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SCOTTISH NATIONAL BLOOD TRANSFUSION SERVICE

Minutes of Meeting of Factor VIII Study Group
held in Headquarters Unit on 21st November 1985

Present: Dr J D Cash (in the chair)
Dr F E Boulton
Dr B Cuthbertson
Dr J Dawes
Dr G S Gabra
Dr D S Pepper
Dr R J Perry
Dr C V Prowse
Mrs E Porterfield (Minutes)

1. INTRODUCTION

An apology had been received from Dr Foster.

2. MINUTES OF PREVIOUS MEETING (7th February 1985)

The suggested amendments were all agreed, with the exception of Mr Farrugia's title; at the time of the previous meeting he had not received his doctorate.

With these amendments the minutes were agreed to be a true record.

3. MATTERS ARISING

(a) Fresh Frozen Plasma Specification

A. (i) The Group noted with pleasure the issue of the Specification for FFP from PFC to Regions as a direct result of their work over the last few years.

(ii) Discussion turned to methods employed in the Regions, if any, for validation of specification values in plasma sent to PFC. Validation was seen as a very important aspect of plasma quality in the face of the requirement for increased plasma input to PFC to compensate for the decreased FVIII yield incurred due to the heat treatment process.

Dr Perry tabled a short paper entitled, "FFP Quality 1985 (Jan-Sept)" which illustrated the FVIII:C and Platelet content of plasma processed during that period compared to the 1984 figures. Material samples had been assayed in both PFC and Edinburgh BTS.

It was agreed that Dr Gabra and Dr Boulton should collect Regional data on validation, where available, and circulate to the Group when complete. It was recognised that Regional

differences might be reflected in the figures obtained. It was also suggested that the BTG assay might provide a better estimate of platelet contamination of thawed plasma than the current "particle count".

It was agreed that the figures obtained in the South East on line/pack samples, which had been the subject of a Vox Sanguinis report, should also be included in the above report.

The possibility of a centralised assay system was discussed briefly, it being remitted to Dr Prowse to liaise with Dr Cuthbertson and Mr McQuillan and report to the next meeting.

B. Development of Source Plasma Specification

It was not envisaged that this specification would differ substantially in structure or layout from the FFP version. A draft specification would be submitted to the Group for comment in due course.

(b) Fractionation Update

(i) Dr Perry briefly summarised the current position. PFC were producing intermediate FVIII concentrate having modified the process with the addition of 2% sucrose, and heating at 68°C/24 hours. As anticipated yield was reduced on heating, the average loss being around 20%. It was noted that the heat treatment regime employed at BPL (80°C/72 hours) had led to losses of only 5-10% in yield; however, when the new (Johnson) process for high purity product was available at PFC, it was hoped yield would be improved.

It was not intended to alter the fractionation process at the present time.

(ii) Calcium ion studies

Studies had continued in the area of the citrate content of donor samples obtained by machine plasmapheresis and standard donations. The results had been submitted to Vox Sanguinis for publication; however, it had been recommended that further cell studies should be conducted prior to publication and this work was now in progress. It was noted that Biotest would make available the necessary number of bags to conduct some of these tests. PFC would carry out comparison studies on these packs for Factor VIII content by core sampling.

It was felt that this project should be treated as a high priority for study because of its potential significance to plasma quality.

It was agreed that as a matter of policy this required careful discussion and planning as it was necessary to carry out autologous infusion studies, probably on a multicentre basis. It was remitted to Dr Prowse to contact Dr David Aronson to ascertain current FDA requirements in this area.

Once the above studies were complete the Group would investigate the establishment of a project team to monitor future progress.

There was a discussion on cryoprecipitation as a method for processing plasma as it was known to incur losses of 30% from starting plasma. It was agreed that it was not opportune at the moment to investigate this. However, once the Johnson project was established to produce high purity product without existing yield losses, this area could be reconsidered.

(iii) NE Plasmapheresis Study

Dr Urbaniak's report on this project had been circulated. Dr Prowse summarised the results obtained during this study which, it was agreed, had been most interesting.

The Group noted (Table 10) the differences between the two systems on the values of FVIII:C obtained. The lower figures recorded for the filtration method of collection could be the result of membrane activation rather than size fractionation during filtration.

Dr Perry summarised for the Group the processing plan for the plasma obtained during this experiment. Four pools would be evaluated as far as the cryoprecipitation stage for FVIII content viz: (a) Haemonetics (V50 + PCS) plasma, (b) Dideco plasma, (c) Recovered plasma and (d) Platelet rich plasma, the latter two as controls. All 4 pools (approx 240 litres each) would be processed to Factor IX, each batch being split into two (heated/unheated) and infused into dogs for assessment.

(iv) White cell/heparan release

Dr Dawes summarised the work to date on the release of heparin in stored red cells and plasma. Ionised calcium levels did not change in stored red cells studied however, when half strength citrate plasma was compared with ordinary plasma it was evident that heparan sulphate was released soon after donation. Progressive release was also noted during storage of standard-citrate red cell concentrates. Work was continuing in this area, in conjunction with Dr Prowse and it was hoped further results would be available in Spring 1986.

(v) Cardiff Studies on Lots NY771 and NY772

These products represent a split batch processed with and without calcium addition. Dr Perry reported that in vivo recovery and half life studies were underway, but no results were yet available from Professor Bloom/Dr Greedharry.

(vi) Products for von Willebrand Disease Patients

Dr Boulton reported that the number of VWD patients requiring treatment was small; most requiring no treatment. The

current product had been shown to transiently shorten the bleeding time in one patient. It was recognised that new high purity heat treated FVIII product might not be so effective for VWD patients and in view of the larger infusion volume required when using cryoprecipitate, and possible problems with infectivity it was likely there might be a small demand for a specific von Willebrand factor produce. Dr O'Brien had tested current products (with and without heating) in his in vitro bleeding time assay and found no differences, but neither was as good as his cryoprecipitate.

(c) Virocidal Update

(i) Overview

Dr Pepper reported that while heat treatment regime used by commercial companies had proved adequate to inactivate HTLV-III, NANB was still causing problems and it was agreed he should conduct studies on product supplied by Dr Smith, BPL, using heat, irradiation (+ 1 control lot).

Dr Pepper summarised findings on acidification which had proved remarkably virocidal and which he recommended as a valid option for products other than FVIII, which was unstable at pH levels below 5.6.

Acidified FIX concentrate heated at 80°C/72 hours was currently being assessed in PFC for water content/viral inactivation.

(ii) Specific Update

Dr Cuthbertson tabled copies of the poster presented at the Oxford BBTS meeting entitled "Viral inactivation in dry heated clotting factor concentrates".

Of particular interest was Experiment 2 when 2 logs of Vaccinia virus had remained after heating, whereas repeat experiments showed a greater inactivation. Dr Pepper had evaluated samples of the same material using irradiation, when all virus was inactivated. There was no explanation for this phenomenon.

Studies were ongoing and an update would be provided at the next meeting of the Group.

(iii) Validation of heat treatment/HTLV-III

Dr Perry explained the difficulties which had been experienced at PFC with regard to health and safety aspects of conducting HTLV-III inactivation studies in conjunction with Dr Robin Weiss. These difficulties had now been overcome and it was agreed that Dr Perry should contact Dr Weiss once more in an effort to re-establish collaboration in this area. Once it was clear when PFC could commence work Dr Perry and

Dr Cuthbertson would liaise on this aspect.

Dr Cuthbertson briefly summarised the efforts which had been made to establish collaboration with the Pasteur Institut.

(d) Factor VIII Assay Group

(i) Dr Prowse had circulated a summary of all QA results to date as well as those of the most recent exercise. The next exercise would take place post Christmas, after which the Group would meet to assess results.

Dr Prowse felt that it was probable that the chromogenic assay kit would be the best option for general RTC use; however this was costly.
It was agreed that Dr Gabra/Dr Boulton would ascertain Regional attitudes on assays (5/week) and assess whether centralization would be worthwhile. (This would be combined with the studies at item 3(a)A(ii) ).

(ii) Publication: Vox Sanguinis

Dr Gabra had prepared a first draft to which the 1985 figures would be added prior to review with Dr Prowse. Once this was done the draft would be circulated to the Group, probably in January or February 1986.

(iii) FVIII Deficient Plasma Substrate

Dr Prowse summarised the problems which has been encountered with artificial substrates produced at PFC. It was agreed that despite problems there was a market for this product and that the production of artificial substrate should be pursued but, in view of Circular 1984 (GEN)1 "Manufacture of Products in the NHS, Scotland" this could not be done in PFC; however, given that the technology/process could be developed within PFC, the question of licensing could be assessed. Dr Perry would discuss this topic with Dr Cuthbertson and Mr McQuillan in order to assess PFC priorities in this area.

Dr Prowse tabled results of immunodeficient adsorption studies using VIII deficient plasma and explained these to the Group. It was agreed these were probably not good enough to warrant clinical evaluation. It was envisaged that by Spring 1986 it would be clear whether or not to pursue the artificial substrate project or the immunodepleted plasma project.

(e) Clinical trials of heat treated factor VIII

(i) Dr Boulton said 3 patients had received heat treated FVIII during the year. Good recovery and half life studies had been reported. In response to a query from Dr Cuthbertson regarding follow-up samples from patients who received heat treated FVIII Dr Boulton said it was probable Dr Ludlam had library samples on which tests could be carried out to

ascertain whether or not seroconversion had occurred.

(ii) There would be a need for discussion and agreement on a protocol for study on the new high purity product when available. As PFC were now looking at the area of downstream processing of the new product it was estimated these discussions would be necessary in mid-January. Dr Perry, Dr Boulton, Dr Cash and Dr Ludlam would liaise on this aspect.

(f) Detection of heat induced damage to protein molecules

Dr Dawes spoke to the notes of the meeting of 9th May, 1985 which had been circulated and gave a brief outline of developments since that time. Dr Dawes and Dr Pepper had carried out experiments using FPLC on Tris extract of cryoprecipitate, both heated and unheated. It seemed from these experiments that the presence of fibrinogen and fibronectin was a significant factor in the formation of complexes. Similar studies had been conducted on real product with similar results/conclusions. PFC had provided paired samples for study, unfortunately from the same batch. Dr Cuthbertson would arrange to send Dr Dawes paired samples from different batches for further appraisal.

Dr Cash agreed to contact Dr Ludlam regarding the possibility of setting up studies of the following immunological aspects of haemophilia patients:

(i) Total IgG
(ii) Rheumatoid factor
(iii) Factor VIII inhibitors
(iv) Immune complexes
(v) Chronic changes in complement levels
(vi) Antibodies to FVIII product, assayed by ELISA

4. PHOSPHOLIPIDS AND FACTOR VIII:C

A draft discussion paper prepared by Dr Pepper had been circulated. Dr Trevor Barrowcliffe had indicated willingness to carry out assays and samples had been dispatched to him but no results were available yet. Dr Pepper would contact Dr Barrowcliffe to ascertain the current status.

Dr Barrowcliffe would also be asked to study existing monoclonal antibodies to ascertain lipid binding activity, if any. Studies would be conducted under the 4 category headings itemised in Table I of the discussion paper. It was thought possible that lipid FVIII competes with inhibitor binding. Therefore at the same time inhibitor assays could be conducted on cryoprecipitate from platelet rich and platelet poor plasma (using e.g. product from NE plasmapheresis project) to assess the effect of lipids on inhibitor activity. Dr Boulton would also liaise with Professor Bloom on this aspect. Dr Pepper would assay existing Factor VIII product supplied by Dr Cuthbertson for evidence of lipid bound VIII.

5. FACTOR IX

(a) Trials had been carried out in 3 Christmas Disease patients using ordinary DEFIX and heat treated DEFIX. Recovery had been the same in each case. Data on fpA was not yet complete but would be written up when available.

The question of studies in liver patients was discussed. Dr Gillon had had a preliminary discussion with Dr Nial Finalyson. It was agreed this should be pursued and it was remitted to Dr Boulton and Dr Gillon to meet Dr Finalyson to discuss the best way forward. Dr Finalyson should be reassured regarding the thrombogenic/infective status of heat treated Fctor IX.

(b) Fractionation matters

(i) In the absence of Dr Perry, who had to attend another meeting, Dr Cuthbertson outlined the current problems in manufacturing PPSB/FVII.

(ii) Substantial losses were incurred during the production of Factor IX which were possibly related to a freeze drying problem. There were sufficient stocks available to meet existing demand but problems could be encountered in the face of any escalation in demand.

(iii) Dr Cuthbertson agreed to send Dr Prowse the results of FIX losses for the 2 batches of DEFIX currently being dried if these showed loss was related to water content. This would allow comment on such losses in the paper currently being written on the initial dog studies.

(c) Thrombogenicity

The Group noted that the dog studies had been transferred from Cambridge to Wellcome Foundation Laboratories at Glasgow Veterinary School.

Dr Dawes was looking at the use of small animal models for the assay of FpA.

ANY OTHER BUSINESS

It was unanimously agreed that it would now be appropriate to rename the Group in view of its increased range of activities. Henceforth it would be called the "Coagulation Factor Study Group".

DATE OF NEXT MEETING

27th February, 1986 at 10.30 am in the Headquarters Unit.