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Comparison between the Doppler flowmetry and the free flow of dog's internal thoracic artery with and without use of norepinephrine

Comparação entre a Dopplermetria e o fluxo livre da artéria torácica interna de cães com e sem o uso de noradrenalina

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Abstract

Objective: **This work aims to study comparatively the free flow and the Doppler flowmetry of the internal thoracic artery in anesthetized dogs, with and without continuous intravenous administration of norepinephrine.**

Methods: **The sample was made up of ten mongrel dogs, which dissected the left and right internal thoracic arteries and evaluated your stream; first, by Doppler flowmetry and then by free flow. The mean arterial pressure and the diameter of the arteries at the beginning of the procedure were registered. The workflow checks by two methods occurred in three times: zero time, 10 and 25 minutes. After the first check in time zero, the continuous infusion of norepinephrine in the right atrium; other checks were made in the same way that the first time, to 10 and 25 minutes, in the same arteries and by two methods, each one in his artery, noting the results, as well as the corresponding average blood pressure.**

Results: **The results of the flowscan, between Doppler flowmetry and free flow, there were similar; being the first, zero times, ten and twenty-five minutes, respectively, 183, 230.1 and 237 ml/min compared to seconds, 168.6, 226.8 and 226.4 ml/min (*P*=0.285). The mean arterial pressures of three times and the average diameter of the arteries, showed no statistically significant differences between the methods, so did not influence on the comparison of the results.**

Conclusion: **The evaluations, both from Doppler flowmetry and free flow, were similar in three times checked.**

Descriptors: **Mammary arteries. Ultrasonography, Doppler. Continuous flow. Norepinephrine. Dogs.**

Resumo

Objetivo: **Este trabalho objetiva estudar comparativamente o fluxo livre e a dopplerfluxometria da artéria torácica interna de cães anestesiados com e sem a administração de noradrenalina endovenosa contínua.**

Métodos: **A amostra foi constituída de 10 cães mestiços, nos quais foram dissecadas as artérias torácicas internas direita e esquerda e avaliado seu fluxo; primeiramente, pela dopplerfluxometria e depois pelo fluxo livre. Foram registrados a pressão arterial média e o diâmetro das artérias no início do procedimento. As verificações do fluxo pelos dois métodos**

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Abbrebiations, acronyms and symbols	
ITA	Internal Thoracic Artery

ocorreram em três tempos: tempo zero, 10 e 25 minutos. Após a primeira verificação no tempo zero, iniciou-se a infusão contínua de noradrenalina no átrio direito; as avaliações aos 10 e 25 minutos foram feitas da mesma forma que na primeira vez, nas mesmas artérias e pelos dois métodos, anotando-se os resultados, assim como a pressão arterial média correspondente.

Resultados: **Os resultados da verificação de fluxo, entre Dopplermetria e fluxo livre, apresentaram-se similares; sendo os primeiros, nos tempos zero, 10 e 25 minutos, respectivamente, 183, 237 e 230,1 ml/min, comparados aos segundos, 168,6, 226,8 e 226,4 ml/min (*P*=0,285). A média das pressões arteriais dos três tempos e o diâmetro médio das artérias não apresentaram diferenças estatisticamente significativas entre os métodos, portanto, não influenciaram na comparação dos resultados.**

Conclusão: **As avaliações, tanto da dopplerfluxometria quanto do fluxo livre, foram semelhantes nos três tempos verificados.**

Descritores: **Artéria torácica interna. Ultrassonografia Doppler. Fluxo contínuo. Norepinefrina. Cães.**

INTRODUCTION

The internal thoracic artery (ITA) is used in coronary artery bypass surgery as a graft, and in the anastomosis of the coronary artery for its long patency and adequate perfusion. Compared to the saphenous vein graft, the artery is clear in 85% of patients, 10 years after surgery, while with the vein, it occurs on average in 60% of the cases [1,2]. ITA dissection is simple in most cases. Due to these characteristics, ITA is the first-choice artery for revascularization of the anterior descending artery, the most important in the cardiovascular structure [3].

Due to the importance of ITA in CABG, we should properly evaluate its patency, especially after dissection to avoid early occlusion, assessing its flow. An ITA with low flow can be used as a free graft.

The evaluation of the ITA free blood flow is composed of its incision and verify it in a container at a given time. This investigation was compared to measurement by transthoracic Doppler of the same artery, in a study by Canver et al. [4] in 1991, which demonstrated adequate correlation between the methods.

Some studies used a scanning method for evaluation of the free flow of the ITA. Wendler et al. [5] in 1999, comparatively evaluated ITA flow dissected with a pedicle and a skeletonized one, comparing them by the free flow. Most studies of ITA aims to analyze their patency over the years and few studies have the objective to evaluate their flow in the intraoperative period, as the present article does, therefore, this is our most relevant aspect.

Experimental study in dogs, conducted by Sasajima et al. [6] demonstrated the histopathological correlation of the ITA in dogs and in humans. Tada et al. [7] have analyzed the dog's ITA through Doppler flowmetry under the effect of inotropic and vasoactive substances and Suematsu et al. [8] defended the epicardial Doppler flowmetry as a good method for evaluating the ITA in CABG in dogs.

This work aims to comparatively study the free flow and ITA Doppler flowmetry in anesthetized dogs with and without continuous intravenous administration of norepinephrine.

METHODS

This work was performed at the Laboratory for Experimental Surgery of the Medical Research Institute from the Graduate Program in Principles of Surgery, Evangelical University of Paraná. The study was approved by the Research Ethics Committee of the Evangelical University of Paraná (nº 4586/01), according to the Declaration of Helsinki.

We followed the principles of ethics in animal experimentation, recommended by the Brazilian College of Animal Experimentation, specifically for dogs, according to the National Research Council [9].

Animal experimentation

The sample consisted of 10 mongrel dogs (*Canis familiaris*), with body weight ranging from 15 kg to 25 kg,

from the kennel of Curitiba City Hall. The sample size was sufficient to be statistically evaluated.

Preoperative period

The animals were fed with dog food (Nuvita® for adult dogs) and had free access to water. The dogs were kept under observation for 15 days, subjected to vaccination and treatment of parasites. They were weighed and fasted for 12 hours before surgery.

Anesthetic technique

The animal was taken to the operating room where the cephalic vein was punctured with Venofix® nº 21 scalp, which was connected to a 500 ml bag of 0.9 % saline solution (B. Braun S/A) to drip until the end of the procedure.

The anesthesia was endovenously induced with sodium thiopental hypnosis (Thionembutal®, Abbott Lab Brazil Ltda.) at a dose of 5 mg/kg. The solution was obtained after diluting 1 g of thiopental sodium lyophilized in 1 ml of 0.9% saline solution, obtaining 25 mg / ml of anesthetic solution.

The animal was considered anesthetized when it was unconscious, with the involuntary movements ceased and no reaction to surgical management. We also performed an orotracheal intubation by direct laryngoscopy with 7.5 mm cannula in diameter, with a ballon of cardiac tamponade (RUNCH probe).

The animal was kept in ventilation controlled by a respirator (Takaoka® model 671) using inhalation anesthesia with halothane (Halothano®, Cristália) for continuous vaporization.

Surgical Technique

1. The animal was placed in supine position with its cranial and caudal limbs fixed.

2. The fur from ventral thoracic region, close to the skin, was shaved with a razor blade.

3. In antisepsis of the surgical region, the povidone-iodine solution was used (Povedine Degermante®, Darrow), and then, povidone-iodine dye solution (Povedine Tintura®, Darrow).

4. The sterile surgical drapes were positioned, delimiting the area to be incised.

5. Median thoracic incision was performed, from the jugular notch to the xiphoid process with a electric scalpel (Wem ® model BC-160), depthened to the breastbone, where the hemostasis was performed.

6. The sternum was sawed in the midline, with Gigli saw, and hemostasis performed with bone wax (2.5 g, Poly Suture) and infrasternal bleeding vessels with a 4.0 USP cotton thread (Algofil®, Cirumédica).

7. The right ITA and left ITA was dissected using skeletonization, starting from its origin in the subclavian artery and continuing until near the xiphoid process [5], linking its branches with 3-0 USP cotton thread (Algofil®, Cirumédica.) After dissection of the ITA, 300 mg of papaverine hydrochloride were injected into the artery wall, in order to avoid spasms.

8. The pericardium was longitudinally incised along its entire length before being fixed to the subcutaneous tissue with three 2.0 USP cotton thread (Algofil®, Cirumédica), rising it and exposing the heart.

9. Suturing was performed in tobacco pouch with braided polyester threads (Ethibond 2.0) in the aorta and right atrium.

10. A catheter was inserted into the aortic pouch and connected to a sphygmomanometer clock, functioning as an arterial pressure checking system.

11. A nº 8 nasogastric tube was introduced in the atrial pouch, connected to a serum catheter with an infusion pump (Nutrimat II®, B. Braun) and a flask with norepinephrine standard glucose solution consisting of 4 mg of norepinephrine diluted in 250 ml of 5% dextrose solution (16 g/ml) [10]. The infusion has not been started yet.

12. Heparin was intravenously injected (400 IU / kg) in the dog, with the purpose of anticoagulation. The dose of heparin injected was directly correlated to the weight (P=0.00001) (Table 1).

13. Verification by the Doppler method (performed by a cardiologist with subspecialty in echocardiography and Doppler) was initiated by placing the transducer on one of ITAs. The first verification was adopted as the zero time (Vd0) assessing the diameter (Table 1) and the velocity of arterial blood flow, and then calculating the arterial volume per minute. Blood pressure (Pd0) was measured by the sphygmomanometer connected to the aorta.

14. The contralateral ITA has its diameters observed (Table 1) by Doppler and then had its distal end transversely incised. The artery was dissected away from its bed and its caudal end clamped with Bulldog forceps.

15. The clamped artery was displaced over a container and then the clamp released. The bleeding was evaluated for 15 seconds. Blood volume was multiplied by four, defining the 1-minute flow, then, we noted the mean arterial blood pressure when the verification started (P0). The collected blood was injected with a 20 ml syringe through the dog's venous access.

16. A continuous infusion of norepinephrine solution was started at a constant flow of 0.3 g / kg per minute, it was then calculated for each dog, according to the weight, in milliliters per hour (Table 1) standard dose [10].

17. After ten minutes of continuous infusion of norepinephrine solution, the methods were evaluated by Doppler (VD10) and the free flow (V10) in the same arteries and the same way as at time zero, verifying the mean arterial pressures corresponding to each method (PD10 and P10).

18. After 25 minutes of infusion of norepinephrine, further measures were taken in the arteries by Doppler (Vd25) and then the free flow (V25) and the blood pressure was observed in each of the two moments (Pd25) and free flow (P25).

19. All the measures were recorded (Tables 2 and 3).

Table 1. The weight of the dogs, the volume of heparin performed on each animal, the amount of noradrenaline performed per hour and the diameter of internal thoracic arteries at zero time.

	Weight	Volume of Heparin	Volume of Noradrenaline volume	Initial diameter of LITA	Initial diameter of RITA
Dog 1	23 kg	1.9 ml	26 ml/h	3.4 mm	3.1 mm
Dog 2	18 kg	1.5 ml	20 ml/h	2.4 mm	2.3 mm
Dog 3	24 kg	1.9 ml	27 ml/h	4.2 mm	4.0 mm
Dog 4	22 kg	1.8 ml	25 ml/h	4.2 mm	4.1 mm
Dog 5	25 kg	2.0 ml	28 ml/h	3.2 mm	3.1 mm
Dog 6	24 kg	1.9 ml	27 ml/h	3.4 mm	3.6 mm
Dog 7	17 kg	1.4 ml	19 ml/h	5.2 mm	4.6 mm
Dog 8	18 kg	1.5 ml	20 ml/h	2.6 mm	2.8 mm
Dog 9	19.5 kg	1.6 ml	22 ml/h	4.1 mm	3.9 mm
Dog 10	17.5 kg	1.4 ml	20 ml/h	2.6 mm	2.5 mm

LITA = left internal thoracic artery. RITA = right internal thoracic artery

Table 2. Results of blood pressures measured by the Doppler method (Pd0, Pd10, Pd25) and the free flow method (P0, P10, P25).

	Pd0	Pd10	Pd25	P0	P10	P25
Dog 1	70	85	90	70	80	90
Dog 2	80	110	120	80	120	125
Dog 3	100	120	120	100	120	120
Dog 4	100	140	130	100	140	130
Dog 5	70	90	80	70	90	80
Dog 6	100	110	120	100	110	110
Dog 7	100	120	130	90	120	120
Dog 8	80	100	100	80	110	120
Dog 9	90	120	110	80	120	110
Dog 10	90	130	120	90	130	130

Note: The unit of measurement used for pressure was millimeters of mercury

Table 3. Results of Doppler flowmetry flow variables(Vd0, Vd10 and Vd25) and free flow variables (V0, V10 and V25).

	Vd0	Vd10	Vd25	V0	V10	V25
Dog 1	146	198	198	132	240	248
Dog 2	126	160	143	136	160	144
Dog 3	285	357	376	240	280	320
Dog 4	287	321	338	280	320	320
Dog 5	102	166	156	130	168	160
Dog 6	172	232	220	132	180	168
Dog 7	264	280	280	236	260	272
Dog 8	124	143	156	120	160	180
Dog 9	200	330	268	200	320	280
Dog 10	126	188	166	120	180	172

Note: The unit of measurement used for volume was milliliters per minute

20. Initially, a four-dog pilot group underwent the experiment described above, for technical development and to test the devices used.

21. ITA was evaluated by one and then by the other method, taking turns from one dog to the next.

Euthanasia

After the completion of the assessments, the animal was sacrificed with 40 ml of 19.1% potassium chloride, endovenously administered.

The Doppler flowmetry

The Doppler used to perform the measurements was the AU3 Partner and the artery was examined by a 5-10MHz linear transducer, and the volume flow calculated by multiplying the diameter by the average velocity of the blood flow.

Variables analyzed

Variables were evaluated in three stages; zero time corresponding to the beginning of the experiment and at two different times, 10 and 25 minutes (Tables 2 and 3):

1. The variables of Doppler evaluation were: Vd0, VD10 and Vd25, and mean arterial pressures corresponding to each verification were; Pd0, PD10 and Pd25.

2. The free flow variables were: V0, V10 and V25, and mean arterial pressures corresponding to each verification were, respectively, P0, P10 and P25.

Statistical Analysis

The analysis was performed by the nonparametric Friedman and Kruskal-Wallis tests. Comparing the results, we adopted the null hypothesis that the flow results measured by the two methods are equal versus the alternative hypothesis

that the results would be different. In all tests, we considered a 5% significance level. The variation of the diameter of the internal thoracic arteries and blood pressure were also analyzed by the same tests, as factors that influence the result of the flow between the two methods. Since the null hypothesis has $P<0.05$, they would be different in the two groups.

RESULTS

The experiments were uneventful. It was easy to dissect the ITA in the animals, presenting a good caliber and a good flow. The average diameter of the ITAs verified by Doppler was 3.54 mm and the free flow of 3.39 mm.

No difference was observed between diameters of ITA in the 2 methods, Doppler and free flow ($P=0.705$).

The minimum diameter measured by the arterial Doppler method was 2.3 mm, and the free flow of 2.4 mm, while the maximum diameter was 5.2 mm for the first and, 4.6 mm for the second method. There was a standard deviation of 0.89 for Doppler, while for the free flow of 0.76. The standard error was 0.28 for the first and 0.24 for second method.

The values for mean arterial pressure measured by the Doppler method correspond to Pd0, PD10 and Pd25 and the free flow to P0, P10 and P25 (Table 2).

The average blood pressure in the Doppler flowmetry method at zero time was 88 ± 12.29 mmHg, with a minimum of 70 mmHg and the maximum of 100 mmHg, whereas in the free-flow method at the same time, the average was 86 ± 11.74 mmHg, with a minimum of 70 mmHg and a maximum of 100 mmHg.

The average blood pressure by Doppler method was 112.5 ± 17.20 mmHg at the 10- minute time, the minimum and the maximum of 85 mmHg and 122.5 mmHg, respectively, at the free flow was 114 ± 17.76 mm Hg, with a minimum of 80 mmHg and a maximum of 140 mmHg. At the 25-minute time, the mean blood pressure in Doppler flowmetry method was 112 ± 16.87 mmHg, the minimum and maximum of 80 mmHg and 122.5 mmHg, respectively, the free flow was 113.5 ± 16.67 mmHg, with a minimum of 80 mmHg and the maximum of 130 mmHg.

The blood pressure measured by Doppler method and the free flow were compared with analyzes of nonparametric variance (Friedman). No significant difference was found, therefore, the pressure in both methods were similar during the three times ($P=0.309$).

The values found for the arterial flow in the Doppler variables correspond to Vd0, Vd10 and Vd25 and for free flow, V0, V10 and V25, and can be verified in Table 3.

The mean arterial flow in the Doppler flowmetry time at zero time was 183 ± 71.6 ml/min, with a minimum of 102 ml/min and a maximum of 287 ml/min, while the free flow method at the same time presented an average of 168.6 ± 59.7 ml/min, with the minimum of 120 ml / min and the maximum of 280 ml/min.

The mean arterial flow in Doppler flowmetry was 237.5 ± 78.7 ml/min at the 10-minute time, with a minimum of 143 ml/min and a maximum of 357 ml/min and the free flow was 226.8 ± 65.2 ml/min with the minimum of 160 ml/min and the maximum of 320 ml/min. At the 25-minute time, the mean arterial flow in Dopplermetria method was 230.1 ± 82.1 ml/min, with a minimum of 143 ml/min and a maximum of 376 ml/min and the free flow was 226 4 ± 68.8 ml/min, with a minimum of 144 ml/min and a maximum of 320 ml / min.

The results revealed no significant difference in blood flow between the methods in the times analyzed ($P=0.285$). Regression analysis shows that blood pressure is the determining factor for the variation in flow ($P=<0.00001$*). By adjusted R^2, approximately 30% of the variation of the flow are due to the pressure variation.

DISCUSSION

The importance of this experiment was evidenced by demonstrating similar results between the standard method (Doppermetry) and the free flow method, equaling them in daily clinical practice and allowing the free flow method as an option in studies, for example, to evaluate the effect of drugs into the blood flow, or to test methods of ITA dissection [11] and others. The free flow is also useful in the daily assessment of ITA dissected in coronary artery bypass surgery.

The animal chosen was the adult dog for having a ITA with good caliber and good flow [12]. The artery of the dog has similar histopathological characteristics with the human's, as demonstrated by Sasajima et al. [6]. All experimental work used the dog to the assessment of ITA flow [6,7,12,13].

Norepinephrine was used in order to compare the two methods in different blood pressures and consequently different blood flow speeds, therefore, it provides other data for the comparison between Doppler flowmetry and free flow [14].

It is important to note that drug was used in a constant volume by means of an infusion pump, not showing significant fluctuations in blood pressure during the analysis of the two types of verification. We used the halothane inhalational anesthetic, since it was continously administered with the same quantity. The continuous inhalation anesthesia is the one which causes less oscillations in blood pressure [15]. In studies analyzed, the maintenance of anesthesia during surgery was performed in the same manner as in this experimental work [6,7,12,13].

The factors that can influence the arterial flow results between the methods are:

1. The difference in diameters between the arteries in both methods. The experiment showed the similarity between the diameters of arteries verified in Doppler flowmetry and free flow, being statistically significant ($P=0.705$).

2. The difference between the blood pressures during

Doppler flowmetry and free flow verification. The experiment showed the similarity between the mean arterial pressure, recorded at the beginning of the Doppler flowmetry and free flow, being statistically significant (*P*=0.309).

3. The decrease in blood flow due to the blood loss during the free flow verification. It would be important if the measurement taken at the 1-minute time were corrected by performing the measurement in 15 seconds, the blood loss was lower, and then injected into the animal.

There are few studies comparing the two methods. Canver et al. [4] separately used the two types of flow verification of the internal thoracic artery, the Doppler flowmetry in the preoperative period and free flow in the intraoperative period, demonstrating the correlation of them with a "*P*" statistically significant, as in this work. In the study of Cagli et al. [16] performed on humans, there was a comparison between the free flow in the intraoperative period with Doppler flowmetry in the pre-and postoperative period, with a smaller flow in the first method. However, there is a strong bias in this study, for comparing a method in which the patient is anesthetized (free flow) whose blood pressure is different from a non-anesthetized patient (Doppler flowmetry), demonstrating a lower flow in the first one, and other problems such as the manipulation of the dissection be present in only one method (free flow).

Authors as Tada et al. [7] used the free flow to assess the inotropic action of substances on the arterial flow, while Choi and Lee [17] and Wendler et al. [5] used to compare the free flow of the dissected ITA with and without the pedicle, demonstrating that both methods are reliable. Hata et al. [18] demonstrated that ITA even with flow under 20 ml/min can be used in CABG, increasing the flow in the coronary artery with severe obstruction, similar to the ITA with greater flow.

The flow verification by Doppler can identify factors of early occlusion of arterial or venous graft in the coronary artery, as the low flow in the coronary bed after the anastomosis. This method is more comprehensive than the free flow. Van der Meulen et al. [19] used the intraoperative Doppler to verify blood flow speed in six internal thoracic artery before and after anastomosed to the anterior descending coronary artery. Suematsu et al. [8] used the Doppler flowmetry compared to histological examination in dogs and then compared to angiography, in humans, in evaluating the flow of anastomoses graft to the coronary arteries, showing good correlation between the methods (*P*<0.001) [8]. Doppler would be an excellent evalution method of ITA *in situ* and after the graft anastomosis in CABG [20,21].

The free flow is currently used on a smaller scale than the Doppler and angiography, but it is still an efficient method in different papers [22,23]. Its easy and simple applicability, no need for a device or an expert in the field, can increase the number of surveys assessing blood flow, being especially useful in countries with deficits of research financial resources, as in Brazil.

CONCLUSION

A comparative evaluation between the free flow and Doppler flowmetry of the ITA in anesthetized dogs with and without administration of norepinephrine presents results without statistically significant difference.

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