

Video Article

Subclavian Vein Puncture As an Alternative Method of Blood Sample Collection in Rats

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Abstract

Blood collection with enough blood volume is essential in animal experiments. Blood collection from the tail vein of rats is popular and less stressful compared to other more aggressive methods such as retro-orbital plexus sample collection. However, this blood collection method is sometimes limited by an unsatisfactory success rate. Here, we introduce a method for blood collection through the subclavian vein puncture. The subclavian vein is located just under the clavicle and this vein is large enough to fulfill the volume requirements of blood collection. Our results show that this method is safe and applicable for blood collection sampling with the required blood volume. Blood collection through the subclavian vein puncture could serve as an alternative blood collection method in case of failed tail vein blood sampling in rats.

Video Link

The video component of this article can be found at <https://www.jove.com/video/58499/>

Introduction

Blood collection is essential in animal research. The target veins for blood collection include the retro-orbital plexus, the jugular vein, the saphenous vein, tail blood vessels, and the carotid artery^{1,2,3,4}. Sometimes, blood could be obtained from the abdominal aorta, vena cava or even the heart^{5,6,7}. On such occasions, the animals must be sacrificed and cannot be used for subsequent observation; thus, these methods are used less in daily experimental work. Blood collection from the tail vein of rats is popular and less stressful compared to the above mentioned methods⁸.

However, blood collection from the tail vein is sometimes limited by an unsatisfactory success rate. Occasionally, it is also difficult to obtain enough blood volume by this method. As the subclavian vein is large enough and is located just under the clavicle bone, subclavian vein puncture can be an alternative method for blood sampling if routine blood collection methods are unsuccessful. Here, we introduce a method of blood collection by means of subclavian vein puncture in rats.

Protocol

This study was approved by the Central South University Ethics Committee for Animal Research from the Second Xiangya Hospital (Changsha, China). The manuscript was prepared according to ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines⁹.

1. Material and Animal Preparation

1. Prepare required materials: adhesive tape, epilating agent, 75% ethanol, blood collection tube, 2.5 mL syringe connected with needle (24 G, 0.6 mm x 25 mm), hair shaver, electronic scale, and gauge (see **Table of Materials**).
 2. Use 20 Sprague-Dawley (SD) rats, aged 8-10 weeks and weighing 153-200 g (see **Table of Materials**). Maintain rats in accordance with the Guide for the Care and Use of Laboratory Animals¹⁰.
 3. House rats under standard conditions with free access to food and drinking water. Keep them in 530 cm² cages with wood-shaving bedding (2 rats per cage). Maintain animals in a temperature-controlled room temperature at around 25 °C.
 4. Induce general anesthesia (sodium pentobarbital 40 mg/kg) in all animals through intraperitoneal injection before the subclavian vein puncture.
 5. Place the rat in a supine position. Fix the hind limbs in a comfortable position and fix the upper limb parallel to the body axis just beside the trunk.
- NOTE: No mechanical ventilation is needed for this procedure (**Figure 1**).

6. Clean both sides of the infraclavicular space to remove fur (with hair shaver and epilating agent, see **Table of Materials**) and any visible dirt. Wipe the neck and thoracic skin with 75% ethanol. Keep the area for puncture clean and dry with gauze.

2. Blood Collection Procedure

NOTE: Both sides of the subclavian vein are suitable for puncture, and here we choose the right side for puncture. The location and direction of the right clavicle are identified by the operator's left thumb (**Figure 2**). The suprasternal fossae are gently touched with the index finger as the suprasternal fossae is in the direction of the needle (**Figure 2**).

1. Rinse a sterile 2.5 mL syringe with heparin solution (10 IU/mL).
2. Locate the puncture site 0.5 mm caudal to the outer 1/3 region of the right clavicle (**Figure 3**). Move the syringe gently into the skin of the puncture site. Once the needle enters the infraclavicular skin, apply negative pressure with the operator's right hand (**Figure 4**).
NOTE: The average and maximal blood volume are 1.0 mL and 1.4 mL, respectively. The direction of the needle is positioned toward the suprasternal fossae and should be almost parallel to the clavicle just posterior to it. Usually, the subclavian vein can be reached by inserting the needle about 2 mm in this direction. Once the needle enters the vein, blood will enter the syringe under negative pressure.
 1. Maintain negative pressure until enough volume of blood is drawn (**Figure 5**).
3. If the subclavian vein blood collection is not successful, withdraw the needle to the subcutaneous area and adjust the direction of the needle slightly interior to the sternum.
 1. If three attempts fail, avoid the subclavian vein from the same side and use the contralateral side for blood sampling.
4. After blood collection, remove the puncture needle, and apply pressure to the puncture site for 1-2 minutes to stop bleeding.
NOTE: The pressure must be very gentle to prevent choking.

3. Blood Sample Processing

1. Transfer the blood sample into an anticoagulant-coated vacuum tube (with EDTA) and centrifuge for 10 min at 1600 x g to collect plasma.
2. Aspirate the supernatant to a clean tube and store at -80 °C (**Figure 6**).

Representative Results

A total of 20 SD rats (male n=10, female n=10) were used here. Blood collection was performed by skilled operators, who have performed more than 20 blood samplings from the subclavian vein in rats, and by the beginner operators, who have no subclavian vein puncture experience in rats or other animals. Before operations, beginners watched at least 3 procedures done by skilled operators.

Blood sampling succeeded in 17 rats through the right subclavian vein access. This procedure failed in 3 rats (1 in the skilled group and 2 in the beginner group), and blood sampling was successful through the left subclavian vein accesses in these 3 rats. The time course and blood volume collected for each case are illustrated in **Table 1**. The success rate was similar between the beginner group (80%) and the skilled group (90%, $p = 0.531$). The success rate was 100% for both groups when including the additional left subclavian vein accesses in the 3 rats. Two tailed Student's *t*-test showed that there was no significant difference on time expended between beginner group and skilled group (102.1 ± 14.9 s versus 84.3 ± 9.7 s, $p = 0.33$, **Figure 7**). No severe complications occurred in all procedures such as pneumothorax, hemopneumothorax, or major bleeding during or after operation. All animals survived and recovered within 30 minutes after the blood sampling.

To demonstrate the difficulties of learning the puncture procedure, we further compared the number of withdrawals between skilled and beginner groups. As shown in **Table 2**, the average number of withdrawals in the skilled group is slightly less than the beginner group (1.6 ± 0.3 vs. 2.5 ± 0.4), but there is no statistical significance ($p = 0.0913$).

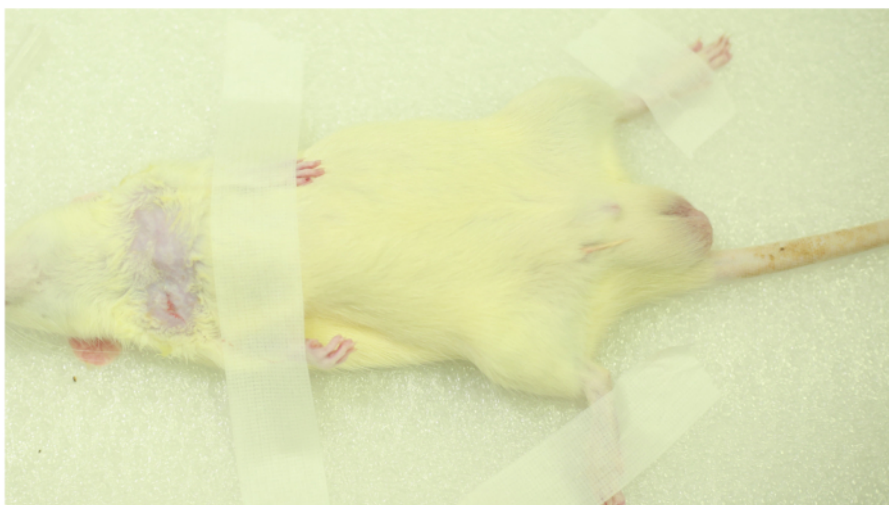


Figure 1: Position of rats. The rats are laid in a supine position, and the upper limbs are fixed parallel to the body axis, just beside the trunk. [Please click here to view a larger version of this figure.](#)

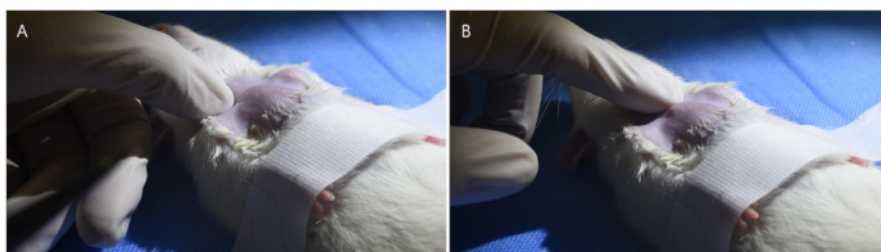


Figure 2: Identify the surface symbols of rats. (A) The left thumb of the operator is on the right clavicle to identify the location and array of this bone; (B) The suprasternal fossae are gently touched with the index finger. [Please click here to view a larger version of this figure.](#)



Figure 3: Location of puncture. The puncture site is located 0.5 mm caudal to the outer 1/3 site of the right clavicle. [Please click here to view a larger version of this figure.](#)

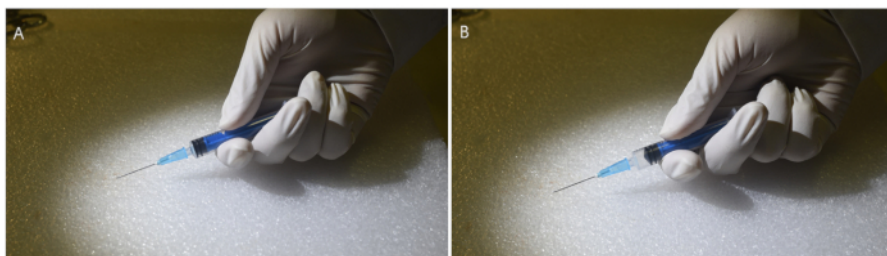


Figure 4: Forming negative pressure in the syringe. Once the needle enters the infraclavicular skin, the fingers of right hand move to create negative pressure in the syringe. [Please click here to view a larger version of this figure.](#)



Figure 5: Withdrawing blood into the syringe. When the needle enters the subclavian vein, blood will enter the syringe under negative pressure. The negative pressure is kept until enough blood is obtained. [Please click here to view a larger version of this figure.](#)

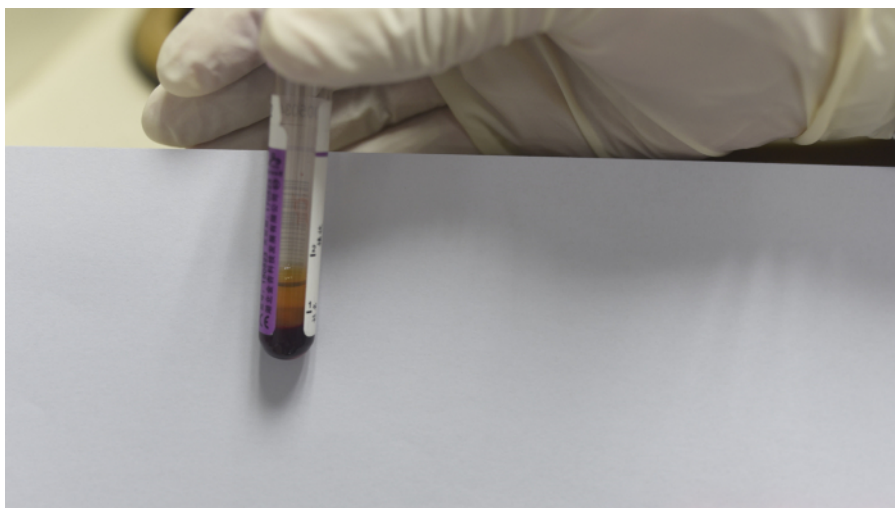
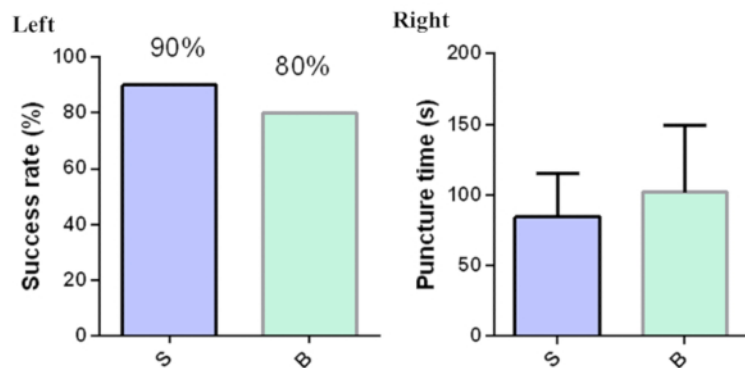


Figure 6: Plasma extraction after centrifuging. After centrifuging, the blood is separated into plasma and cells. [Please click here to view a larger version of this figure.](#)



S: the skilled group
B: the beginner group

Figure 7: The time course and success rate between different groups. This chart shows no significant differences in the time spent (left panel) or success rate (right panel) between the skilled group and the beginner group. The time course in the beginner group and the skilled group are 102.1 ± 14.9 s and 84.3 ± 9.7 s, respectively, $p = 0.33$ between groups. [Please click here to view a larger version of this figure.](#)

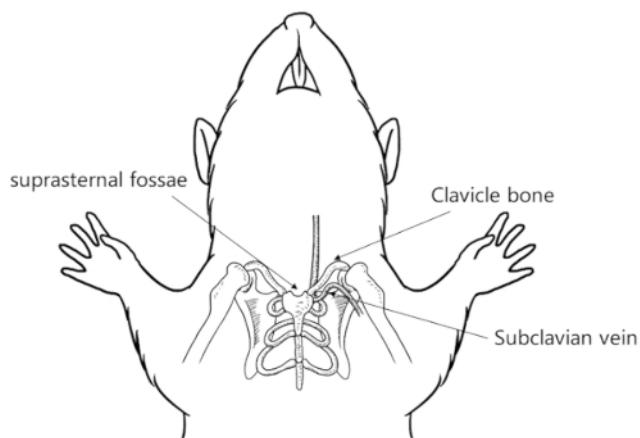


Figure 8: The anatomy of subclavian vein. The subclavian vein is located just posterior and caudal to the clavicle. It joins with the internal jugular vein to form the brachiocephalic vein and drains into super vena cava, just beneath the suprasternal fossae. [Please click here to view a larger version of this figure.](#)

skilled group					Beginner group				
	Gender	Body weight (g)	Blood volume (mL)	Time course (S)		Gender	Body weight (g)	blood volume (mL)	Time course (S)
S1	male	153	1.6	70	B1	male	149	1.6	72
S2	male	168	1.6	60	B2	male	158	1.6	68
S3	female	183	1.6	75	B3	female	190	1.6	83
S4	female	170	1.6	74	B4	female	160	1.6	77
S5	female	159	1.6	65	B5	female	153	1.6	200
S6	male	200	1.6	83	B6	male	187	1.6	92
S7	male	178	1.6	86	B7	male	164	1.6	99
S8	male	187	1.6	168	B8	male	200	1.6	82
S9	female	192	1.6	83	B9	female	198	1.6	70
S10	female	157	1.6	79	B10	female	175	1.6	178

Table 1: The blood volume and time course from puncture to a finished blood collection. S: skilled group; B: beginners.

Group	n	Mean	SE	95% Conf. Interval	
B	10	2.5	0.4	1.59	3.41
S	10	1.6	0.3	0.91	2.29

Table 2: The number of withdrawals between the two groups. B: beginners; S: skilled group. $p = 0.0913$

Discussion

Here, we introduce an alternative method for blood extraction from the subclavian vein and we show that this method is feasible, safe, and applicable for blood sampling in rats. This method is derived from the subclavian vein puncture in pacemaker lead implantation for patients¹¹. The subclavian vein is located just posterior and caudal to the clavicle and is a continuation of the axillary vein. It runs from the outer border of the first rib to the medial border of anterior scalene muscle¹². From here, it joins with the internal jugular vein to form the brachiocephalic vein and drains into super vena cava, just beneath suprasternal fossae (**Figure 8**). The clavicle is easily identified and there is a clear anatomic relationship between the subclavian vein and the clavicle. Here, we applied this clinical skill to animal experiments. Due to the different orientation of the upper limb in humans and rats, the upper limb is placed just beside the trunk during the procedure to expose the space caudal to the clavicle, which will facilitate the puncture (**Figure 1**).

The subclavian vein is much larger than the tail vein. Therefore, it is easier to get enough blood from the subclavian vein than from the tail vein. The total sample volume could reach 10% of total circulating blood volume each time¹. If conventional tail vein blood sampling or another route fails, researchers can consider the subclavian vein puncture. General anesthesia is required for this procedure, and this is a major limitation of this procedure. Here, we use pentobarbital sodium for general anesthesia. Compared to other agents, pentobarbital sodium is easy to inject, and no other special instruments are needed. Tsukamoto *et al.* reported that isoflurane inhalation had the most stable SpO₂ than other agents and had short recovery times. In centers with inhalation equipment, isoflurane can be considered¹³. Moreover, as the subclavian vein is located near to the thoracic cavity, we must be careful to prevent severe procedure-related complications such as pneumothorax and hemopneumothorax. The direction of the needle must be carefully directed to avoid these complications. Here, the needle is always positioned parallel to the clavicle and just posterior to it and moved gently towards the suprasternal fossae. The results show that use of above technique can avoid potential complications. Avoid any downward direction of the needle toward the thoracic cavity in this procedure. Also, the depth of the subclavian vein must always be kept about 2 mm from the puncture site.

Occasionally, attempts from one side may fail. We do not suggest more than 3 attempts from the same side. In such conditions, use the other side for puncture, which may improve the success rate of blood sampling. To further illustrate the feasibility of our method, we tested the success rate and time expended between a beginner group and a skilled group. Interestingly, there were no statistical significance between these groups ($p = 0.33$), which proved the practicability of this newly developed method.

As discussed here, the main limitation is the success rate on one side. If the procedure fails, the other side of the subclavian vein must be considered. General anesthesia is used in this procedure and the recovery time averaged about 30 min with pentobarbital sodium. Isoflurane inhalation anesthesia has a much shorter recovery time and the adoption of new anesthesia methods could be helpful in reducing the duration of the procedure. We show that this method is applicable to rats, but we have not tested its applicability to smaller animals such as mice.

In conclusion, the study results indicate that the subclavian vein puncture method for blood sample collection is safe and feasible in rats. This method is especially valuable when large amounts of blood are needed. This method is an optimal alternative if conventional blood sampling procedures fail.

Disclosures

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