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ROUTINE BLEEDING TECHNIQUES IN LABORATORY RODENTS

Madhuri Oruganti* and Sudesh Gaidhani

Central Council for Research in Ayurvedic Sciences, Dept. of AYUSH, Ministry of Health & Family Welfare, Janakpuri, New Delhi, India

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Correspondence to Author:

Dr. Madhuri Oruganti

SRF (Pharmacology), Central Council for Research in Ayurvedic Sciences, Dept. of AYUSH, Ministry of Health & Family Welfare, Janakpuri, New Delhi, India

ABSTRACT

Collecting blood from Lab animals is necessary for a wide variety of scientific studies, and there are a number of efficient methods available. It is important to remember that blood collection can stress the animals, and may have an impact on the outcome of research data. Potential adverse affects viz. Hypovolemic shock, anemia, stress, haemorrhage, bruising, thrombosis; infection at the site of needle entry, phlebitis, scarring, and nerve damage should be avoided. It is essential to be able to recognize the clinical signs of shock and to take appropriate action. The purpose of this article is to review the different methods of blood collection, their advantages, limitations, monitoring, restraints, possible adverse effects, their prevention and control in laboratory rodents.

INTRODUCTION: Rat, mice, guinea pig, hamster & gerbils are the rodents used in laboratories for scientific studies in different fields such as applied research, xeno transplantation, embryo technology, cancer research, toxicity studies.etc. Collecting blood from Lab animals is necessary for a wide variety of scientific studies, and there are a number of efficient methods available. It is important to remember that blood collection can stress the animals, and may have an impact on the outcome of research data. In addition, it is extremely important that those who collect blood become skilled in the techniques they employ, and seek to stress the animal as little as possible. Blood collection techniques can broadly be classified as (**Fig. 1**). Blood collection not requiring anesthesia: Saphenous vein, dorsal pedal vein. Blood collection requiring anesthesia: Tail vein, Orbital sinus, Jugular vein. Terminal procedures: Cardiac puncture, Posterior vena cava, Axillary vessels, Orbital sinus [1, 2].

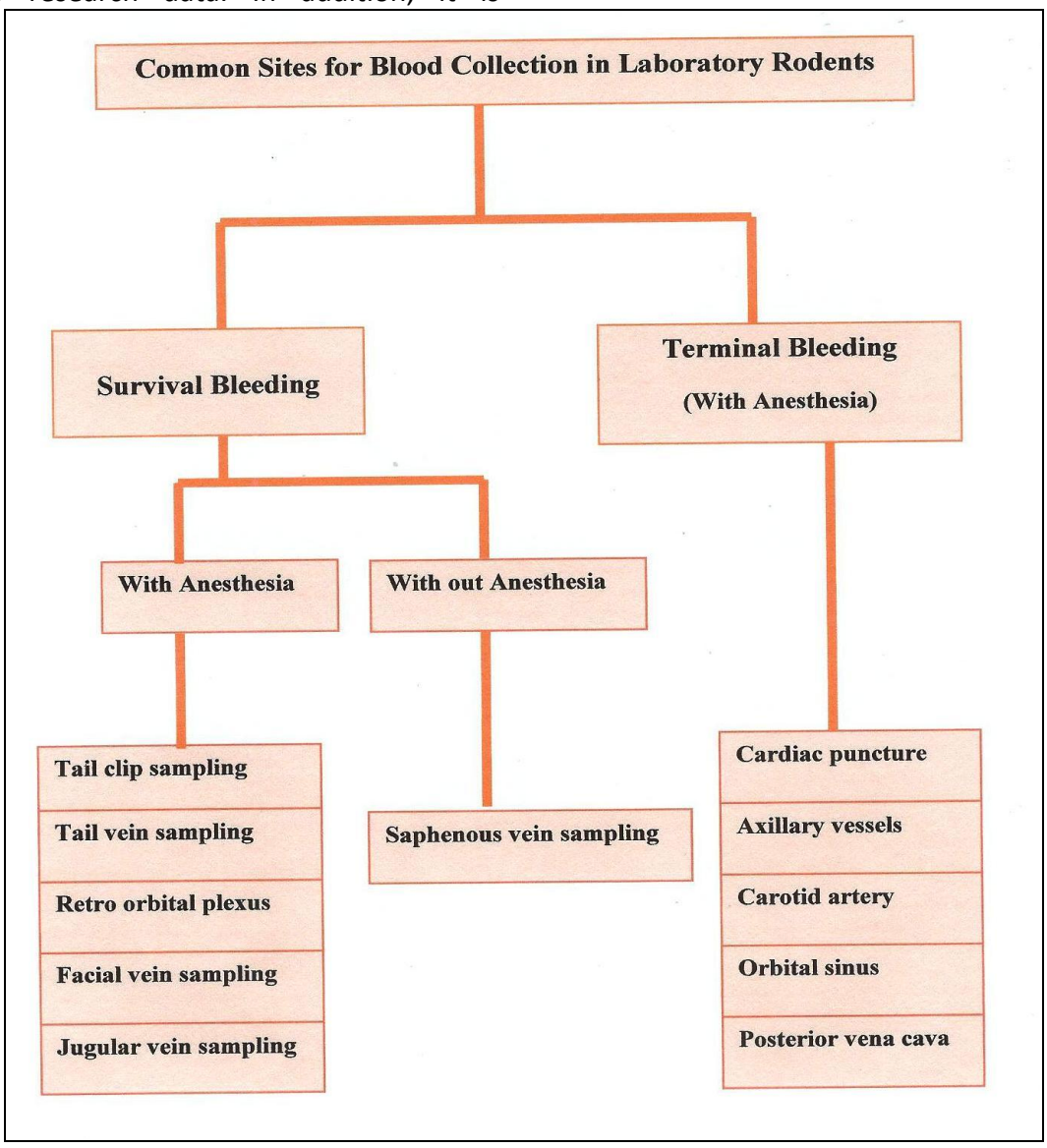

FIGURE 1: COMMON SITES FOR BLOOD COLLECTION IN LABORATORY RODENTS

Choosing the blood withdrawal technique appropriate for the purpose at hand includes consideration of several factors such as: The species to be bled, size of the animal to be bled, type of the sample required (e.g. serum, whole blood, Plasma, etc.), quality of the sample required (sterility, tissue fluid contamination, etc.), quantity of blood required, frequency of sampling, Health status of the animal being bled, training and experience of the phlebotomist, effect of restraint or anesthesia on the blood parameter measured [3].

Policy for Blood Collection: All Survival (non-terminal) blood collection without replacement fluids is limited to 10% of the total circulating blood volume of a healthy animal at 2 week intervals. On average, the total circulating blood volume is equal to 5.5 -7.0 % (~66 ml/kg) of the animal's body weight [3]. If larger amounts are needed, then up to 15 % of the total circulating blood volume may be withdrawn (replacement fluids are given at the time of blood withdrawal).

MATERIAL & METHODS:

Materials required: The following materials are used in the collection of blood from the laboratory rodents. Clippers (surgical blade, size 40), tourniquet of a suture looped through a syringe, 22 gauge needle, silicon gel, micro- hematocrit tubes, alcohol, swabs, & styptic powder.

Handling and Restraint: Firm, empathetic handling is very important, as is the time required to withdraw the sample. Both these parameters can affect the degree of stress for the animal and consequently the quality of the sample and research data. Physical restraining of animals is necessary to prevent any movement that would result in lacerating the blood vessel or other potentially serious complications. Blood may be collected from conscious animals that are appropriately restrained provided that persons performing the procedure are skilled. The animal should be restrained by an experienced person (preferably one known to the animal). The correct level of restraint is that which allows a satisfactory sample with drawl at the first attempt but which does not cause the animal to become unnecessarily distressed. Inanimate restrainers can be used, although these may not always be the best method for individual animals. Manual restraint facilitates recognition of distress more effectively [4].

A vein will collapse if a sample is taken too quickly, so care should be taken to ensure that blood is withdrawn at an appropriate rate. Consideration should be given to offering a reward after each bleed, depending on the species.

Anesthesia: Anesthesia is required if blood collection is being performed either via the retro-orbital sinus or by cardiac puncture due to the distress and pain which can be caused and for the serious complications (injury to the eye, cardiac tamponade and death) associated with these routes. For survival procedures requiring anesthesia, isoflurane is recommended as it is short-acting and allows replacing the rodent in its cage within minutes. The use of topical analgesics has been advocated [5]. The disadvantage of incorporating local analgesics may include increased procedure time and lack of impact on adverse factors other than pain.

Needle Size: A sterile needle (or lancet) should be used to puncture the skin and underlying blood vessel. The size of the needle (length and bore) is very important. It is recommended to use as large a bore as possible to ensure rapid blood withdrawal without collapsing the vein, within the constraint of avoiding hematoma (i.e. the bore should be just less than the diameter of the vessel).

Monitoring: If the animal is being bled routinely, the red blood cell packed volume (PCV) should be

checked weekly to determine when blood collection should be suspended in order for the animal to recover from potential anemia. While healthy adult animals can recover their blood volume within 24 hours, it may take up to 2 weeks for all the other blood constituents (i.e. cells, proteins) to be replaced. By monitoring the hematocrit (Hct or packed cell volume- PCV) and/or hemoglobin of the animal, it is possible to evaluate whether the animal has sufficiently recovered from a single or multiple blood draws. After a sudden or acute blood loss, it takes up to 24 hours for the hematocrit and hemoglobin to reflect this loss. In general, if the animal's hematocrit is less than 35% or the hemoglobin concentration is less than 10 g/dl, it is not safe to remove blood. The normal PCV values of laboratory rodents were given in **table1**.

TABLE 1: NORMAL PACKED CELL VOLUME (PCV) FOR SOME LAB RODENTS (%) [6]

Mouse	39-49
Rat	36-54
Gerbil	43-60
Hamster	40-61
Guinea Pig	37-48

Volume of blood to be collected: The volume of blood removed and the frequency of sampling will be based on the purpose of the scientific procedure and the total blood volume of the animal. For reasons of good animal welfare and science, serious consideration must be given to the combined effect of sample volume and the frequency of sampling. If too much blood is withdrawn, too rapidly or too frequently without replacement, an animal may go into short-term hypovolaemic shock and/or in the longer-term anaemia. Data interpretation and scientific validity may be confounded if excessive sampling is employed. Approximate blood sample volumes for a range of body weights are given in the **table 2**.

TABLE 2: APPROXIMATE BLOOD SAMPLE VOLUMES FOR A RANGE OF BODY WEIGHT [3]

Body weight (g)	***CBV(ml) Circulating Blood Volume**	**1% CBV (ml)** every 24 hrs†	**7.5% CBV (ml)** every 7 days†	**10% CBV (ml)** every 2 wks†
20	1.10 - 1.40	.011 - .014	.082 - .105	.11 - .14
25	1.37 - 1.75	.014 - .018	.10 - .13	.14 - .18
30	1.65 - 2.10	.017 - .021	.12 - .16	.17 - .21
35	1.93 - 2.45	.019 - .025	.14 - .18	.19 - .25
40	2.20 - 2.80	.022 - .028	.16 - .21	.22 - .28
125	6.88 - 8.75	.069 - .088	.52 - .66	.69 - .88
150	8.25 - 10.50	.082 - .105	.62 - .79	.82 - 1.0
200	11.00 - 14.00	.11 - .14	.82 – 1.05	1.1 - 1.4
250	13.75 - 17.50	.14 - .18	1.0 – 1.3	1.4 - 1.8
300	16.50 - 21.00	.17 - .21	1.2 – 1.6	1.7 - 2.1
350	19.25 - 24.50	.19 - .25	1.4 – 1.8	1.9 - 2.5

*Circulating blood volume

†maximum sample volume for that sampling frequency

As a rough guide, up to 10% of the total blood volume can be taken on a single occasion from a normal, healthy animal on an adequate plane of nutrition with minimal adverse effects; this volume may be repeated after 2-3 weeks. For repeat bleeds at shorter intervals, a maximum of 1.0% of an animal's total blood volume can be removed every 24 hours; the effects of stress, site chosen and anesthetic used must be carefully considered. If frequent samples are necessary, the use of cannulation as a less stressful alternative to repeated vein puncture should be considered. As a general rule, total blood volume can generally be estimated as 55 - 70 ml/kg body weight. However, care should be taken in these calculations as the percentage of total blood will be lower (-15%) in obese and older animals. Information on total blood volumes and safe blood sample volumes for laboratory animals is given here. As a general principle, sample volumes and number of samples should be kept to a minimum [7]. Practical blood sample volumes for laboratory animals are given in the **table 3** [8].

TABLE 3: PRACTICAL BLOOD SAMPLE VOLUMES FOR DIFFERENT LABORATORY ANIMALS [8]

Species	Reference weight (g)	Blood volume (ml/kg)$	Total blood volume (TBV), normal adult (ml)	Safe volume for single bleed (ml)*	Bleed out volume (ml)
Mouse	18 - 40	58.5	Male 1.5 - 2.4 Female 1.0 - 2.4	0.1 - 0.2	Male 0.8 - 1.4 Female 0.6 - 1.4
Rat	250 - 500	54 - 70	Male 29 - 33 Female 16 - 19	Male 2.9 - 3.3 Female 1.6 - 1.9	Male 13 - 15 Female 7.5 - 9
Hamster	85-150	78	Male 6.3 - 9.7 Female 7.1 - 11.2	Male 0.6 - 0.9 Female 0.7 - 1.1	Male 2.9 - 4.5 Female 3.3 - 5.2
Gerbil	55 - 100	66 - 78	Male 4.5 - 7 Female 3.8 - 6	Male 0.4 - 0.7 Female 0.4 - 0.6	Male 2.2 - 3.5 Female 1.9 - 2.9
Guinea pig	700 - 1200	69 - 75	Male 59 - 84 Female 48 - 63	Male 6 - 8 Female 5 - 6	Male 29 - 42 Female 24 - 31

$ - blood volume estimate for a single species may not reflect differences among individual breeds or variations due to age, sex, or illness.
Single bleed 10% of blood volume

Venous collection: Choose a site that is fit for purpose and one which causes minimal stress to the animal. Samples taken from different sites may have differences in biochemical/hematological values. Time should be spent accurately locating and dilating the vein before puncturing the vessel. In general, anesthesia is not required for venous access, since the associated stress would probably be greater than the discomfort of a needle prick or of a puncture with a lance.

Dilation of the vein: In conscious rodents, blood can be more easily obtained if the animal (or part of the animal the sample is taken from, e.g. tail) is warmed first. Animals may be placed in a thermostatically controlled warming box for a brief period during which they should be kept under constant observation in order to prevent hyperthermia (indicated by breathing more rapidly, panting or salivating). Use of topical irritants such as xylene is not recommended. Xylene tends to cause leucocytosis and if it comes in contact with blood, will cause hemolysis. Adequate dilation of vessels can generally be achieved by use of a heat lamp, alcohol or gentle massaging. In anaesthetized animals, vasodilation may occur as a result of the anesthetic. Anaesthetized animals may not, therefore, need warming so consideration should be given to bleeding when animals are anaesthetized for another purpose.

Arterial collection: The main reason for collecting blood from arteries is that large samples can be obtained rapidly and relatively easily. Many of the principles described above for venous collection also apply to removing blood from arteries.

Common sites for bleeding:

1. **Tail Clip Sampling:** This technique involves clipping (e.g. amputating) of the distal end of tail not more than 1mm in mice or 2mm in rat. This technique produces a sample of variable quality that may be contaminated with tissue and skin products. Sample quality decreases with prolonged bleeding times and "milking" of the tail. If a topical hypothermic anesthetic is used, blood will flow as the tail re-warms. If a local anesthetic is applied, adequate contact time should be allowed for it to take effect [9]. In most cases warming the tail with the aid of a heat lamp or warm compresses will increase obtainable blood volume. Tail clip sampling generally used to collect small volume of samples. Repeated collections were possible with this technique.

2. **Tail vein sampling:** This is a reliable and safe method for serial collection of small blood volumes. This can be used in both rats and mice by cannulating the blood vessel, or, by superficially nicking the vessel perpendicular to the tail. Sample collection by nicking the vessel is easily performed in both species, but produces a sample of variable quality that may be contaminated with tissue and skin products. If tissue analysis requires tail tip amputation, it should be done under general anesthesia for mice older than 21 days of age. Care should be taken to take the smallest piece of tissue possible and stop bleeding by application of gauze pad or silver nitrate, if necessary. Animals should not be placed in a cage with other animals immediately after performing this procedure as the presence of blood on the tail can cause mutilation and further trauma. Use finger pressure to assure hemostasis. Repeated collections are possible with tail vein nicking (the clot/scab can be gently removed for repeated small samples if serial testing is required e.g., glucose measures, etc.). Wipe off the site with hydrogen peroxide (H_2O_2 3%) to eliminate remaining blood residues. Tail artery sampling yields larger volumes but requires the animal to be anesthetized and placed in dorsal recumbency. Good hemostasis is also required, as always whenever an artery is incised.

3. **Retro-orbital Sinus/Plexus Sampling:** This is a reliable and relatively safe method for serial blood sampling that yields reasonable sample volumes in rodents. The eye and health of the animal are seemingly unaffected when the procedure is properly done by experienced personnel. This technique should only be performed under general anesthesia. The technique involves application of slight pressure (thumb) to the jugular vein to occlude venous return from the orbital sinus followed by penetration of the retro-orbital sinus (medial to the eye) with a small glass capillary tube or Pasteur pipette. Blood flow is terminated by release of pressure on the jugular vein and removal of the capillary tube.

Retro-orbital sampling can be used in both mice and rats (though usually not a method of choice in the rat). Repeat sampling from the same orbit may be difficult (10 days to 2 weeks recommended between successive bleeds). However, alternating orbits should not be attempted until the phlebotomist is proficient with the technique in the same orbit. Retro orbital bleeding may damage the eye, causing blindness, and may be a painful procedure. In g. pig, Infra-orbital sinus is not a preferred route, should be used only when absolutely necessary [10].

Procedure: Anesthetize the animal. Stabilize the head against the tabletop with one hand. Insert a micro hematocrit tube below the eye and slightly behind it, midway between the medial and lateral canthus of the eyelids. Blood fills the tube by capillary action. Remove the tube, and the bleeding stops. Blot the blood with a sponge. Apply an ophthalmic ointment to the eye. Finally, check the eye to make sure that bleeding has stopped.

4. **Facial Vein Sampling:** Blood collection from the submandibular facial vein is a safe and fast technique in mice. It requires momentary restraint and approximately 200ul of blood can be obtained easily from a healthy adult mouse. The vessel is located just beneath the skin immediately caudal to the facial vibrissae (whiskers) at the corner of the jaw. Repeated sampling is possible by alternating sides of the face. Materials needed include a 20 or 22 G hypodermic needle, blood collection tubes and sterile gauze. Training is necessary before this procedure is performed [11].

5. **Jugular Vein Sampling (limited to the rat):** Jugular vein/anterior vena cava (either side may be utilized but vessels on the right are often more accessible) sampling can be conducted without anesthesia, although the use of anesthesia greatly facilitates the procedure. This technique yields medium to large volume samples of high quality. Jugular Vein Sampling does not easily lend itself to repeated serial sampling.

   Procedure: Place the anesthetized animal in dorsal recumbancy and locate the area of the thoracic inlet (dorsal to the junction of the uppermost portion of the sternum and the first rib). Insert a 22 to 25 gauge, 5/8 to 1" needle at a 30 to 45 degree angle, maintaining gentle aspiration pressure upon entry. Direct the needle toward the midline of the thorax to a depth of 10 to 16 mm until blood appears in the hub of the needle; the sample may be collected in accordance with accepted volume guidelines. Apply gentle pressure to the site for 45 seconds to prevent hematoma formation [12].

6. **Saphenous Vein Sampling (medial or lateral approach): (both rats & mice):** Saphenous vein is a tortuous vessel which courses over the lateral aspect of the hock joint is the only visible collection site in hamsters, guinea pigs & gerbils and can be prone to hematoma formation. Animal may be sedated or manually restrained and the area over the vessel clipped and mobbed with alcohol. The restrainer may facilitate vessel dilation and stability of the leg by grasping the loose skin just in front of or behind the leg as it meets the body.

   A light coating of sterile ophthalmic ointment is used over the vessel to cause blood to "well" on the skin instead of dispersing; a 22 or 23G [22 G - 20 G in g. pig] needle is utilized to simply puncture the vessel. Be sure to apply gentle pressure to the site for approximately 45 seconds after collection has been completed to assure hemostasis [13, 14, 15]. Accessing the lateral saphenous vein does not require anesthesia. But, this technique may be practiced under sedation to reduce animal struggling due to distress.

7. **Cardiac puncture:** This technique is limited to terminal collections due to the difficulty of the technique and the danger of lung injury or cardiac tamponade and death when serial samples are taken. Cardiac puncture should be done only under general anesthesia or on euthanized animals [16].

   Procedure: Insert the needle into left of the sternum and directed 45^0 approximately

towards the heart. Put index finger below the sternum but avoid placing too much pressure on the animal, which would impair its breathing. Pull slightly on the plunger to create a little negative pressure in the syringe. Advance the needle to the midpoint of the chest cavity. When the needle reaches the heart, blood will flow into the syringe. Maintain gentle aspiration pressure. Squeeze the thorax rhythmically to pump blood from the peripheral circulation back to the heart so that we can get a faster flow of blood into the syringe. This is a terminal procedure which must be accomplished under general anesthesia and followed by an assurance of death. Euthanize the mouse before it regains consciousness. Cardiac puncture as a method of survival blood collection carries a high risk in guinea pigs as it requires anesthesia; hemorrhage into the pericardium also may result in death due to cardiac tamponade.

8. **Collecting Blood from the Axillary Vessels:** This is a terminal procedure. The mouse must be in a plane of surgical anesthesia. Incise axilla, deeply severing the major vessels with sharp scissors or a scalpel. Cut through tendons and other structures to open the area. In this technique we are able to collect a large volume of blood using a pipette. This blood loss may not be sufficient to assure death by ex-sanguinities. Therefore, euthanize the animal immediately [16].

9. **Collecting Blood from the Carotid Artery:** This is also a terminal procedure. The mouse must be in a plane of surgical anesthesia. Clip the fur from a large area around the neck. With sharp scissors or a scalpel, incise deeply in the neck, severing the major vessels as deep as the carotid. You should be able to collect a large volume of blood using a pipette. Euthanize the animal immediately after blood collection.

Potential Adverse effects: Potential adverse effects [17] viz., hypovolaemic shock, anemia, stress, haemorrhage, bruising, thrombosis; infection at the site of needle entry, phlebitis, scarring, nerve damage should be avoided. It is essential to be able to recognize the clinical signs of shock and anaemia and to be able to take appropriate action.

Haemorrhage: Due to poor hemostasis is not a common problem, unless the animal has a clotting defect, and in some cases gentle continuous pressure applied for several minutes may be all that is needed to stop the bleeding. In arterial sampling, longer compression of the puncture site is required to stop bleeding.

Bruising: Is due to subcutaneous bleeding at the time of vein-puncture or after the animal has been placed in its cage or pen, when the site might be aggravated by the animal itself through licking or rubbing.

Thrombosis: (clotting) and phlebitis (inflammation of the vein) are usually caused by dirty technique or leaking of an irritant substance (e.g. alcohol-based chemicals) around the vein.

Shock: Signs of hypovolaemic shock include a fast and thready pulse, pale dry mucous membranes, cold skin and extremities, restlessness, hyperventilation, and a sub-normal body temperature. If more than 10% of the total blood volume has been removed, a routine replacement with the same volume of warm (30-39^{o}C) normal buffered saline would constitute good animal care. Lactated Ringer's Solution (LRS) is recommended as the best for fluid replacement. For mice administer 1 ml of warmed LRS IP or SC. For rats administer 5 -10ml warmed LRS ½ via IP and ½ via SC administration.

Signs of anaemia: Include pale mucous membranes of the conjunctiva or inside the mouth, pale

tongue, gums, ears or footpads (non-pigmented animals), intolerance of exercise and, at the more extreme level, an increased respiratory rate when at rest. Where there is concern about the development of anaemia, packed cell volume, hemoglobin level, red blood cell and reticulocyte counts should be monitored throughout the series of bleeds using the results from the first sample from each animal as the baseline for the animal. [18]

Limitations: The limitations for blood collection preserve the health status of the animal and maintain the validity of experimental results based on blood samples. The guidelines provided here are for healthy, normal adult animals. Animals that are young, aged, stressed, have undergone experimental manipulations, or/are suffering from cardiac or respiratory disease may not be able to tolerate this amount of blood loss.

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