The Use of Protamine Titrations and Clotting Time

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Introduction

This article is presented for the benefit of clinical perfusionists and describes a method for rapid analysis of the degree of heparinization of patients before, during and after open-heart surgery.

In a series of twenty-five cardiopulmonary bypass procedures, we have found that as the heparin level decreases, there is a loss of circulating platelets.

Protamine is a basic protein; heparin is acidic and forms a salt compound with protamine. Protamine in excess may act as an anti-coagulant; however, with the addition of the proper amount of protamine to heparinized blood, normal blood coagulation is restored.[1]

Equipment needed:

a) 12 x 75 test tubes with #12 corks
b) 50mg. Protamine, 250ml. normal saline
c) 10ml. pipet, 1ml. pipet
d) 50ml. mixing flask
e) 10ml. Vacutainer test tube
f) Rack for test tubes

Procedure

Dilute 50mg. of Protamine (5cc) with 45 ml. of normal saline in a 50 ml. flask. This Protamine solution will be utilized for subsequent dilutions.

STEP 1—Number seven 10 ml. test tubes (1-7).

STEP 2—Using the 10 ml. pipet, add 1 ml of Protamine solution to test Tube #1 and repeat the process each time increasing the amount in each test tube by 1 ml. Example:
#1—1 ml.
#2—2 ml.
#3—3 ml., etc.

STEP 3—Add 9 cc of normal saline to test tube #1 and repeat the process each time so that each test tube has 10 ml. Example:
#1—9 ml.
#2—8 ml.
#3—7 ml., etc.

STEP 4—Set up a rack with seven 12 x 75 sample test tubes and number these 1-7. From the master tube add 1/10 ml. to each sample tube corresponding with the master tube. Generally, six sets (1 set = 7 test tubes) will be sufficient for one case.

Procedure During Bypass

In our series, the patient is initially heparinized with 3mg. of heparin per kg. of body weight. The pump oxygenator is primed with one-third of the initial patient dose of heparin and 25 mg. per unit of blood.

Ten minutes after initiation of perfusion, 10 ml. of blood is sampled from the pump oxygenator. One ml. of blood is placed in each protamine diluent test tube and is capped with the properly labelled cork. The seven tubes are tilted 75° every 30 seconds.

The tubes are observed for clot formation and the tube that contains the most solid clot first is considered the titration level. The desired level should be tube #4.

Subsequent to the initial dose of heparin, one-sixth of this dose is given to the patient via the pump circuit every half hour. If not on bypass within the first half hour, the first dose should be given through an intravenous line to maintain the level of heparinization.

After 10 minutes on bypass, obtain a titration sample and the next half-hour dose is determined by the tube number. If the titration is tube #5 or 6 do not give heparin and after a half hour take another titration in order to maintain a constant tube #4 level. Always wait at least 10 minutes after any heparin has been given before taking the next sample. If the titration is tube #4, give a regular (one-sixth of initial) dose.

Generally, one sample is taken at 10 minutes on bypass and subsequently every hour after that, or at the discretion of the perfusionist. Usually, the best results occur when the titration level is held at tube #4 during the bypass. Toward the end of the pump run, try to omit the last one-half hour dose which should result in a tube #3 level at the termination of bypass.

Discussion

Coming off pump at a tube #3 level means less protamine will be required to return the patient to normal clotting times. This is desirable as protamine has other effects on the coagulation system. In addition to its action to neutralize heparin, Protamine clumps and destroys platelets.[2]

It is rare to require more protamine than a 1:1 ratio. Our experience reveals that an inital 1/2:1 ratio is usually effective, though additional Protamine may be required within the subsequent 3 hours. Our results indicate that levels below tube #4 during bypass result in decreased circulating platelets. It is likely that this occurs from platelet adhesion and clumping due to inadequate heparinization. The higher the concentration of anticoagulant the less adhesive are the platelets.[3]

Determination of Clotting Times (Quantitatively)

Protamine Clotting Set

Draw 3cc. from master titration tube #4 with a syringe and a #20 gauge needle and label this syringe with a "P". Draw 5cc. of topical thrombin in a syringe with a #20 gauge needle and label this syringe "T".

Place 5 (12 x 75) test tubes in a rack and proceed as follows:

Put one drop of topical thrombin in the first tube and label it "T"

Put one drop from "P" syringe in the second 12 x 75 test tube and label it P^1.
Put two drops from P syringe in the third test tube and label it P^2.
Label the remaining two test tubes S_1 and S_2. These are regular Lee White clotting tubes.

Draw a 2 cc blood sample from the patient and discard the entire sample and syringe. Then draw 7 cc. of blood into a fresh syringe. Put 1 cc. of blood in each test tube and discard the remaining 2 cc. Start timing as soon as the first cc. of blood enters the T tube. The thrombin "T" tube should clot firmly within 30 seconds. Tilt tubes 75° every 30 seconds.

If enough protamine has been given to bring the patient to normal, the Lee White tubes (S_1 and S_2) should clot before the protamine tubes (P_1 and (^{2}). If the difference between S_1, S_2 and P_1 is less than one minute, more protamine should be given. No more than 25 mg. should be given without another clotting time.

It is of interest to note, in many cases, the valuation amount of heparin used and protamine required following bypass. Thus, using preconceived and rigid ratios of heparin and protamine may *not* be the most appropriate method.

NOTES—Our determinations of hemolysis are adapted from the method as written in the chapter entitled "Total Heme Pigments", pp. 318-322 of the book *"Diagnostic Laboratory Hematology"* by George E. Cartwright, M.D., fourth edition.

Dr. Lewis Bosher, Jr., Thoracic and Cardiovascular Surgery, Medical College of Virginia, and Dr. Lyman Fisher, Chief of Hematology, designed this system and I became exposed to it while working for Dr. Lewis Bosher as a technician.

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REFERENCES

[1 2] Blood coagulation, Hemorrhage and Thrombosis (Chapter entitled "Heparin Methods of Assay" by L. B. Jacques), p. 383, edited by Leandro M. Tocantins, N.D., and Louis A. Kazal, Ph.D.

[3] Blood Coagulation, Hemorrhage and Thrombosis (Chapter entitled "Estimation of the Adhesiveness of Blood Platesets (Method of Wright)" adapted by R. R. Holburn), p. 61, edited by Leandro M. Tocantins, M.D., and Louis A. Kazal, Ph.D.

Symposium

Answers to earlier SYMPOSIUM questions are trickling in and will be printed as they arrive. In order to give everyone a chance to respond, we are repeating all of these questions this issue with the assurance that any contributions in answer to early questions will be utilized.

The following are a new set of problems to replace the old.

1. *Given: A infant requires intra-cardiac surgery.*
   Question: Describe in detail the rationale and pump oxygenator circuitry your team prefers during such cases. How does this vary with each type of defect?
2. *Given: Concern for the mental outlook of the chronic dialysis patient.*
   Question: Explain the outlook your team wishes to develop in the patient, how this is accomplished by the medical personnel, and the effect upon the patients.
3. *Given: Patients are to be trained to accomplish dialysis at home.*
   Question: Summarize your training program emphasizing what you feel are its strongest points.
4. *Given: The aortocoronary bypass graft has probably become the most frequent cardiac procedure of late.*
   Question: Describe the rationale, technique and pump-oxygenator circuitry your team favors for this procedure. A discussion of results may also be included, if you like.
5. *Given: Organ preservation systems are many and diverse.*
   Question: Describe the preservation system you use and why this particular technique was chosen.
6. *Given: The increasing amount of interest shown in the impedance plethysmograph (sometimes called the impedance cardiograph).*
   Question: Outline the concepts upon which it operates and its potential value to the study of hemodynamics, or a summary of your experience with this unit, if you prefer.

Please reply by letter, include any illustrations you might desire, and send your reply to:

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