PILOT STUDY FOR LARGE SCALE PLASMA PROCUREMENT USING AUTOMATED PLASMAPHERESIS

INTRODUCTION

In recent years the National Blood Transfusion Service of England and Wales have been faced with an ever increasing demand for larger amounts of "source plasma" for the preparation of stable therapeutic plasma products. The two products making the greatest demand upon human blood resources are albumin and Factor VIII. It has been estimated that the clinical demands for Albumin are approximately 200 gm for every 1,000 total population. To establish the actual Factor VIII requirement for all the Haemophilia A sufferers in this country is extremely difficult, but it has been suggested that we should aim for 1.5×10^6 units of Factor VIII for each million of the population. For the Yorkshire Region with a population of approximately 3.5 million this means the procurement of 28,000 L of Plasma 17,000 L of which must be fresh.

This volume of plasma is greater than that achievable using every donation from our current donor panel, the annual collection being 136,000 donations. To date there has been an increasing gap between the amount of Factor VIII concentrate produced by the National Blood Transfusion Service of England and Wales and the therapeutic amount utilised. Currently this gap is filled by commercially produced concentrates and the present debate is whether it is economically and practically feasible to become nationally self sufficient in Factor VIII production.

The major problem to resolve is whether it is economically possible to increase the supply of source plasma to a sufficiently high level to meet the demand. This problem can be approached in three ways.

1. Increase the number of whole blood donations
2. Increase the clinical use of red cell concentrates
3. Introduce plasmapheresis programmes

Each Regional Transfusion Centre in England and Wales has to meet the clinical demands of its own region and also contribute sufficient source plasma to the National Blood Products Laboratory for the preparation of Factor VIII concentrates, albumin and other fractionated products. Each Region is subject to individual variation and must meet the problem of plasma self sufficiency by a method most suited to the Region's other requirements, i.e. whole blood, fresh frozen plasma, platelets and cryo-precipitate.

For the Yorkshire Region methods 2 and 3 were deemed the most applicable. Method 1 was considered impractical as a gross surplus of red cells would accrue which would be an unneccessary wastage and also a new sessional team would be needed.

Following a Regional survey to assess the clinical acceptability of concentrated red cells it was ascertained that a maximum of 50% concentrated red cell issue was all that could be achieved. To reach the goal of plasma self sufficiency therefore the third method available, plasmapheresis, was investigated. An automated system of plasmapheresis as opposed to a manual programme was chosen as in terms of donor time and safety it was more attractive. Initially it appeared cost prohibitive but if contemplated on a large scale and Factor VIII yields can be optimised then it becomes a more economic proposition.

This pilot study shows that large scale automated plasmapheresis programmes could readily produce the source plasma necessary for National self sufficiency provided adequate fractionation facilities are available.

METHODS & EQUIPMENT

Plasmapheresis was performed using the Haemonetics model 50 automated plasmapheresis machine (Haemonetics Corporation, Braintree, Massachussetts). This is an automated, single vein, closed cyclic system using a disposable pre-connected pheresis harness. Plasma separation is achieved by high speed centrifugation with return of the concentrated red cells to the donor at the end of each cycle.

The machine was preset to collect 500 ml of plasma so that the maximal extra - corporeal blood volume experienced by the donor did not exceed 700 ml. Two anticoagulants were used in this study.

1. 4% trisodium citrate (as recommended by the manufacturers)
   (4% C-50)
   each 1,000 ml contains
   trisodium citrate dihydrate 40 gm
   citric acid monohydrate 0.055 gm

2. Citrate Phosphate Dextrose (C.P.D. - 50)
   This formulation was calculated to give the same final citrate molar concentration when used at a ratio of 15:1 as that obtained using the Committee of Experts recommended CPD formula at a ratio of 7:1 in standard CPD blood donation packs.

   each 1,000 ml contains

Trisodium citrate dihydrate	39.5 gm
Citric acid monohydrate	4.9 gm
Sodium dihydrogen phosphate	3.76 gm
Dextrose Monohydrate	50 gm

   Kindly supplied by J.G. Watt, Director, Protein Fractionation Centre, Edinburgh.

CPD - 50 was used in an attempt to improve Factor VIII yields from this source of plasma as several reports have indicated the superiority of CPD plasma over ACD plasma.

The anticoagulant is automatically delivered at a fixed ratio of 15:1 during the bleed cycle of the donation.

PLACE AND STAFF

This pilot study was carried out in the Cell Separator Unit at the Leeds Regional Transfusion Centre. The unit is sited in a Hospital Ward in the same grounds as the Transfusion Centre. 4 Haemonetics model 50 machines were used. 2 machines were placed adjacent with a bed on either side, so one member of staff could supervise 2 donors simultaneously.* The minimum number of staff required was 3 donor attendants, one State Registered Nurse, a part-time clerk and a Medical Officer, i.e. 1 donor attendant per two machines, 1 haemoglobin/tea nurse, and 1 supervisory State Registered Nurse.

DONOR SAFETY

All donors under 9 stone (60 kgm) were rejected as the extra corporeal blood volume at the end of the 500 ml collection would exceed 15% of their total blood volume and increase the liklihood of hypovolaemic faints.

No donor was accepted unless their general practitioner had confirmed their fitness to donate.

To ensure adequate fluid replacement donors were encouraged to drink prior and during the procedure if considered necessary.

560 procedures have been analysed for the incidence of donor and/or machine problems.

Donors who had given twice in the preceding two months were asked to consent to the taking of blood samples pre and post plasmapheresis to investigate the effects of automated monthly plasmapheresis. The post samples were taken from a separate venepuncture site from the opposite arm to avoid any sampling error. (See TableI)

* See Fig. 2.

DONOR RECRUITMENT

Plasmapheresis donors were initially recruited from the existing voluntary blood donor panel. A recruitment letter was sent to selected donors at local sessions asking them if they were interested in becoming "A Special Plasma Donor". A brief explanation of the procedure followed, and a reply slip to be filled in if the donor was prepared to come to a discussion evening.

The donors selected were between the age of 18 - 50 years, had given at least two previous donations and were group A or O Rh Positive. Discussion evenings were arrangedonce fifty or more donors had indicated their willingness to attend. At the discussion evenings a short slide show was given to illustrate the need for more plasma and the workings of the plasmapheresis machine explained. A staff donor was then put on the machine and a question time followed. If still interested the donor took a form to indicate his willingness to donate, times of availability and the name and address of his General Practitioner. An existing panel of white cell donors were also asked to join the plasmapheresis panel, as call up for white cell donations is very sporadic and by joining a three monthly call up system the donor can be regularly checked for fitness, and their interest maintained.

An experimental recruitment drive was also carried out at the Leeds and Bradford permanent Blood Donor Centres to assess the ease of recruiting a large panel of regular plasmapheresis donors from the existing panel. An illustrated publicity layout was placed at the entrance to the session, explaining the why's and wherefore's of plasmapheresis.* Experienced donors were asked to attend and donate on the machine so that interested new donors could view the procedure. A clerk was available to collect the names and addresses of all suitable donors who wished to join the 'Special Plasma Panel'. Towards the end of the pilot study, when more donors were required to assess the maximum daily capacity of each machine and the staffing level required, experienced donors were directly recruited by a full explanatory letter. They were asked to temporarily join the plasmapheresis panel for a three month period to enable this pilot study to go ahead, at the end of which they would be returned to the normal panel.

During the three month trial period donors were bled monthly, to enable sufficient plasma to be collected for experimental fractionation and assessment of the Factor VIII yield from the two anticoagulants used. The eventual aim is to recruit sufficient donors to allow for a three month call up period. Prior to acceptance of any new donor a letter was sent to each donor's general practitioner to ascertain the fitness of the donor to participate in such a programme.

* See Fig. 1

TABLE I

Pre and Post Pheresis Donor Investigations.

Investigation	Number of Donors Investigated	
	4% C - 50	CPD-50
Full blood count	16	19
Platelet count	16	19
Coagulation screen (PT, PTT, TT)	10	12
Platelet Aggregation *	4	5
Anti-Thrombin III **	10	10
Fibrinogen ***	10	10
Serum calcium & phosphate	10	10
Total serum proteins Albumin & Fibrinogen	10	10
Post serum citrate levels ****	10	10

* PT = Prothrombin time PTT = Partial Thromboplastin time TT = Thrombin time Platelet aggregation was measured using a twin channel platelet aggregometer with ADP at 4, 2 and 1 ugm/ml, collagen and adrenalin at 3 ugm/ml.

** Anti-Thrombin III was assayed by inhibition of proteolytic activity of Thrombin upon a chromogenic substrate (Boehringer)

*** Fibrinogen was assayed using Radial Immunodiffusion plates (Behring)

**** Citrate was measured by a citrate lyase technique 4. All other measurements were carried out by standard routine laboratory techniques.

The initial and final protein levels of 22 donors who had given 3 consecutive monthly donations were subjected to a paired T test to determine whether a significant fall in their serum proteins had occurred.

Investigations for Donor Erythrocyte Damage

The erythrocytes from four donors were investigated by sampling directly from the machine via the donor red cell return line near completion of the final return cycle. Pre-pheresis samples were taken to act as a comparative control.

1. Erythrocyte morphology was carried out by phase contrast light microscopy after fixation in 1% glutaraldahyde.

2. Osmotic fragility was measured, based on the method of Parpat et al

3. Erythrocyte ATP levels were assayed by the enzyme system of Bücher

These studies were kindly undertaken by M White, Department of Biology, York University.

4. Scanning electron microscopy studies after fixation in 0.5% glutaraldehyde using a Joel T20 scanning electron microscope, Kindly undertaken by the Department of Forensic Medicine, Leeds University.

Plasma Collection

500 ml donations were collected from each donor. Each donation was snap frozen in a bath of CO_2 ice and ethanol within three hours of collection. Each Litre plasma pack containing 0.5 Litres of plasma was held flat during the freezing procedure, by clamping the pack between aluminium plates to allow for a rapid even rate of cooling. The frozen packs were then stored at - 40°C and kept in this state during transit prior to fractionation. Well mixed samples were taken for analysis prior to freezing and the results compared with analyses of other sources of plasma, see Table II.

Table II

Comparison of Source Plasma

Investigations	Source of Plasma and Number of Samples Tested					
	4% C-50	CPD-50	ACD 5 L Pool	CPD 5 L Pool	ACD* Manual	
Platelet count	42	26	50	NT	10	
β Thromboglobulin **	10	10	10	10	NT	
Cytospin	10	10	10	NT	10	
Total Proteins Albumin & Globulin	43	35	11	10	10	
Calcium	43	35	11	NT	10	
Phosphate	NT	35	NT	10	NT	
Citrate	35	35	11	10	10	
P H	43	35	11	10	10	
Factor VIII ***	16		12	NT		

N.T. Not Tested

* Plasma collected by conventional manual plasma pheresis techniques into acid citrate dextrose anticoagulant for the preparation of anti-D immunoglobulin.

** β Thromboglobulin was assayed using the Amersham Radio Chemical Kit

*** Factor VIII was measured using the standard two stage assay system

RESULTS

The Haemoneticsmodel 50 machine proved a reliable easy machine to use, simple to set up and rapidly dismantled. Sufficient safety devices are incorporated within the system to protect the donor from any technical mishap, with a manual override system to stop the procedure at any time the donor or operator wishes.

Once a sufficient level of experience is reached by both donors and staff, a time of 45 minutes per donor can be achieved. This allows for an average of 30 mins donor time on the machine with a mean of $3\frac{1}{2}$ cycles per donor and a bleed and return rate of 80 ml per minute for a 500 ml plasma collection. The remaining 15 minutes allows for donor reception, the taking of a finger prick blood sample for the haemoglobin and haematocrit estimation and some fluid imbibition by the donor, pre-donation.

As a result of these findings each donor is asked to allow an hour for the donation to ensure a rest and refreshment period afterwards.

From the staff point of view it takes five minutes to harness the machine, five minutes for donor preparation and venepuncture and five minutes to strip down the machine.

For problems encountered see Table III.

TABEL III

Problems Encountered In 560 Procedures

Problem	Number	%
Dizziness	15	2.7
Faints	3	0.5
Faulty Harnesses *	4	0.7
Machine Faults **	8	1.4
Red Cell Spill Over	16	2.8
TOTAL	30	5.3
No. Procedures abandoned due to problems	13	2.3

* 2 minor faults were detected during the harnessing procedure but 2 harnesses developed leaking bowls during plasmapheresis.

** The majority of these faults were due to derangement of the pump ratios leading to inadequate delivery of anticoagulant and subsequent clotting of the plasma collection.

1 simple but serious fault of a new machine was the malalignment of a pump tubing support leading to haemolysis of the donor blood. This was immediately recognisable in the plasma collection and the procedure was terminated before the return cycle started.

The commonest problem was the occurrence of red cell and platelet spill over into the collection plasma despite correct setting of the donor haematocrit into the machine. This was probably due to the donor haematocrit rising during plasmapheresis, resulting in the plasma having to be respun prior to freezing which was an undesirable extra step in the procedure.

Donor Recruitment

No difficulty was encountered in recruiting sufficient donors for this pilot study. There was a 57% response rate from donors invited to attend a discussion evening, the attendance rate at these meetings was 83%, and 93% of these donors agreed to donate by plasmapheresis. All existing white cell donors readily agreed to join the plasmapheresis panel provided the distance involved was not inhibitory. As a fortunate by product many newly recruited plasmapheresis donors also wished to join the white cell donor panel. Only three experimental recruitment drives at permanent donor centres were necessary to show that a large panel of plasmapheresis donors could be rapidly recruited by this method. Direct letter recruitment to local donors yielded a 28% response rate and only 8% of these donors requested a discussion evening prior to their first donation. It therefore proved easy to recruit donors by all methods used, the level of interest aroused and the willingness to participate in such a programme proved overwhelming. 95% of these specially recruited donors gave more than one donation during the three months trial period.

The sessional attendance rate was 72% and the next appointment was made at the session, thereby avoiding the expense of postal call ups.

75% of missed appointments were remade by the donor telephoning the Centre, again demonstrating the high level of interest generated by this study.

The only difficulty encountered was returning the temporary recruits to the normal donor panel as many wished to continue as plasmapheresis donors.

As a result of this pilot study a preliminary cost analysis has been done for siting a plasmapheresis unit at one of our permanent city centre blood donation units.

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