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THROMBOGENICITY TESTING RESULTS FOR CONTROL LEGALLY MARKETED COMPARATOR DEVICES (LMCD): COMPARISON BETWEEN TRADITIONAL NON-ANTICOAGULATED VENOUS IMPLANT (NAVI) ASSAY AND AN *IN VITRO* OVINE BLOOD LOOP TEST

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ABSTRACT

Legally marketed comparator devices (LMCD) are required by many regulatory bodies in as a control for thrombogenicity testing when evaluating new devices. It is assumed by both the medical device manufacturing industry and regulatory bodies that these LMCD's have good clinical history and should perform with no to minimal thrombus accumulation and thereby serve as valid negative controls for the assay. APS regularly performs these assays for many medical device manufacturers, all of whom select a predicate comparator device (required by FDA to be an LMCD), for both the in vivo Non-Anticoagulated Venous Implant (NAVI) assay as well as a custom in vitro blood loop AVI. In this retrospective analysis, we have compiled thrombogenicity scores of control/predicate devices (limited to assays which used LMCD's), both the discrete score from the classification standard scoring scheme and the continuous values obtained from the percent surface area associated with thrombus. We have compared results from 37 NAVI studies and 22 in vitro blood loop studies. These compiled results show ~25% of LMCDs score ≥3 (>50% of the surface covered in thrombus) in the NAVI model while <5% of LMCDs score ≥3 (>50% thrombus) in the Blood-Loop assay. In addition, the median score and mean % thrombus for LMCD in the blood loop assay is substantially lower than the median and mean scores for LMCD in the NAVI assay. This retrospective assessment highlights a high proportion of false-positive scores for LMCD in a large number of NAVI assays.

Keywords: hemocompatibility, thrombogenicity, medical devices.

1. INTRODUCTION

ISO 10993-4 thrombogenicity testing is widely used for meeting regulatory requirements for approval of blood-contacting medical devices [1]. We are continuing to develop an in vitro thrombogenicity assay using minimally heparinized ovine blood that has been successfully used in lieu of the non-anticoagulated venous implant (NAVI) assay in recent submissions with the US FDA. Our blood loop assay is primarily used for testing thrombogenicity of test articles (catheter-shaped devices typically less than 18 Fr) and comparing them to similar predicate devices. We also frequently perform the traditional NAVI model in canines. The NAVI test requires 2-3 naïve animals, typically large dogs, and also compares the performance of a test article (a new product or new manufacturing procedures/materials in development) to a predicate. In both the blood loop and the NAVI assays, predicate devices are typically legally marketed comparator devices (LCMD). One observation that is rarely discussed is the high thrombogenicity scores of LCMD's in the NAVI assays. Ideally, it is expected that the test article will have an equal or lower relative thrombus score than the LCMD. Using the ISO 10993-4, Table C.2 scoring scheme, a score of 2 or lower (<50% of the surface covered with thrombus) is associated with minimal to no risk for thrombogenicity in clinical use. We performed this retrospective analysis to further characterize the cumulative performance of the NAVI assay and also to contrast this assay to a new alternative in vitro assay based on a circulating blood loop.

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2. METHODS

2.1 Materials

Legally Marketed Comparator Devices (LMCD) were used in all the assays surveyed for this report. For the blood Loop assay, negative control catheters, CBAS® Heparin-Coated Catheters, and positive control catheters, Solomon Polyurethane uncoated Medical Grade 5Fr tubing, were both from Instech Laboratories (Plymouth Meeting, PA).

2.2 Humane Use of Animals

The use of all animals in this study was with the review and approval of the American Preclinical Services Institutional Animal Care and Use Committee (IACUC). In Vitro Blood-Loop studies were performed under an approved protocol using a selected group of sheep as donors for the blood loop assay. The Non-Anticoagulated Venous Implant (NAVI) protocol followed ISO-10993 guidance in canines for deployment and assessment of both control and test devices.

2.3 *in vitro* BLOOD LOOP ASSAY

2.3.1 Blood Sourcing

Blood was obtained from donor sheep ranging in age from 1-4 years. For the blood loop assay, fresh ovine blood (~500 mL) was collected from healthy donor sheep allowing a minimum of two weeks for recovery between draws. During venipuncture and collection, porcine heparin was added to a final concentration of 1 IU per mL of blood in the collection bag (IntraVia™ Container, 1000mL Capacity, Baxter). All donor animals were free from aspirin, ibuprofen, acetaminophen, heparin, Coumadin™ or any other anticoagulant medication for a minimum of 14 days prior to obtaining blood.

2.3.2 Loop Preparation

For typical assays, four independent blood loops were prepared [2, 3]. Two loops were prepared by inserting up to 3 positive controls in one loop, and 3 negative controls into another loop. Test articles were inserted into the third loop and a control (LMCD) device was inserted into the fourth. The blood was then introduced and each loop was filled to capacity (total volume 100±5 mL/loop), the loops were sealed, and the blood was circulated using a peristaltic pump. Typical flow in the system was measured at 0.5 L/min. Finally, the loop was partially submerged in a heated chamber and held in place for the duration of the incubation.

2.3.3 Device Retrieval and Preparation for Assessment

After 4 hours, the pump was stopped, the loop was removed, opened and the blood was drained. The tubing segments containing either the control or test articles were cut longitudinally and evaluated for thrombus associated with the study article.

Table 1: Thrombogenicity Scoring

Score	Thrombus formation score description
0	**Thrombus non-existent or minimal and, if present, appears to be associated with implant venotomy site or loop insertion site**
1	**Thrombus minimal, observed to be covering 1 % to 25 % of material surface.**
2	**Thrombus moderate, observed to be covering 26 % to 50 % of material surface.**
3	**Thrombus severe, observed to be covering 51 % to 75 % of material surface.**
4	**Thrombus extensive, covers 76 % to 100 % of material surface.**

2.3.4 Thrombogenicity Scoring, *in vitro*

A digital image was obtained and used to document the visual assessment quantifying relative % of thrombus on the surface of the study article. Surface area of the thrombus was determined by measuring each region of thrombus with a calibrated ruler or a digital caliper. Percent thrombus was recorded for all study articles and in addition, a thrombus score was determined on a scale of 0-4 using the scheme in Table 1.

2.4 *In vivo* NAVI ASSAY

2.4.1 Surgical Preparation and Anesthesia Induction

In preparation for the surgical procedure, the dogs were fasted beginning overnight prior to anesthesia which was maintained throughout the procedure by inhaled Isoflurane [3]. Jugular vein or iliac vein access sites were identified and a percutaneous access was obtained by the surgeon and a guide wire introduced. The study articles (either test article or LMCD) was deployed into the vein to the desired depth, the devices were then fixed in place and allowed to remain undisturbed for ~4 hours. Blood samples were collected via the cephalic IV access pre- and post-device implantation for determination of Activated Clotting Times (ACT). In addition, a complete blood count (CBC) was also performed to assess the health of the animal (prior to implant). At termination, heparin was administered IV at a total dose of 20,000 IU to assure no post-mortem coagulation. The animal was then euthanized by administration of IV Euthasol™ and transferred to the necropsy suite.

2.4.2 Device Dissection and Assessment

The deployment sites were exposed and the vein containing the deployed study articles was photographed *in situ*. After explantation, the isolated vessels were photographed. The veins were opened lengthwise, gently rinsed with saline to remove

remaining blood. The *in situ* devices were exposed and gently rinsed with saline.

2.4.3 Thrombogenicity Scoring, *in vivo*

A digital image was obtained and used to document the visual assessment quantifying relative % of thrombus on the surface of the device, catheter or tubing Scoring was performed as described for the *in vitro* model above (see **Table 1**).

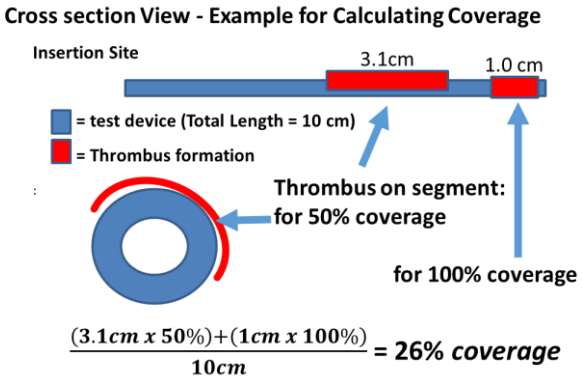

Figure 1. Calculation of thrombus coverage was performed by measuring each segment with a caliper then determining the approximate circumference of the thrombus formation around the study article. The total coverage of an identified area will be calculated by taking the length of the thrombus formed, multiplied by the percentage of the study article covered and divided by the total length implanted. The coverage of each segment will be summed.

3. RESULTS

Data for this retrospective analysis was taken from a series of 25 in vivo NAVI tests and 12 in vitro blood loop assays all using predicate LMCDs as control articles. These LMCD's were from a wide range of catheter-like medical devices, but all of similar shape and the length evaluated was between 15 and 30 cm in length. All assays were performed over a period of ~20 months. All NAVI assays used ISO 10993-4 compliant protocols. All surface thrombus measurements were performed according to the procedures described in Figure 1. In addition to the observed values for percent thrombus formation on the device surface, a thrombogenicity score was assigned using the scoring scheme in Table 1. The NAVI tests do not have any internal positive or negative controls to aid in assay consistency and animal-to-animal variability other than acceptance levels for platelet count and ACT. However, the in vitro blood loop assay does have both positive and negative control materials exposed to the same blood circulating in identical configurations to the test articles and LMCDs with similar assessments performed at the 4 hour endpoint. These positive and negative control materials from the blood loop are evaluated in an identical manner as the test or comparator article and their corresponding thrombogenicity scores were also assigned.

The observed thrombus scores for all LMCDs from the assays under evaluation along with a similar group of LMCDs in the blood loop model are shown in Figure 2. The percent thrombus formation values for the positive and negative controls associated with the blood loop assays are also shown in Figure 2. The assigned thrombogenicity scores for all articles are shown in Figure 3.

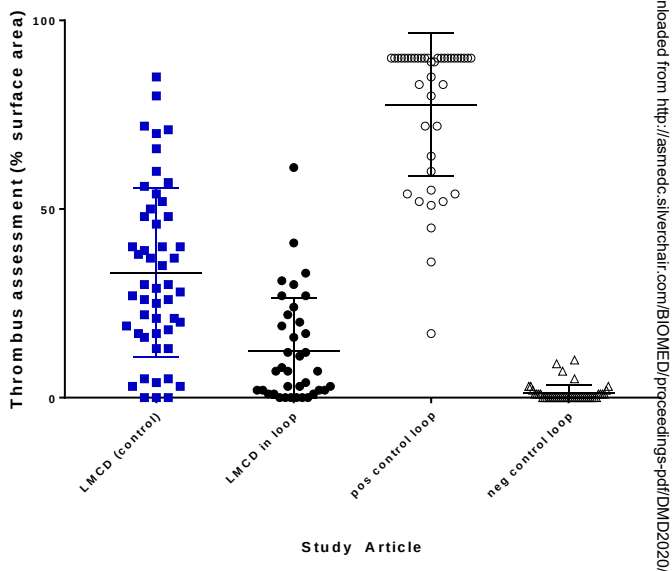

Figure 2: Comparison of thrombus values (per cent surface) for LMCD in the NAVI and the blood-loop assays along with and positive and negative controls from the blood loop. Lines and bars indicate mean and standard deviation.

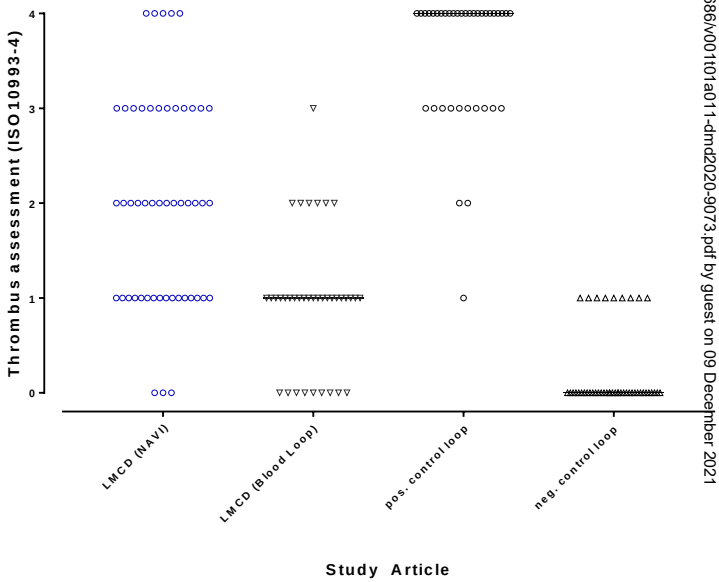

Figure 3: Comparison of thrombus scores (assigned from Table 1, ISO10993-4 guidance) for LMCD in the NAVI and the

blood-loop assays along with and positive and negative controls from the blood loop.

4. CONCLUSIONS

APS performs a large number of both the NAVI assay and the *in vitro* blood loop assay for thrombogenicity assessment of catheter-like blood-contacting medical devices. Both of these assays are performed following standardized protocols consistent with the ISO-10993 standards and developed over several years incorporating recommendations from the FDA. All these tests were performed under Good Laboratory Practices (GLP) and were typically submitted for regulatory review. There are many sources of variation historically associated with the NAVI assay and they have been well characterized in the review by Wolf and Anderson [4]. The protocols at APS strive to minimize these potential sources of variability, nonetheless, a substantial number of assays resulted in outcomes where the LMCD scores surprisingly higher than expected.

As a general rule, all new catheter-like test articles requiring thrombogenicity assessment were evaluated in either the *in vivo* or the *in vitro* assay, dependent on each Sponsor's regulatory strategy for the specific program. In all cases, due to regulatory requirements, an LMCD was used as a control or comparator in the NAVI assay. In addition, the positive and negative controls were deployed in the *in vitro* assay. These LMCDs were expected to result in none to minimal thrombus formation (from 0-25% of the surface area of the device and 0-2 thrombogenicity score) and therefore support the validity of the assay. We obtained permission from a group of sponsors to compile data on LMCDs evaluated in both assays over a 20 month period and performed this retrospective analysis. Although ISO guidelines typically use scores (0-4) to rank materials we also used the actual percent thrombus formation (a continuous variable) on the LCMD surface in addition to the thrombus score obtained from each individual study. Use of these continuous data allowed for calculations of means for each assay and with continued assessment of the data, a statistical assessment can be performed allowing normal ranges for performance of LMCDs in both assays along with 95% confidence intervals.

These compiled results show a substantial percentage of LMCDs (~25%) score ≥3 (a value associated with >50% of the surface covered in thrombus) in the NAVI model. However, a much lower frequency of LMCDs (<2%) scored ≥3 (>50% thrombus) in the Blood-Loop assay. In addition, the median score and mean % thrombus for the group of LMCDs in the blood loop assay is substantially lower than the median and mean scores for LMCD in the NAVI assay (median 2 and mean 33.2 ±22 for the NAVI vs. a median 1 and mean 12.3±14 % for the blood loop). If the LCMD scores a 3 or higher (>50% of the surface covered with thrombus), the interpretation of the test becomes problematic even if the test article is equivalent to the LCMD and may result in difficulty when reviewed by regulatory bodies. The high number of false negative results in this analysis is an alarming observation; that a standardized assay so frequently required in regulatory submissions yields scores for LMCDs in a range that would appear to predict risk for clinical use (>50% of the material surface covered with thrombus). If these scores were obtained from unapproved new test articles, they would likley encounter regulatory difficulty based on this assay outcome alone.

We evaluated a broad range of catheter-like LMCDs in two assays for thrombogenicity. The requirement for use of LMCDs, by their nature, having regulatory approval and with an assumed low frequency of clinical occurrences of thrombus formation and low clinical risk of thrombogenicity is a sensible means of controlling what has been known to be difficult and problematic assay process. The high frequency of the observations of percent thrombus formation values and associated thrombogenicity scores of ≥3 for LMCDs highlights the deficiency in the performance NAVI assay. In comparison, the in vitro blood loop assay results where assay performance of LMCDs was much more consistent with their clinical performance and regulatory history. In addition, the blood-loop assays also carries the enhanced support by concurrent use of well-behaved positive and negative controls to address the variability of the *in vitro* assay. Overall, these results are strong supportive evidence for the superiority of the Blood Loop assay over the *in vivo* alternative NAVI.

ACKNOWLEDGEMENTS

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