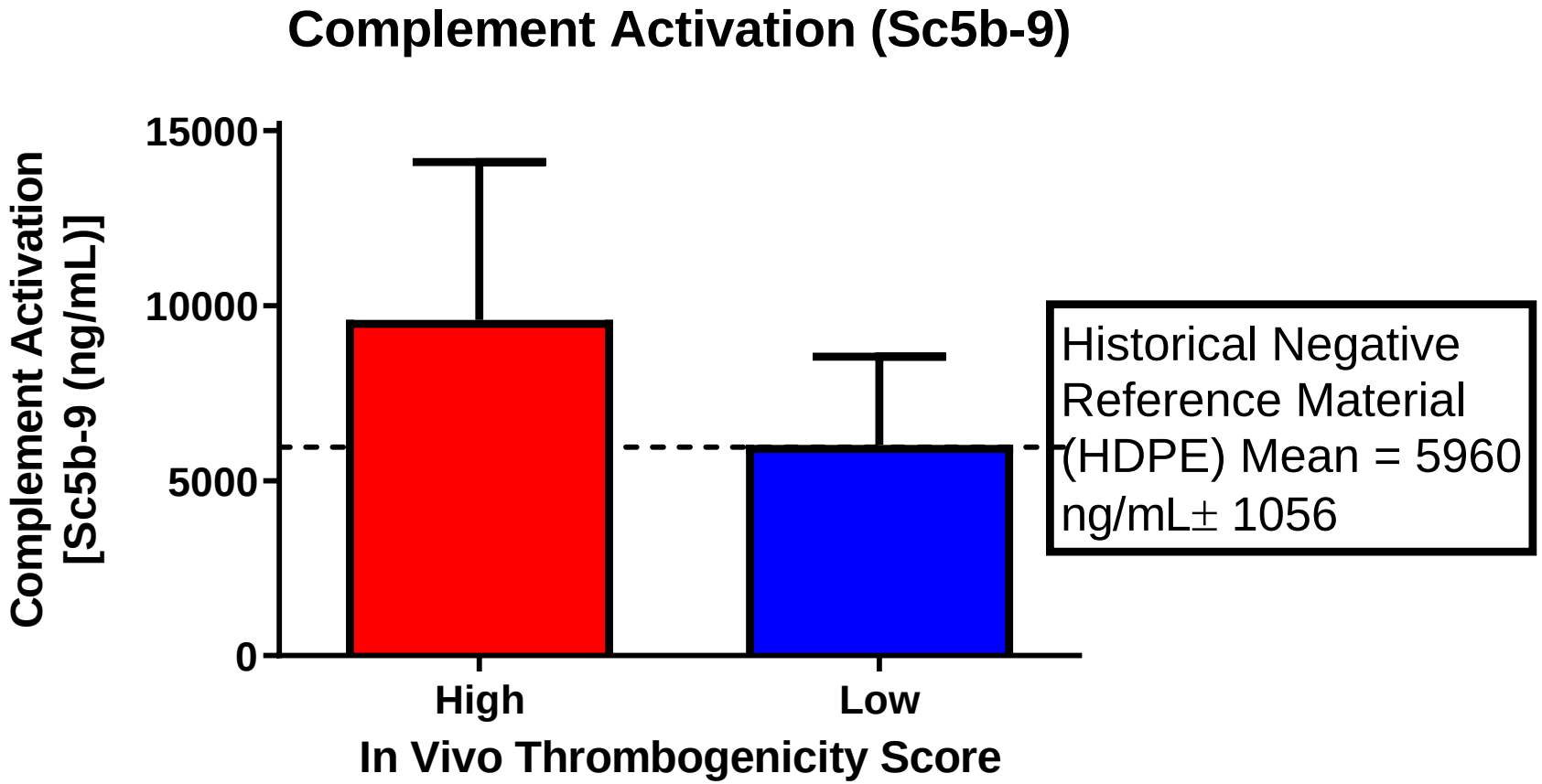
- *In vivo* NAVI test/control article results were split into 2 categories:
  - HIGH with scores of ≥ 3 in at least 2 animals
  - LOW with scores of ≤ 2 in at least 2 animals
- The corresponding *in vitro* results were evaluated
- Results were statistically analyzed

PTT vs. NAVI



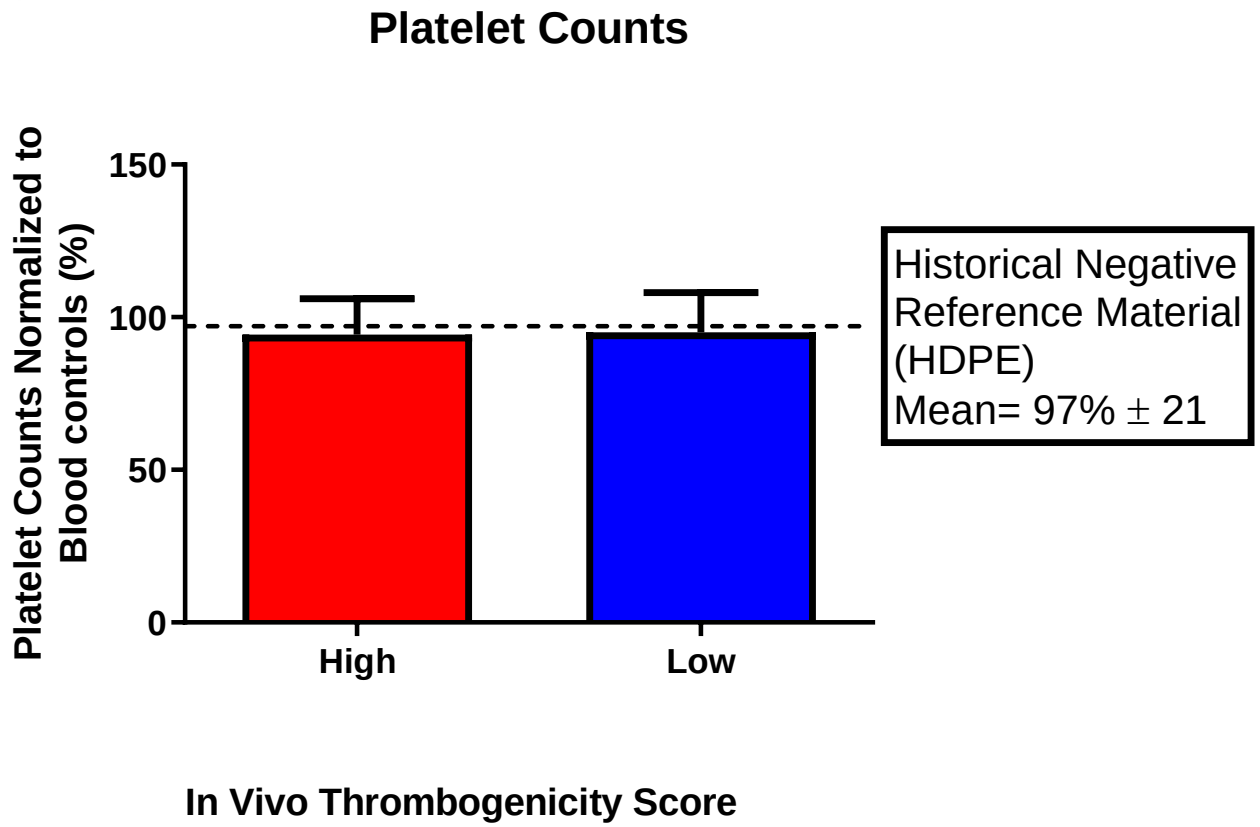
PTT results (clotting time normalized to plasma) were on average 20% lower in devices associated with high NAVI scores.

SC5b-9 vs. NAVI



SC5b-9 levels were statistically significantly higher in devices associated with high NAVI scores.

PLATELET COUNTS vs. NAVI



No association between platelet counts and elevated thrombus scores was observed. Citrated human blood was used in these studies.

CONCLUSION

- Variable results and elevated thrombus scores are commonly reported in NAVI studies - Inconsistent thrombogenicity predictions
- Reduced PTT and elevated SC5b-9 activation may be associated with *in vivo* thrombogenicity
- PTT, CAA and P&L are useful in assessing material-mediated thrombogenicity
- These *in vitro* assays conducted in static conditions, possibly combined with *in vitro* flow loop to cover dynamic, flow-mediated thrombosis, may provide a feasible alternative to NAVI

Key Takeaways

- Since there is no absolute pass/fail criteria for a clinically acceptable level for PTT or CAA, it is important to include a marketed comparator device to assess biological relevance of the *in vitro* results
- PTT testing should be performed with one of FDA's "preferred" analyzers
  - New analyzer is more sensitive
  - Reduced PTT (50-80% of negative control) was found in 24% (13/54) of test articles and 19% (7/36) of marketed comparator at WuXi using new analyzer and updated methods. HDPE was about 96%. Positive control was 47%.
- Follow latest version of ASTM F2382-18 PTT and ASTM F2888-19 for P&L

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- ASTM (2019) Standard Practice for Platelet Leukocyte Count—An *In-Vitro* Measure for Hemocompatibility Assessment of Cardiovascular Materials. ASTM F2888-19. American Society for Testing and Materials.
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THANK YOU!

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