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Comparison of Direct and Indirect Methods of Measuring Arterial Blood Pressure in Healthy Male Rhesus Macaques (*Macaca mulatta*)

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Blood pressure is a critical parameter for evaluating cardiovascular health, assessing effects of drugs and procedures, monitoring physiologic status during anesthesia, and making clinical decisions. The placement of an arterial catheter is the most direct and accurate method for measuring blood pressure; however, this approach is invasive and of limited use during brief sedated examinations. The objective of this study was to determine which method of indirect blood pressure monitoring was most accurate compared with measurement by direct arterial catheterization. In addition, we sought to determine the relative accuracy of each indirect method (compared with direct arterial measurement) at a given body location and to assess whether the accuracy of each indirect method was dependent on body location. We compared direct blood pressure measurements by means of catheterization of the saphenous artery with oscillometric and ultrasonic Doppler flow detection measurements at 3 body locations (forearm, distal leg, and tail base) in 16 anesthetized, male rhesus macaques. The results indicate that oscillometry at the forearm is the best indirect method and location for accurately and consistently measuring blood pressure in healthy male rhesus macaques.

Abbreviations: DAP, diastolic arterial pressure; MAP, mean arterial pressure; SAP, systolic arterial pressure

Quantitative measurement of blood pressure is a staple of anesthetic management in both human and veterinary medicine. Mean arterial pressure (MAP) is calculated from the systolic pressure (SAP) and the diastolic pressure (DAP).[8] Maintaining adequate blood pressure is essential for continued tissue perfusion, and a MAP of less than 60 mm Hg in most mammalian species can lead to hypoperfusion, potentially resulting in renal failure, neurologic abnormalities, and shock.[6]

Arterial catheterization for direct measurement of blood pressure is the 'gold standard' in both human and veterinary medicine.[8] However, placement is fairly invasive and can be difficult to execute rapidly and consistently, thus potentially precluding the use of direct blood pressure measurement during procedures requiring only brief sedation. Less-invasive methods, including oscillometry and ultrasonic Doppler flow detection, have practical advantages over direct monitoring but have distinct limitations and decreased reliability at high (that is, greater than 160 mm Hg) and low (that is, less than 80 mm Hg) values.[5] Both oscillometry and Doppler flow detection determine blood pressure according to the air pressure required to occlude arterial blood flow by using compression from an externally placed inflatable cuff. Oscillometry detects the vibrations in arteries associated with restricted blood flow, which is caused when the external cuff exerts pressure below SAP but above DAP.[17] Doppler flow detection uses a piezoelectric ultrasound crystal placed over an artery to detect blood flow, which is converted into an audible sound.[9]

The shortcomings of indirect blood pressure methods are well documented and species-dependent. Studies performed in dogs have shown significant variation in indirect blood pressure measurements taken at different anatomic locations and lack of consistency between indirect and direct blood pressure measurements.[2,7] Some authors recommend that indirect methods should not be used, given the inaccuracy of those methods when compared with direct measurement.[2] Variation between Doppler measurements and direct measurements has been demonstrated in cats,[4] whereas Doppler measurements in rabbits reportedly agree with direct SAP measurements.[9] In addition, the size and location of the blood pressure cuff significantly influences blood pressure measurement. A cuff that is too large leads to underestimation of pressure and vice versa.[17] The width of the cuff should be approximately 40% of the circumference of the limb.[8,14] In addition, blood pressure readings vary depending on peripheral anatomic location and recumbency.[15] In dogs, SAP was significantly higher when measured at the tarsus than at the carpus.[1] In addition, arterial pressure increases with increasing peripheral distance from the aortic arch.[15] Collectively, these studies reveal that blood pressure measurements vary depending on the technique, species, and anatomic site. Species-specific validation of indirect measures against direct measures of blood pressure is essential for accurately monitoring patients and informing clinical decisions.

Rhesus macaques remain the most commonly cited NHP model in biomedical research.[11] Under most circumstances, NHP require sedation or general anesthesia for routine clinical care, diagnostics, and research-related procedures. Many of these anesthetic regimens include α2 agonists, opioids, phenothiazine derivatives, and inhalant gases. These agents have been associated with moderate to severe hypotension under a variety of clinical and experimental circumstances, making blood pressure monitoring a critical component of NHP patient care. One group of investigators demonstrated the accuracy of high-definition oscillometry for identifying drug-related

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changes in blood pressure in cynomolgus macaques,[16] but the authors did not compare multiple indirect methods and, to date, indirect and direct methods for measuring blood pressure in rhesus macaques have not been compared systematically.

In this study, we compared indirect ultrasonic Doppler flow detection and oscillometric blood pressure measures with direct measurement after catheterization of the saphenous artery in healthy rhesus macaques. Specifically, we evaluated both the consistency and accuracy of each indirect measurement to determine which method best predicts direct blood pressure measurement. In addition, we evaluated the effect of anatomic location of the blood pressure cuff on the accuracy and consistency of measurements. Collectively, these data can be used to determine whether noninvasive methods can be applied to accurately measure blood pressure both in sedated and awake NHP.

Materials and Methods

Animals. Subjects were 16 Indian-origin male rhesus macaques (*Macaca mulatta*; age, 3 to 16 y; average weight, 9.7 kg). Ten of the 16 macaques were housed in 6.0-ft^2 individual cages arranged in blocks of 4 (quads) within a 715-ft^2 room maintained on a 10:14-h dark:light cycle. These 10 animals were part of an unrelated, ongoing, noninvasive study with an IACUC-approved exemption from social housing. The remaining 6 macaques were housed in indoor–outdoor enclosures as harem breeding groups at the Johns Hopkins University NHP breeding facility. All macaques were fed a standard commercial diet (2050 Teklad Global 20% Protein Primate Diet, Harlan Laboratories, Madison, WI). Commercial diets were supplemented with a variety of food enrichment items, including foraging mix, fresh fruits, and vegetables. Water was freely available. This protocol was approved by the Johns Hopkins University IACUC in compliance with the Animal Welfare Act, federal regulations, and the *Guide for the Care and Use of Laboratory Animals*.[10]

Calibration. Prior to initiation of the study, all direct and indirect blood pressure monitoring systems were calibrated by using a mercury manometer. For the direct system, noncompliant tubing was filled with a heparinized saline solution (1 L 0.9% NaCl containing 20,000 IU heparin) and replaced between measurements in consecutive subjects. The tubing was connected to a pressure transducer (TruWave Pressure Monitoring Set, Edwards Lifesciences, Irvine, CA), which was connected to a data acquisition system (model PM-9000Vet, Veterinary Portable Multi-Parameter Patient Monitor, Mindray, Shenshen, China). The system was connected to a bag of heparinized saline pressurized to 300 mm Hg and used for flushing throughout the study period. Transducers were zeroed to atmospheric pressure before each use and assessed for accuracy against a mercury manometer. The system was inspected regularly and flushed throughout the procedure to prevent air bubbles and clots. The waveform was analyzed and determined to be normal before study initiation.

A single oscillometric device (PM-9000Vet Veterinary Portable Multi-Parameter Patient Monitor, Mindray) and a single aneroid manometer (Ri-san Palm-style Aneroid Sphygmomanometer, Riester, Ventura, CA) were used throughout the study and calibrated prior to beginning measurements on each macaque. The instruments were calibrated by comparing measurements with a mercury column pressurized to 200 mm Hg. Calibration was accepted when displayed values were within 2 mm Hg of the value from the mercury column. The Doppler flow detector was not calibrated.

Study design. All macaques enrolled in the study were considered to be in good clinical health as defined by specified inclusion and exclusion criteria. The inclusion criteria were: an unremarkable physical exam, CBC, and serum chemistry (assessed at study initiation and previously on a semiannual basis); seronegativity for SIV, simian retroviruses 1 and 2, simian T-lymphotropic virus, measles virus, and *Macacine herpesvirus 1*; and a negative tuberculin skin test for *M. tuberculosis*. Exclusion criteria were: acute disease during the 6 mo prior to study initiation; history of chronic disease; and clinical or experimental drug treatment within the past 6 mo, except for specified anesthetics necessary to perform this study.

All macaques were food-fasted for 12 h before anesthesia; water remained freely available. Ketamine HCl (20 mg/kg IM; Zetamine, VetOne, Boise, ID) was administered to facilitate handling, intubation, and subject preparation. The distal leg, distal arm, tail base, and perianal region were shaved bilaterally. Macaques were maintained in a stable surgical plane under general inhalant anesthesia by using isoflurane (0.5% to 1.5%; Forane, Baxter Healthcare, Deerfield, IL) and oxygen (1 to 2 L/min). Heart rate, respiratory rate, SpO_2, end-tidal CO_2, and ECG were monitored throughout the experimental period. Indirect methods were evaluated on the arm, leg, and tail base, with the animals positioned in dorsal recumbency. After data collection in this position, the animal was moved to lateral recumbency to determine whether blood pressure measured at the tail base differed due to pressure on the cuff. Sufficient anesthesia was confirmed through the lack of a palpebral reflex and lack of a withdrawal response to toe pinch. The skin over the distal aspect of the saphenous artery was disinfected with an alcohol swab, and a 22-gauge arterial catheter was placed between the ankle and knee. The catheter was then connected to the pressure transducer by using noncompliant tubing filled with heparinized saline. The transducer was placed at the level of the heart.

Consistency regarding the location of cuff placement is important, given that blood pressure changes depending on the distance from the heart.[15] Therefore, defined locations were selected in light of multiple factors, including the availability of clear anatomic landmarks and the ability to place the cuff and secure it with equal contact across the width of the cuff. We have found that a palpable pulse was unreliable at sites where a cuff could be placed in NHP.

To select the correct cuff size, the circumferences of the forearm, distal leg (contralateral to the arterial catheter), and tail base at defined locations were determined (Figure 1 A through C), and 30% and 40% of the circumference was calculated, indicating the correct width of the bladder on an appropriate cuff.[8] Cuffs ranging from neonatal size 2 to child size (Mindray) were used. If 2 cuffs were deemed appropriate, the larger cuff was selected. Cuffs were placed at each location, and wedges were used to elevate the legs and arm to the level of the heart (Figure 2).

Oscillometric measurements. Prior to taking measurements at each location, the noncompliant tubing was checked for the presence of air bubbles, the arterial catheter was flushed with heparinized saline, and the pressure bag was checked to confirm pressurization to 300 mm Hg. Beginning with the forearm, the noninvasive blood pressure oscillometric data acquisition system was connected to the cuff, and measurement was begun. The same system (model PM-9000Vet Veterinary Portable Multi-Parameter Patient Monitor, Mindray) was used to measure direct blood pressure, and both direct and oscillometric measurements were displayed on the screen. Within 2 s of when the oscillometric measurement appeared, the screen was photographed to simultaneously capture the 2 values. The measurements were recorded, and this process was repeated

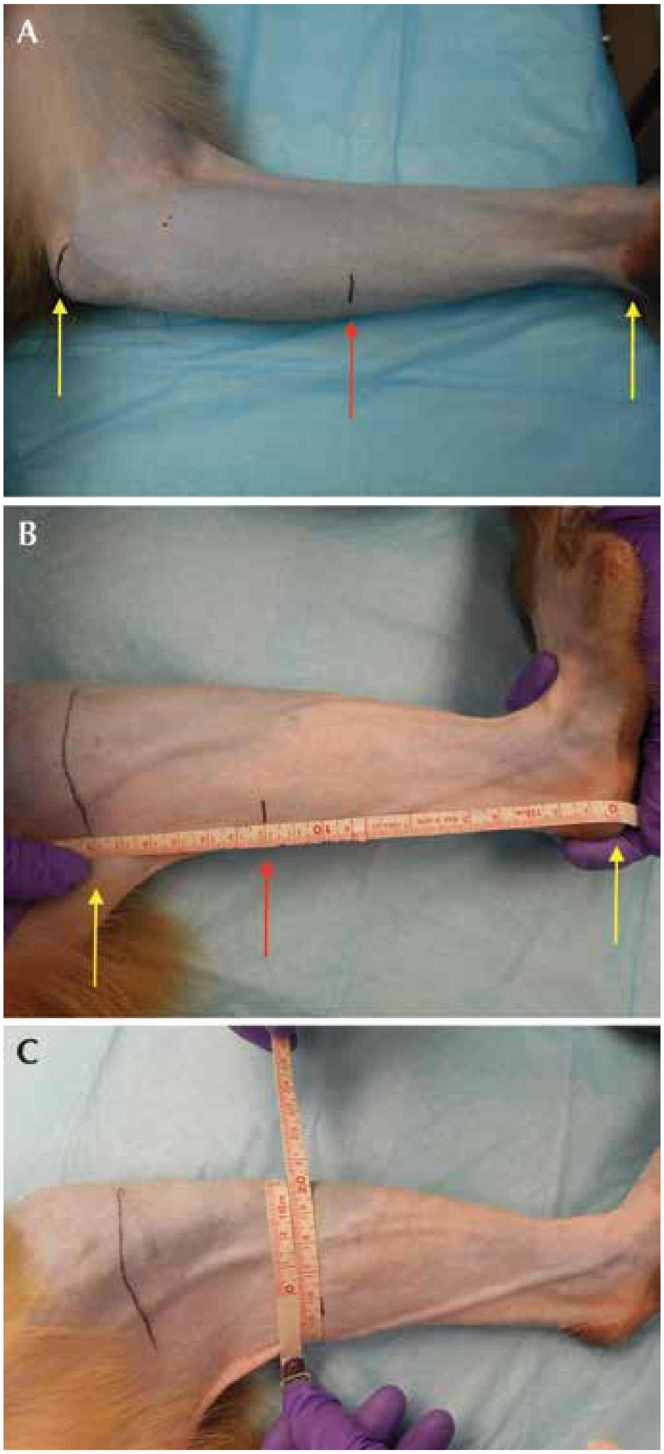

Figure 1. (A) The arm landmarks for determining the length of the forearm are indicated by yellow arrows, and the midpoint where circumference was measured is indicated by a red arrow and black line. (B) The leg landmarks for determining the length of the lower leg are indicated by yellow arrows and the 2/3 point where circumference was measured is indicated by a red arrow and black line. (C) The circumference was measured at the determined location by using a flexible tape measure.

twice on the arm at 2-min intervals. This process was repeated for measurements from the leg and tail base.

Doppler measurements. Doppler measurements (model 811-B Doppler Flow Detector, Parks Medical Electronics, Las Vegas, NV) were taken at the arm, leg, and tail base, with the cuffs in the same locations as used for oscillometric measurements.

Figure 2. Rhesus macaque in dorsal recumbency, with blood pressure cuffs on left forearm, left leg, and tail base. The arterial catheter is placed in right saphenous artery.

Prior to measurement at each location, the noncompliant tubing was again checked for the presence of air bubbles, the arterial catheter was flushed with heparinized saline, and the pressure bag was checked to confirm pressurization to 300 mm Hg. Beginning with the arm, the sphygmomanometer was attached to the preplaced cuff. Ultrasound gel was applied to the medial aspect of the wrist, distal to the cuff. The ultrasound probe was placed over this region to identify strong, pulsatile sounds. The cuff was inflated to 180 mm Hg, ensuring the loss of audible pulsatile sounds and then deflated at 2 to 3 mm Hg per second. When pulsatile sounds returned, the value on the sphygmomanometer was recorded. Within 2 seconds of the sounds returning, an observer photographed the direct blood pressure measurement displayed on the monitor to correspond with the Doppler measurement, which only provides one value, the systolic pressure. The direct and Doppler blood pressure measurements were recorded, and this process was repeated twice on the arm at 2-min intervals. This process then was repeated for measurements on the leg and tail.

After measurements were completed, the sphygmomanometer was disconnected, the cuffs removed, the arterial catheter removed, and the macaque recovered from anesthesia.

Statistical analysis. Because simultaneous measurement of blood pressure by direct, oscillometry, and Doppler methods was infeasible, each indirect measurement was compared with its own contemporaneously recorded direct measurement. Comparison of direct blood pressure measurements taken during Doppler data collection revealed significant differences from indirectly measured SAP over time. Therefore, indirect measurements were normalized to corresponding direct measurements to compare indirect measurements taken at different times and locations. Matched one-way ANOVA was used to compare blood pressure measurements between anatomic sites; significant ($P < 0.05$) differences were further analyzed by using a Bonferroni posthoc test. Pearson correlation coefficients were calculated for paired direct and indirect blood pressure measurements at each anatomic site; 3 measurements were taken from each site and animal and were treated as separate data points for our analyses. Bonferroni correction was performed on Pearson correlation analyses. For all analyses, a corrected P value of less than 0.05 was considered statistically significant. Prism 7 (GraphPad Software, San Diego, CA) was used for all statistical analyses. Throughout the manuscript, data are reported as mean ± SEM.

Results

Direct blood pressure measurement during oscillometric measurement. Given that indirect blood pressure measurements were taken at different times over an approximately 2-h period in 16 rhesus macaques, we needed to determine whether directly measured blood pressure changed throughout the study. A significant difference between the direct blood pressure measurements taken in parallel with oscillometric measurements over time would preclude comparison of the measurements obtained by means of oscillometry at different time points. Direct measurements of SAP ($P = 0.2973$), DAP ($P = 0.7637$), and MAP ($P = 0.4013$) did not differ during oscillometric data collection. Therefore, we compared the indirect blood pressure measurements taken at different time points by using oscillometry.

Indirect blood pressure measurement across locations. When comparing blood pressure by using Doppler, cuff placement on the arm or tail provided similar measurements. However, placement of the cuff on the leg resulted in greater ($P < 0.05$) underestimation compared with both the arm and tail (Figure 3). This same degree of variation was seen when we compared SAP by using oscillometry on the arm, leg, and tail. MAP and DAP differed by location when the values obtained by using oscillometry on the arm, leg, and tail, were compared.

Indirect compared with direct blood pressure measurements. Compared with direct SAP, Doppler measurements obtained through placement of a cuff on the arm, leg, and tail of rhesus macaques underestimated the blood pressure by 11.4, 25.9, and 11.5 mm Hg, respectively (Table 1). SAP obtained by oscillometry at the arm, leg, and tail underestimated blood pressure by 6.0, 11.6, and 3.8 mm Hg, respectively, compared with direct measurement of SAP (Table 2). MAP obtained by using oscillometry at the arm, leg, and tail consistently underestimated the pressure by 4.8, 13.3, and 9.2 mm Hg, respectively, compared with direct measurement of MAP. DAP obtained by using oscillometry at the arm, leg, and tail consistently underestimated the pressure by 5.6, 16.2, 10.2 mm Hg, respectively, compared with direct measurement of DAP.

Correlation between indirect and direct measurements. The indirect blood pressure measurements from the arm correlated most closely with the corresponding direct measurements. When blood pressure obtained by using Doppler was compared with direct measurement of SAP, cuff placement on the arm yielded an R^2 value of 0.4569 (Figure 4 A). Comparison of SAP by oscillometry with direct measurement of SAP, cuff placement on the arm yielded an R^2 value of 0.5678 (Figure 4 B). When compared with blood pressures obtained by using oscillometry, MAP measurements were most strongly correlated with corresponding direct measurements ($R^2 = 0.6519$, $P < 0.0001$), and DAP measurements correlated least ($R^2 = 0.2309$, Figure 4 C and D). These trends are similar to but greater than those for the leg (Figure 4 E through H) and tail (Figure 4 I through L).

Effect of animal position on indirect blood pressure measurement. Depending on the procedure, animals must be positioned differently, potentially resulting in variable compression of peripheral vasculature and alteration of blood pressure measurements. For example, compression of the cuff placed on the tail is most likely when the macaque is dorsally recumbent. To evaluate the effect of animal position on blood pressure, we obtained direct and indirect blood pressure measurements from macaques in dorsal or lateral recumbency. Animal position did not affect SAP as measured by either direct or indirect methods. The mean DAP by direct measurement was higher ($P = 0.019$) when macaques were in lateral recumbency (50.4 ± 1.7 mm Hg) compared with dorsal recumbency (48.0 ± 1.1 mm Hg); the same was true for oscillometric measurements (42.9 ± 2.1 mm Hg, 37.7 ± 1.4 mm Hg, respectively, $P = 0.027$). Likewise, the mean MAP by direct measurement was higher ($P = 0.033$) when animals were in lateral recumbency (64.2 ± 2.0 mm Hg) than dorsal recumbency (61.6 ± 1.3 mm Hg); the same was true

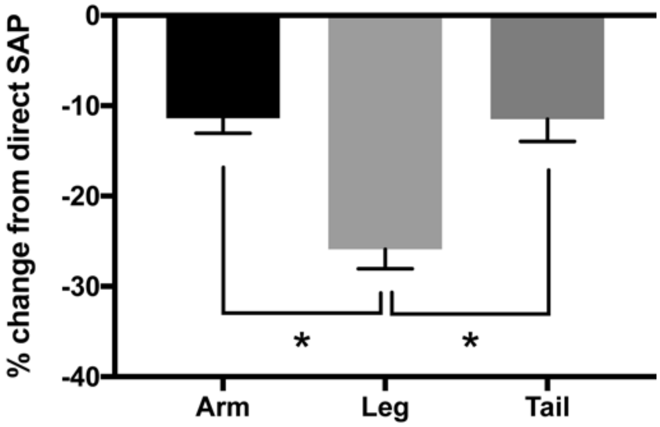

Figure 3. Doppler measurements significantly underestimate corresponding direct SAP measurements. A comparison of Doppler blood pressure measurements from the arm, leg, and tail base (values normalized to corresponding direct SAP measurements). *, $P < 0.05$

Table 1. Comparison of SAP measured directly from the leg with Doppler SAP measurements

	Arm	Leg	Tail
Direct SAP (leg)	92.2 ± 1.9	95.8 ± 2.4	93.8 ± 2.0
Doppler	80.8 ± 2.2[a]	69.9 ± 2.6[a]	82.4 ± 3.1[a]
Difference relative to direct measurement	−11.4 ± 1.7	−25.9 ± 2.2	−11.5 ± 2.5

Data are given as mean ± SEM in mm Hg.
[a]Significant ($P < 0.05$) difference between direct measurement from the leg and corresponding indirect measurement.

Table 2. Comparison of direct blood pressure measurements from the leg with oscillometric measurements of SAP, DAP, and MAP on the arm, leg, and tail

	Direct measurement (leg)	Oscillometric measurement	Difference relative to direct measurement
Arm			
SAP	90.3 ± 1.4	84.4 ± 1.7[a,b]	−6.0 ± 1.2
DAP	48.4 ± 0.9	42.8 ± 1.6 [a,b,c]	−5.6 ± 1.4
MAP	62.5 ± 1.0	57.7 ± 1.8 [a,b,c]	−4.8 ± 1.1
Leg			
SAP	88.4 ± 1.6	76.8 ± 1.8 [a,d]	−11.6 ± 1.4
DAP	47.9 ± 1.1	31.7 ± 1.3[a,d]	−16.2 ± 1.0
MAP	61.4 ± 1.2	48.1 ± 1.5 [a,d]	−13.3 ± 0.9
Tail			
SAP	88.7 ± 1.7	84.9 ± 2.1[a]	−3.8 ± 1.5
DAP	48.0 ± 1.1	37.7 ± 1.4[a]	−10.2 ± 1.4
MAP	61.6 ± 1.3	52.4 ± 1.5[a]	−9.2 ± 1.2

Data are given as mean ± SEM in mm Hg.
The difference between the direct blood pressure measurements and the indirect blood pressure measurements were determined at the arm, leg, and tail for oscillometric measurements. Significant ($P < 0.05$) differences between [a]direct measurement from the leg and corresponding indirect measurement or between indirect measurements on the [b]arm and leg, [c]arm and tail, or [d]leg and tail are indicated.

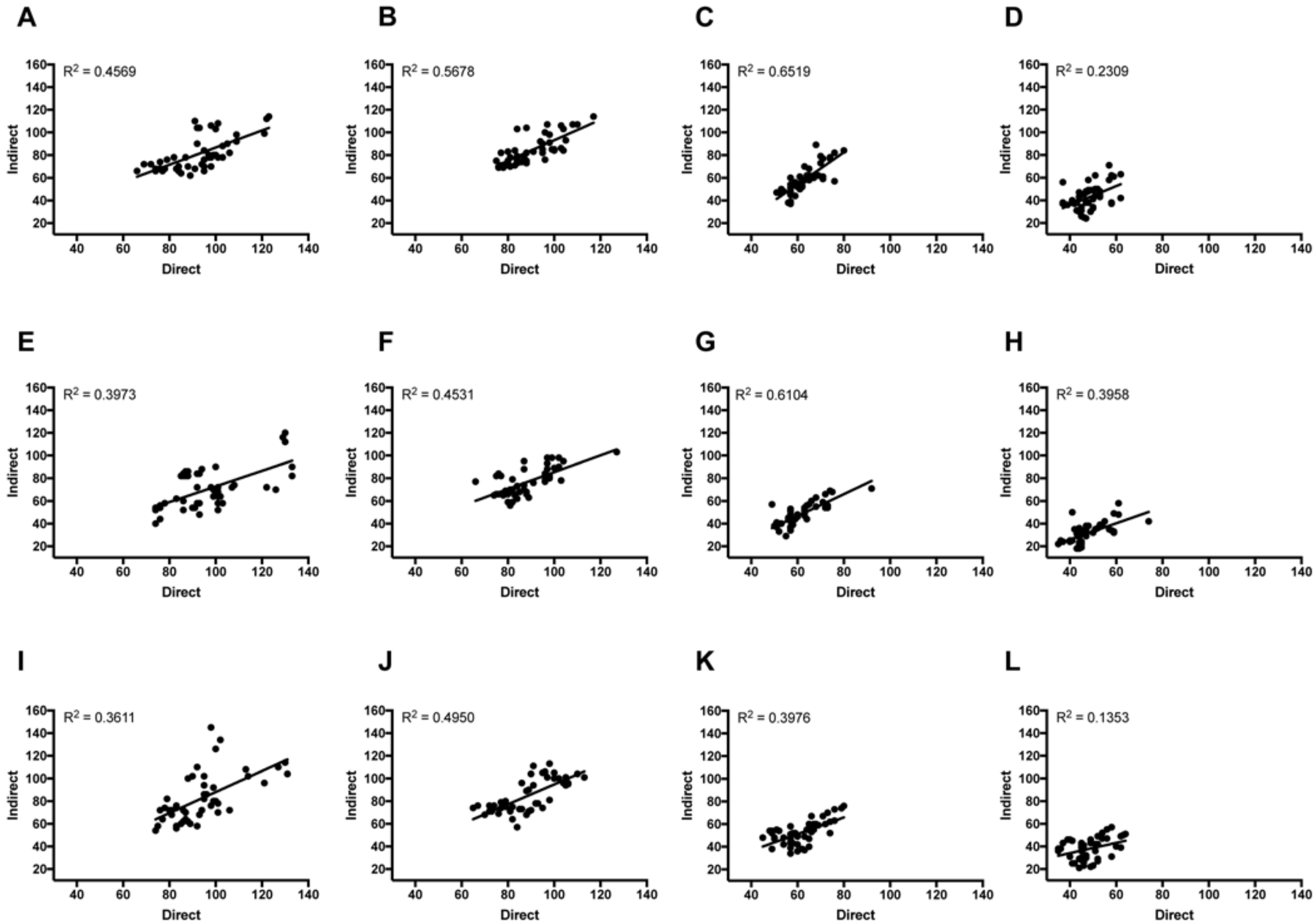

Figure 4. Indirect blood pressure measurements from the arm correlate most closely with corresponding direct measurements. (A) Correlation between the direct measurement of blood pressure and Doppler measurements on the arm was determined. Oscillometric (oscillo) measurements on the arm of (B) systolic pressure, (C) mean arterial pressure, and (D) diastolic pressure were correlated with corresponding direct measurements obtained from the catheter. (E) Correlation between the direct measurement of blood pressure and Doppler measurements on the leg was determined. Oscillometric measurements on the leg of (F) systolic pressure, (G) mean arterial pressure, and (H) diastolic pressure were correlated with corresponding direct measurements obtained from the catheter. (I) Correlation between the direct measurement of blood pressure and Doppler measurements at the tail base was determined. Oscillometric measurements at the tail base of (J) systolic pressure, (K) mean arterial pressure, and (L) diastolic pressure were correlated with corresponding direct measurements obtained from the catheter. Pearson correlation coefficients were calculated for paired direct and indirect blood pressure measurements at each anatomic site. The lines represent the linear regression.

for oscillometric measurements (57.8 ± 2.6 and 52.4 ± 1.5 mm Hg, respectively; $P = 0.022$).

Discussion

The major finding of this study is that indirect blood pressure measurements in rhesus macaques vary depending on the location and method used. This information highlights the importance of identifying a location and indirect method that are most accurate when compared with the direct method.

Interestingly, the indirect blood pressure measurements obtained by using both oscillometry and Doppler were consistently lower than the corresponding direct blood pressure measurements at all locations. When comparing Doppler results with the direct measurement of SAP, measurements in the arm provided the closest approximation, with the leg providing the greatest difference between direct and Doppler measurements. The reason for this pattern is unclear and might be due to the distance between the cuff and the Doppler crystal; the change in pressure was consistent among animals. For oscillometry, values obtained from the tail provided the closest approximation of SAP, whereas values obtained from the arm most closely approximated the MAP and DAP values. Still, SAP measured by oscillometry of the arm provided more accurate values than Doppler measurements on the arm. Therefore, we conclude that oscillometry at the arm is the best indirect method for general-purpose blood pressure measurement in male rhesus macaques. In regard to anesthetic safety, a falsely low blood pressure measurement would be preferred over a falsely high blood pressure measurement, because the aberrantly low value will prompt corrective measures sooner. However, given the error in indirect estimates of arterial pressure, blood pressure measurement by oscillometry should be limited to anesthetic and surgical monitoring, and the gold standard of direct arterial catheterization should be implemented when more accurate measurement is required, such as when prescribing treatments for high and low blood pressure.

The accuracy of indirect blood pressure measurements in other species has been evaluated over the years. One group compared direct blood pressure measurement with 2 indirect methods, high-definition oscillometry and conventional oscillometry, in awake cynomolgus macaques and dogs.[12] Consistent with our findings, they demonstrated that the indirect methods underestimated the blood pressure when compared with direct measurement. In anesthetized cats, oscillometry underestimated the blood pressure and yielded more inaccurate values as the pressure increased.[3]

When measuring blood pressure, it is well-established that the location being measured should be positioned at the level of the heart. Throughout our study, we positioned the arms and legs of each animal to fit this criterion. However, the tail could not be positioned at the level of the heart when the macaque was in dorsal recumbency. In addition, we speculated that the pressure of the body and ischial callosities on the tail-base cuff might affect measurements obtained from the tail in dorsal recumbency. Therefore, we evaluated the influence of body position on indirect measurements obtained from the tail during dorsal and lateral recumbency. When the macaque was in lateral recumbency, the tail was positioned at the level of the heart. We found that although animal position did not influence SAP measured directly or indirectly at the tail, it significantly affected MAP and DAP detected by both direct and oscillometric measurement. For both MAP and DAP measurements, lateral recumbency resulted in higher values than dorsal recumbency obtained by direct and oscillometric measurement. It is important for the limb in which pressure is being measured to be at the level of the heart. Although we adjusted the animal by using wedges, a slight change in the position of the catheter and the cuff might have resulted in an alteration in blood pressure measurements. However, this situation does not completely explain the lack of change in SAP and the change in DAP and MAP when moved to lateral recumbency. Although we are unable to fully explain the change in pressures, we conclude that a change in animal position does influence blood pressure.

A limitation of this study is that the findings are specific to the oscillometric device used. Each oscillometric device uses an empirically derived algorithm to calculate SAP, DAP, and MAP according to the recorded oscillations.[13] Therefore, algorithmic variations should be considered whenever different oscillometric devices are used. In conclusion, we determined that oscillometry at the forearm was the best method and location for accurately and consistently determining blood pressure in healthy male rhesus macaques.

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