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Thromboelastography Values from Pigtail Macaques (*Macaca nemestrina*): Effects of Age and Sex

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Thromboelastography is a clinical laboratory test used to assess global hemostasis. With technologic advances and the test's reemergence in human medicine, its utility in veterinary medicine is being explored. Because assays for PT, aPTT, and d-dimers require platelet-poor plasma, whereas thromboelastography is performed on whole blood, thromboelastography provides a more accurate representation of coagulation and allows the identification of hypocoagulable, hypercoagulable, and hyperfibrinolytic states. Conflicting information has been reported about the effects of age and sex on thromboelastography in humans and animals. Human studies have reported significant effects of age and sex on thromboelastography more often than have animal studies, but few publications are available about thromboelastography in the nonhuman primate and laboratory animal literature. We used a sample of 50 pigtail macaques (*Macaca nemestrina*) to determine whether age or sex influence thromboelastography values. Of 5 measured and 2 calculated variables produced by thromboelastography, sex had a significant effect only on the lysis-30 parameter, which also showed significant interaction between age and sex; values increased with age in male macaques but decreased with age in female macaques. In addition, we used the data to define reference intervals for thromboelastography parameters in pigtail macaques.

Abbreviations: α, α angle of thromboelastography tracing; CI, coagulation index; G, clot strength; K, time from R to a 20-mm spread on the thromboelastography tracing; LY30, percentage of clot formation at 30 min after MA; MA, maximal amplitude; R, reaction time (to clot formation); RoTEM, rotational thromboelastometry.

Thromboelastography is a clinical laboratory test that measures the viscoelastic properties of clotting whole blood to assess global hemostasis. Although it was developed more than 60 y ago, thromboelastography was used only rarely in human medicine until the 1980s. Since the development of the associated assays, coagulation traditionally has been monitored though PT, aPTT, fibrinogen level, and concentration of d-dimers. However, the use of thromboelastography has increased recently due to technologic advances and the need to monitor coagulation during complex surgeries, such as liver transplantation and cardiac bypass. Thromboelastography is now used often to assess causes of hemorrhage and to guide transfusion decisions,[41,59] and this tool is a better indicator of hypercoagulability than is PT or aPTT.[45] Because PT and aPTT are performed on platelet-poor plasma, they measure secondary hemostasis only, which involves the activation of coagulation factors leading to crosslinked fibrin formation. In contrast, because thromboelastography is performed on whole blood, it measures both secondary hemostasis and the platelet aggregation component of primary hemostasis. Plasma lacks other blood components, in addition to platelets, that have important roles in hemostasis. For example, neutrophil serine proteases help trigger blood coagulation and stabilize thrombi.[39] Therefore, compared with PT and aPTT, thromboelastography provides a more accurate and representative picture of hemostasis, and allows for the identification of hypocoagulable, hypercoagulable, and hyperfibrinolytic states.

Figure 1 shows a typical thromboelastography tracing with 4 labeled parameters: reaction time (R), K time (K), α angle (α), and maximum amplitude (MA). R is the time (in minutes) to initial clot formation. K is the time (in minutes) from R to a standardized spread of 20 mm on the thromboelastography tracing. The α angle is created by the maximum tangent to the tracing and is a measure of the rate of clot formation. MA is the maximal width of the thromboelastography tracing and is a measure of clot strength. Lysis 30 (LY30) is the percentage of clot lysis at 30 min after MA. In addition, numerous reports have used calculated values of clot strength (G) and coagulation index (CI).[5,11,13,19,26,32,46,48,51,62,64] In veterinary medicine, reference intervals for thromboelastography have been established in dogs, cats, horses, and pigs,[1,5,16,57,61,63] and data from limited sample sizes have been reported in rats, mice, and rabbits.[23,28,43] Researchers have assessed thromboelastography in small numbers of nonhuman primates for specific disease model comparisons,[23,55] and there have been only 2 publications with substantial sample sizes regarding thromboelastography reference values in nonhuman primates.[13,52] The first study evaluated thromboelastography reference values in 36 rhesus macaques (*Macaca mulatta*; age, 3 to 5 y) in China,[13] whereas the second used rotational thromboelastometry (RoTEM), an alternative form of thromboelastography currently approved in Europe, to examine reference values in 40 cynomolgus macaques (*Macaca fascicularis*; age, 3 to 10 y). However, RoTEM values are not interchangeable with those obtained by using thromboelastography.[56]

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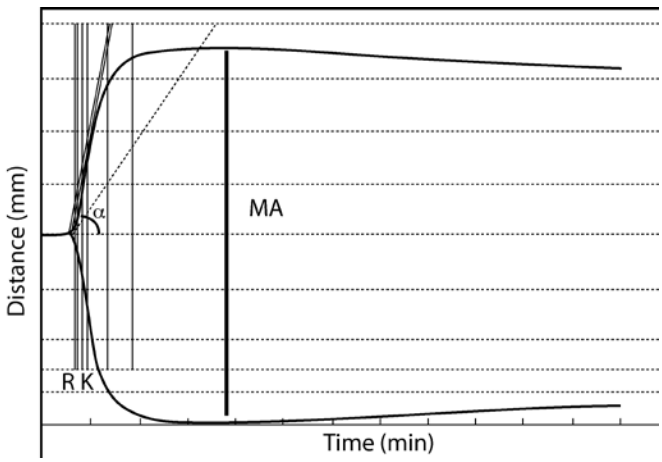

Figure 1. An example of a standard thromboelastography tracing from a 3.58 y male pigtail macaque in our study with the following labeled parameters: R is the time to initial clot formation, K is the time from R to a standardized spread of 20 mm, α is the angle created by the maximum tangent to the tracing, and MA is the maximal width between the lines.

In various species, investigators have reported conflicting results of the effects of age and sex on thromboelastography values.[5,11,13,19,34,46,48,56] In the aforementioned rhesus study,[13] the authors found an effect of sex on LY30 but did not have an appropriate age distribution to study its effects. The RoTEM study in cynomolgus did not find any effects of age or sex.[52] Dogs, like nonhuman primates, did not demonstrate any statistically significant effects of sex on thromboelastography.[5] More studies in humans have reported significant effects of age and sex on thromboelastography than have animal studies,[11,19,34,46,48] although these effects are not seen consistently. No differences were noted among age groups of healthy children or between children and adults.[11] Similarly, aging was not associated with hypercoagulability,[48] although other authors reported that hypercoagulability increased with aging in normal healthy adults.[46] In terms of sex-associated effects, one study revealed significantly higher coagulability in every thromboelastography result, except R, for women compared with men.[46] Other researchers reported increasing coagulability in women compared with men in all thromboelastography values.[19,48] Finally, in a human RoTEM study, coagulation trended toward hypercoagulability in women compared with men and with increasing age.[34]

The goal of the current study was to provide a more comprehensive examination of thromboelastography in nonhuman primates, with a larger and more diverse sample in terms of age and sex. It is the first report of thromboelastography in pigtail macaques (*Macaca nemestrina*) and the first study to determine the effects of age on thromboelastography in nonhuman primates. Based on the human literature, our hypothesis was that age and sex would affect thromboelastography in pigtail macaques. We expected to find increased coagulability in female and older animals. In addition, we present reference values for thromboelastography in pigtail macaques.

Materials and Methods

Research was approved by the IACUC of the University of Washington and was conducted in accordance with the *Guide for the Care and Use of Laboratory Animals*.[22] The macaques were singly, paired, or group-housed indoors at 2 facilities at the Washington National Primate Research Center (Seattle, WA). Samples were excluded retrospectively for abnormalities in CBC or chemistry screens within 6 mo prior to sampling, with particular emphasis on platelet count, PCV, and albumin. Other exclusion criteria included pregnancy, medication administered within 6 half-lives of the drug, and experimental history with permanent biologic effects. None of the macaques were currently assigned to research projects. Animals (n = 50) were selected from our colony to ensure even distributions of both age and sex (Table 1). Ages to the nearest month were calculated at the time of blood collection. The average age of male macaques was 7.44 y (range, 2.08 to 19.33 y) whereas that for female macaques was 8.12 y (range, 2.00 to 16.75 y). Blood collection was scheduled to coincide with sedation for semiannual tuberculosis testing to reduce the number of sedations and venipunctures. Animals were fasted for at least 12 h prior to sedation with ketamine hydrochloride (10 mg/kg; Fort Dodge, Fort Dodge, IA) admixed with atropine (0.04 mg/kg; Baxter Healthcare, Deerfield, IL). Physical exams were performed on all sedated animals as part of routine colony health. In addition, CBC and chemistry screens were collected on 36 of the 50 macaques for routine blood screening. Samples for CBC were collected into 2- or 4-mL K_2-EDTA vacuum phlebotomy tubes (Becton Dickinson, Franklin Lakes, NJ), and those for chemistry screens were collected into 3.5-mL serum separator vacuum phlebotomy tubes (Becton Dickinson). Six members of the veterinary staff collected samples for thromboelastography from the region of the femoral triangle (vein or artery), a standard site of venipuncture in nonhuman primates. Blood was collected by using 10-mL syringes with preattached 21-gauge 1-in. needles (Becton Dickinson) or vacuum phlebotomy tube sets (Push Button Blood Collection Set, Becton Dickinson) and placed into 4.5-mL vacuum phlebotomy tubes containing 3.2% buffered sodium citrate (Becton Dickinson). All tubes were filled to the specified volume to maintain a consistent ratio of blood to sodium citrate. With the exception of one sample that had a transit time of 21 min, transit times varied from 1 h 14 min to 2 h 56 min from time of blood collection to time of sample entry at the University of Washington Medical Center Clinical Coagulation Laboratory. Samples were stored and transported at room temperature. Samples were collected from 24 June to 31 August 2010.

In general, thromboelastography is performed on 0.34 mL of native whole blood (as a point-of-care test) or citrated blood, which enables storage for 4 to 6 h.[7,34] Activators, such as tissue factor and kaolin, typically are added to the blood to promote initiation of coagulation. Blood then is placed into a heated cup that is oscillated around a pin at an angle of 4°45′ for 10-s cycles. The pin measures clot formation, which is electrically transduced into a thromboelastography tracing. Different methods of performing thromboelastography, such as comparing citrated and whole blood or tissue-factor compared with kaolin activation, affect thromboelastography values.[4,7,11,24,46,65] In our study, 20 µL 0.2 M $CaCl_2$ (Haemonetics, Braintree, MA) was pipetted into a sample cup. Then 1 mL citrated blood was added to a vial of kaolin (Haemonetics) and mixed briefly; 340 µL activated blood then was added to the sample cup and analyzed (model 5000 Thromboelastograph Hemostasis Analyzer, Haemonetics). Three analyzers were used; electronic quality control and internal controls were run daily. In addition, the analyzers are serviced every 6 mo. Samples were run at 37 ± 0.5 °C by 4 or 5 trained operators.

CI originally was derived as a discriminant analysis to determine the weights given to different thromboelastography values to identify hypercoagulability in humans.[8] CI is centered on 0 so that values less than −3 indicate hypocoagulability, and those greater than 3 indicate hypercoagulability. By using the

Table 1. Demographics of the pigtail macaques selected to examine the effects of age and sex on thromboelastographic values obtained by using kaolin- activated, citrate-stored whole blood from pigtail macaques

	Age (y)			
	0–5	5–10	>10	Total no. of macaques
Male	9	10	7	26
Female	7	10	7	24
Total no. of macaques	16	20	14	50

There is a relatively uniform distribution of age and sex.

formula from the human literature, CI was calculated as CI = $-0.1227 \times R + 0.0092 \times K + 0.1655 \times MA - 0.0241 \times \alpha - 5.022$.[19,20] G (kdyn/cm^2) was calculated by using the formula $G = 5000 \times MA/(100 - MA)$.[20,30] All statistical analyses were performed by using Systat version 7.0 (Systat Software, Chicago, IL). Skewness and kurtosis were examined to test for normality of the data, and F_{max} tests were used to test for homogeneity of variance. All data were normally distributed. Age was considered a continuous predictor variable, whereas sex was categorical; hypothesis tests of relationships between these variables and blood measures were conducted by using general linear model techniques, including interaction between age and sex. A Student *t* test was used to determine effects of time to sample analysis. A Pearson correlation analysis was used to examine the relationship between platelet count and LY30. Null hypothesis probabilities less than 0.05 were considered statistically significant.

Results

Of the 57 pigtail macaques, 7 were excluded from the study: 1 because she was determined to be pregnant and the remaining 6 because of low (less than 2.5 g/dL) albumin. The remaining 50 animals had albumin concentrations (mean ± 1 SD) of 3.16 ± 0.32 g/dL. Therefore, the threshold for defining hypoalbuminemia corresponds well with the lower reference range that would be calculated by mean ± 2 SD (2.52 g/dL). Of the 50 macaques, only 4 had received any medication within 1 wk of venipuncture, and all had withdrawal periods than exceeded 6 half-lives of the drugs. Furthermore, 13 of the 50 macaques had been assigned previously to a research project but had been returned to the colony without significant effects on health. The remaining 37 animals were either blood or bone marrow donors, breeders, or pending research assignments. Of the 50 macaques examined, 36 had CBC and chemistry screens performed concurrently with thromboelastography for routine health screening. The average platelet count was 488 ± 102.5 × 10^3/μL and the average hematocrit was 40.00% ± 3.35%. The remaining 14 animals had CBC and chemistry screens performed within 7 mo of venipuncture (11 of the 14 were within 1 mo). Average platelet counts (492 ± 79.3 × 10^3/μL) and hematocrit values (41.14% ± 2.54%) for these 14 macaques were similar to those of the other 36 macaques in the study.

Time between venipuncture and sample entry at the laboratory varied from 1 h 14 min to 2 h 56 min (except for one sample with a 21-min transit time). However, this timing cannot be used to determine time from venipuncture to thromboelastography analysis, given that 5 samples were logged into the computer as received in less than 4 h but were marked by the laboratory as processed more than 4 h after sample collection. Therefore, 45 of 50 samples were run from 1 to 4 h after venipuncture. A Student *t* test demonstrated there were no significant differences in thromboelastography values between samples run in 4 h or less compared with more than 4 h. Samples evaluated in 4 h or less ($n = 45$) and more than 4 h ($n = 5$) after collection yielded the following results (mean ± 1 SD), respectively: R, 3.22 ± 0.60 min compared with 3.42 ± 0.55 min; K, 0.85 ± 0.09 min compared with 0.88 ± 0.11 min; α, 78.67° ± 1.98° compared with 77.84° ± 2.01°; MA, 69.67 ± 5.59 mm compared with 67.9 ± 3.74 mm; LY30, 7.58% ± 9.45% compared with 4.5% ± 3.49%; G, 12.06 ± 3.25 kdyn/cm^2 compared with 10.75 ± 1.90 kdyn/cm^2; and CI, 4.23 ± 0.91 compared with 3.93 ± 0.62.

Reference values established in this study are shown in Table 2. There were no significant differences found for age or sex on R, K, α, MA, G, or CI. Sex had a significant ($P < 0.05$) effect on LY30, which also showed significant ($P < 0.001$) interaction between age and sex (Figure 2). In male pigtail macaques, LY30 increased with age, whereas in female pigtail macaques, LY30 decreased with age. Macaques with high LY30 values had unusual thromboelastography tracings, which slowly angled back toward but did not fully return to baseline (Figure 3). The Pearson correlation coefficient between platelet count and LY30 was $r = 0.019$ ($P = 0.89$).

Discussion

Whereas reference values for thromboelastography have been reported for other common laboratory animal species,[1,5,57,61,63] reference values for thromboelastography and RoTEM in nonhuman primates have been reported only once each.[13,52] Although a previous thromboelastography study examined the effect of sex,[52] the current study is the first to examine the effects of age on thromboelastography in macaques. In addition, sample size was much larger in the current study ($n = 50$) than in either previous nonhuman primate study.[13,52] Values from the previous cynomolgus study[52] cannot be compared directly with our current data, because RoTEM and thromboelastography values are not interchangeable.[56] However, our results for R, K, and LY30 in pigtail macaques differ markedly from those reported for rhesus macaques.[13] Multiple explanations could account for these different results, including differing methodologies. The rhesus study[14] used citrated blood and the same model of analyzer but did not provide further details about their methods, such as which activator was used. For venipuncture, the rhesus macaques were conscious whereas our animals were sedated with ketamine. Although ketamine reportedly does not affect coagulation in humans and nonhuman primates,[18,21] conscious compared with sedated venipuncture could still affect coagulation due to potential stress or trauma. An additional possible explanation is species-specific differences between pigtail and rhesus macaques. Although species-specific differences may exist, we have performed thromboelastography in small numbers of rhesus and cynomolgus macaques at our institution and found them to yield values similar to those of pigtail macaques.

Our thromboelastography values indicate that coagulability is greater in pigtail macaques (CI, 2.44 to 5.76) than in humans (published reference ranges of –5.1 to 3.6 and –4.3 to 1.3).[12,48] Others have reported a CI reference range of –1.93 to 0.56 for men and 0.16 to 2.29 for women.[19] The relative hypercoagulability in our study is in accordance with the 2 other nonhuman primates thromboelastography studies. CI reference values of 3.47 ± 1.33 have been reported for rhesus macaques also.[13] In addition, relative hypercoagulability (according to RoTEM) was noted in cynomolgus macaques;[52] the authors speculated that increased platelet counts and mean plasma factor VIII levels may contribute to the relative hypercoagulability.[52] Fibrinogen was lower in macaques than in humans and therefore could

Table 2. Means, standard deviations, and ranges of thromboelastographic values obtained by using kaolin-activated, citrate-stored whole blood from male and female pigtail macaques (n = 50; age, 1.83 to 19.33 y)

	Mean ± 1 SD	Reference interval (mean ± 2 SD)	Range
Reaction time (R; min)	3.19 ± 0.60	1.99–4.39	1.8–4.5
K time (min)	0.85 ± 0.09	0.67–1.03	0.8–1.2
α Angle (°)	78.52 ± 2.05	74.42–82.62	70.8–81.6
Maximal amplitude (mm)	68.85 ± 5.02	58.81–78.89	57.3–80.3
LY30 (%)	7.97 ± 9.93	0–27.83	0–46.3
Clot strength (G; kdyn/cm^2)	11.47 ± 2.71	6.05–16.89	6.71–20.38
Coagulation index	4.10 ± 0.83	2.44–5.76	2.15–5.94

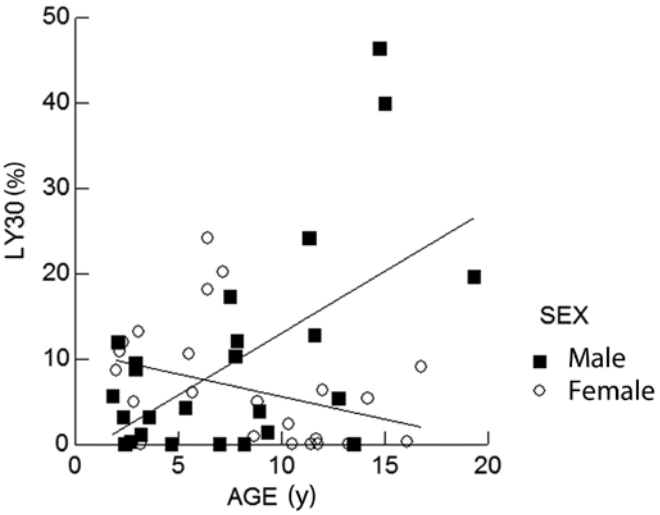

Figure 2. The interaction of the effects of age (y) and sex on LY30 (%) values. The solid lines represent lines of best fit, demonstrating the increase in LY30 values with increases in age in male pigtail macaques and the decrease in LY30 values with increases in age in female pigtail macaques. Data were analyzed by using general linear model techniques with age as a continuous predictor variable and sex as a categorical variable.

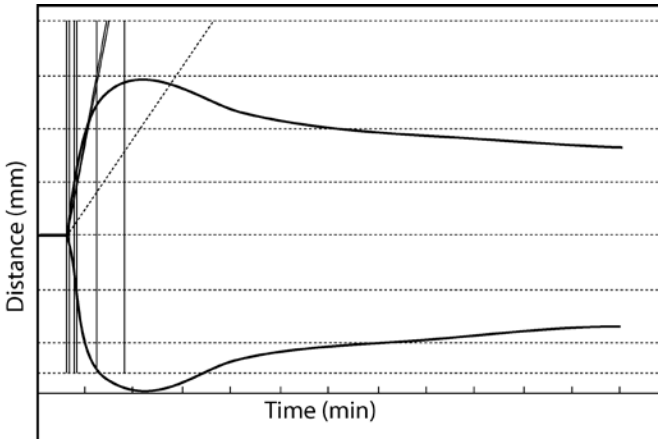

Figure 3. An example of thromboelastograph from a 6.42-y-old female pigtail macaque with a low MA of 58.0 mm and a high LY30 of 24.1%. The fibrinolytic part of the tracing appears different from those of humans with hyperfibrinolysis in that the macaque tracing shows a slow return toward baseline without ever reaching it. This type of tracing was present in 14 of 50 TEGs and is suggestive of clot retraction rather than hyperfibrinolysis.

not explain the difference in coagulability. Of the 50 macaques examined in our study, 36 had concurrent CBC and thromboelastography. The average platelet count in our macaques was $488 \times 10^3 \pm 102.5/\mu L$, which is higher than the human reference range of 150 to $400 \times 10^3/\mu L$. Therefore, higher platelet counts could potentially explain the relative hypercoagulability in our study. Another possible mechanism is that pigtail macaques, compared with other macaques, have increased incidence of gastrointestinal diseases and diarrhea.[47] At our institution, gastrointestinal tissues often have histopathologic changes consistent with inflammatory bowel disease, which has been associated with a hypercoagulable state.[50] Although only 2 animals in our study had a history of diarrhea within the previous year, subclinical gastrointestinal disease may be present. Little is known about coagulation in nonhuman primates, and why they may be more hypercoagulable than are humans is unclear. However, relative hypercoagulability could have important practical implications for nonhuman primate research models, such as transplantation.[52]

In addition, our pigtail macaques had a higher reference range for LY30, 0 to 27.83 than human ranges of 0 to 10.0 and 0.8 to 8.6.[11,48] LY30, which is the amount of clot lysis 30 min after MA, generally is considered to be a measure of fibrinolysis. However, another potential mechanism for the higher LY30 is clot retraction, which is platelet-mediated and has been shown to increase LY30 with increasing platelet counts.[29] The higher average number of platelets in pigtail macaques could lead to greater clot retraction and higher LY30. However, we found no correlation between platelet counts and LY30 (r = 0.019, P = 0.89). In addition, the cynomolgus RoTEM study showed a higher increased platelet count without a higher LY30.[52] The fibrinolytic tracings of thromboelastography with higher LY30 values are different than standard tracings from human patients with hyperfibrinolysis (Figure 2). Human hyperfibrinolytic tracings usually show complete clot lysis, so that both sides of the tracing return to baseline.[6,12,36,49] Prior to the fibrinolytic stage, the thromboelastography tracings are normal in appearance. We estimate that 14 of the 50 tracings obtained in the current study contained these characteristics. These abnormal tracings suggest that the higher LY30 in macaques may reflect clot retraction and that nonhuman primates may exhibit different platelet and coagulation properties from those in humans.

Although RoTEM and thromboelastography have been used to guide antifibrinolytic therapy during liver and cardiac surgeries and in trauma patients, few reports in the literature address thromboelastography and hyperfibrinolysis.[6,25,27,36,49] Artifactual hyperfibrinolysis occurred when a heparinase-containing sample cup was used,[42] but our thromboelastography used a plain cup. Another potential confounding factor is that fibrinolysis has a natural circadian rhythm and increases several-fold during the day.[2] In our study, all blood was collected in the morning. The RoTEM study in cynomolgus and thromboelastography study in rhesus macaques did not find higher LY30.[13,52] However, other studies have suggested that nonhuman primates have active fibrinolytic systems. For example, rhesus macaques were reported

to clear experimentally induced thrombi in as few as 4 d.[10] In Japanese macaques (*Macaca fuscata*), levels of plasminogen, a main determinant of fibrinolysis, were significantly higher than those in humans.[53] In a report comparing urokinase, an inducer of fibrinolysis, among multiple species, macaques had the highest level of fibrinolysis.[37] Hyperfibrinolysis may be viewed as a natural counterbalance to the relative hypercoagulability in nonhuman primates. The mechanisms underlying the increase in LY30 and the unusual thromboelastography tracings in pigtail macaques merit further study. For example, using abciximab, a glycoprotein IIb/IIIa inhibitor that blocks the contribution of platelets to coagulation, would help to differentiate clot retraction from hyperfibrinolysis.

In the current study, only sex had a statistically significant effect and then only on LY30. Similarly, investigators from the 2 previous nonhuman primates studies of thromboelastography and RoTEM combined only found one value that was affected by age or sex.[13,52] In rhesus macaques, sex had an effect on LY30, but age-associated effects were not examined.[13] LY30 values were 1.93 ± 1.52 and 0.77 ± 0.94 in female and male rhesus macaques, respectively, whereas we found LY30 values of 6.39 ± 6.59 and 8.78 ± 11.64 in female and male pigtails, respectively. Therefore, our result of higher LY30 in male compared with female macaques is contradictory to the findings from rhesus macaques. Despite reports of sex-associated differences in the fibrinolytic system in humans,[3,33] a literature review does not consistently support higher fibrinolysis in either sex. One study demonstrated that, compared with men, women have lower levels of tissue plasminogen activator and plasminogen activator inhibitor 1, which may correspond to lower levels of fibrinolysis.[3] In response to exercise, men have larger reductions in plaminogen activator inhibitor 1 levels than do women, and these differences may be associated with changes in body composition.[33] Otherwise, little information is available about sex-associated differences in fibrinolysis. We found a significant interaction between age and sex on LY30 (Figure 3). As female pigtails increased in age, LY30 values decreased, but as male pigtails increased in age, LY30 values increased. In humans, fibrinolysis decreases with age,[9] but 2 major factors of the fibrinolytic system, tissue plasminogen activator and plasminogen activator inhibitor 1, both increase with age.[3] Further studies of the effects of age and sex on fibrinolysis in nonhuman primates are required to confirm our findings. With the exception of LY30, the general lack of effects of age and sex on thromboelastography parameters has important implications for future research involving this technology in nonhuman primates. Unless thromboelastography is used to study LY30 and fibrinolysis, we agree with other authors[52] that nonhuman primates do not require age- and sex-specific reference ranges. Therefore, animals in experimental groups would not have to be matched for age and sex, allowing other facilities to decrease the numbers of animals and tests needed to establish institutional reference values. Furthermore, institutions that have few nonhuman primates may be able to establish appropriate in-house reference intervals and use thromboelastography.

Because of multiple logistical issues, we were unable to standardize the amount of time between venipuncture and analysis by thromboelastography. For the purpose of refinement, samples were scheduled to coincide with semiannual tuberculosis testing to minimize the number of sedations and venipunctures necessary. In addition, to acquire the appropriate demographics, macaques were sampled from 2 facilities, which required transportation of biohazardous materials. Finally, as previously mentioned, a human clinical coagulation laboratory was used, such that human samples received priority and the schedule of open thromboelastography ports could not be predicted. Although citrated samples for thromboelastography reportedly are stable for as long as 8 h, the time to analysis could still lead to variation.[7] Regardless, we found no significant difference between samples analyzed in 4 h or less after collection and those evaluated more than 4 h afterward. In general, prolonged storage leads to greater coagulability.[7,66] However, samples analyzed more than 4 h after collection had lower CI and G mean values than did those tested sooner than 4 h. Therefore, prolonged storage time from venipuncture to analysis does not appear to explain the relative hypercoagulability we noted in the current study.

Venipuncture predominantly was performed by using vacuum phlebotomy tubes, but syringes were used if there was difficulty in acquiring blood. The method of sample collection was not recorded, but more than 90% of samples were estimated to be collected by using vacuum phlebotomy tubes. The different methods of venipuncture could lead to different shear forces and consequently different amounts of contact activation of coagulation. Wall shear stress can be estimated by using the formula $T_W = (32\mu Q/\pi d^3)$, where Q is flow rate, d is the diameter of the blood vessel, and μ is blood viscosity.[17] Shear stress is exponentially inversely proportional to the diameter. Therefore, diameter is the largest independent variable in determining shear stress. Given that vacuum phlebotomy tubes and syringes both used needles of the same diameter, differences in shear likely were minimized. In addition, the use of kaolin as an activator probably decreased potential differences in contact activation due to sample collection. However, 2 recent abstracts have reported differences in thromboelastography according to the method of sample collection of blood in dogs.[31,58] A future study might compare data from samples collected from nonhuman primates by using vacuum phlebotomy tubes with those from syringes to determine whether the method of collection affects thromboelastography parameters.

There were additional limitations to the current study. Standard coagulation panels and other coagulation tests, such as measuring fibrinogen or specific coagulation factors, were not included to determine that these animals were normocoagulable and therefore appropriate for the establishment of reference ranges. However, platelet counts and hematocrits for the previous 6 mo all were within normal reference ranges, and no evidence of gross coagulation abnormalities was noted on physical examinations. None of the macaques we assessed were assigned to research projects, and naturally occurring coagulopathies are rare at our institution. Blood typically is collected from the femoral vein in nonhuman primates. However, due to anatomic proximity, blood might rarely have been collected from the femoral artery instead of the femoral vein. Thromboelastography values vary based on arterial compared with venous sampling.[38] However, a follow-up study from the same group attributed the results to differing shear forces due to different diameters in catheter lumens rather than to the diverse oxygen saturations of arterial and venous blood.[17] Finally, multiple phlebotomists, operators, and thromboelastography analyzers were used to perform thromboelastography. However, all phlebotomists were experienced with venipuncture in nonhuman primates, all operators were trained to run thromboelastography samples, and all analyzers were monitored through daily control runs.

Although thromboelastography is not a well-known test in laboratory animal medicine, the method is now being used to assess hypocoagulability, hypercoagulability, and hyperfibri-

nolysis; monitor anticoagulant therapy and platelet disorders; and guide blood transfusions in humans.[26,35,45,49,54,59,60] One of the most promising uses is the ability to detect hypercoagulability in animals with disseminated intravascular coagulation, neoplasia, parvovirus, or immune-mediated hemolytic anemia and in humans after severe injury.[32,44,45,51,62] Thromboelastography reportedly can predict postoperative thrombosis, although a systematic review found the results to be variable.[15,26,40] As with many new tests, the standardization of thromboelastography methodology has been a challenge to its widespread implementation. Researchers did find consistent RoTEM results among 6 centers, indicating that a universal reference range is possible.[34] However, this finding was not replicated in an international study among thromboelastography run in 4 laboratories and RoTEM run in 6 laboratories on previously frozen plasma.[14] Continuing investigation of the potential applications of thromboelastography likely will lead to increased use in clinical medicine and research. Therefore, laboratory animal veterinarians should be cognizant of this reemerging global coagulation test. In addition, thromboelastography is currently available only at research institutions, and laboratory animal veterinarians and researchers can still contribute to its development as a diagnostic tool.

In conclusion, we recommend that each institution establish its own reference values, due to the lack of research about thromboelastography in nonhuman primates and the diverse methodologies. However, apart from LY30, the demonstrated lack of effects of age and sex may reduce the animals and resources needed to determine reference values. Compared with human reference ranges, macaques had higher LY30 and CI reference values. These differences may merit additional study to determine how these variables might affect animal models.

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