FVIII - HAEM DIRECTORS,
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REPORT ON THE 38TH ANNUAL MEETING

of the

INTERNATIONAL SOCIETY ON THROMBOSIS AND HAEMOSTASIS SCIENTIFIC AND STANDARDISATION COMMITTEES

MUNICH JULY 1992

C PROWSE

Contents

1. Summary and Proposals

Factor VIII

- Meta-analysis shows 50% success in obtaining tolerance in inhibitor patients with daily doses of 100 u/kg for 3 to 12 months.

- Although updated no really new data on inhibitor induction by various concentrates was presented. Inhibitors arising from treatment with the two recombinant products vary in cross-reactivity with porcine VIII.

- Licensing of recombinant VIII in the USA is expected before the autumn and has been submitted in the UK. Canada are delaying for now and continue to use intermediate product. **SNBTS needs to consider the implied impact further.**

- Standards: the 3rd plasma IRP calibration exercise has been done and the proposed 4th concentrate Int Standard will be recalibrated. The likely outcome is a 10% devaluation of the concentrate unit and a 10% enhancement of the plasma unit (hence bringing the two units together). **Need to discuss the potential impact on 'yield' of this with PFC.** Availability of concentrate and plasma samples from pharmacokinetic studies with Dr Barrowcliffe who suggested SNBTS (rather than NIBS) do chromogenic assays (in hand). **Dr Mertens may be worth contacting.** Also possibility of undertaking recovery definition post-infusion with isotopically labelled factor VIII (recombinant). Moves towards chromogenic assay as reference method continue and impact of this being used for (only) calibration of standards need consideration. Dr Barrowcliffe was asked to seek definitions of intermediate and high purity.

- The Dutch described their experience with late onset high titre inhibitors with pasteurised CPG factor VIII and the subsequent withdrawal of this product.

- Dr Morfini and others are progressing in moves towards assessing B19 parvovirus (and ? HAV) infectivity during PUP studies.

- Baxter are involved in developing gene therapy for haemophilia but are also developing plastic implants containing current producer cell lines (insulin, VIII, IX) as a more practible interim solution involving subcutaneous implantation.

- A lot of important studies (prophylatic dosage, recombinant licensing, inhibitor surveillance, etc) appear to be due for presentation at WFH, Athens.

- Both BPL (80° 72h) and Lille (100° 30 min) are assessing terminal heat treatment.

vWf

- Mannucci questions efficacy of high purity vWf concentrates (Lille and Behring) in type III vWd. Nilsson and Savidge disagree.

- Some moves towards use of vWf-deficient plasma as prediluent for assay of such products.

FIX

- CVP should contact Dr Barrowcliffe to assist him in the task of comparing available animal thrombogenicity models, for which Dr Kasper volunteered him.

- Batch testing of HP-IX in animal models is unlikely to be recommended.

Protein C and S

- Dr Walker's studies suggest incidence of 1 in 1,000 C and 1 in 400 S deficiency in Glasgow donors.

Fibrinogen

- Immuno produce steam treated fibrinogen which is used in Austria, Germany and Italy for treatment of acquired deficiency (vial = 1g=50ml). Behring have a pasteurised product.

Other

- BPL now using Helvoet bungs which retain less water.
- Mr Walker has moved T Snape from previous post in BPL.

2. Factor VIII

2.1 Inhibitor Tolerance

Dr Mariani summarised the current state of this registry commenced in Autumn 1989:

(1) 215 patients from 35 centres entered of which 194 evaluable.
(2) 47% (n=91) cured, 5% (10) still have <1BT, 5% (10) 1 to 10 BT and 17% failures.
(3) Success associated with younger age (<20) and higher dose (probably > 100 u/kg/d), though some centres report success with lower doses. HIV and responder status have no significant effect.
(4) Questions that may be answered by further analysis:

- Is a dose of 100 u/kg/d optimal?
- How long does tolerance last and is it associated with general immunosuppression (eg Gomperts suggests assess vaccine response)?
- Do higher purity concentrate work (most to date on intermediate)?
- If failed, does a second higher dose treatment work?
- How long is needed for induction of tolerance (? ~ 12 months)?
- Is continued prophylaxis necessary to maintain tolerance?
- Is addition of cyclophosphamide or IVIgG beneficial?

Comments from audience:

(1) Stuttgart cured 3 of 10 patients on 10-20 u/kg/d and 2 of the 7 initial failures by a second treatment at 300 u/kg/d.

(2) Frankfurt have cured 17/20 in 3 to 4 months of 100 to 200 u/kg/d and see no recurrence in patients continuing on 'on demand' home therapy for <10 years.

(3) Above register does not include Malmo patients (7/11 cured) treated with Nilsson's protocol, who remains tolerant < 7 years on prophylaxis [Malmo have also now tolerised 6 of 7 IX-deficient inhibitor patients using HP-IX, not PCC].

2.2 Porcine VIII

Dr Hay presented his register of pVIII surveillance against published studies reporting reactions after 4 to 7% of infusions (6 to 32% of patients).

The prospective register now includes 1,675 infusions (~ 13 Miu pVIII) given for 357 bleeds to 132 inhibitor patients.

(i) 261/375 bleeds were evaluated clinically:

10% excellent response
65% good
18% poor
10% none or worse

(ii) 55/1675 infusions (in 39/132 patients) gave reactions varying from 49 mild (urticaria, etc) to 11 moderate to 1 severe (anaphylactoid after a dose of 640 u/kg: no reaction on retreat at lower dose) mainly at doses > 100 u/kg.

(iii) 147/162 infusions were associated with a decrease in platelet count (mean from 247. to 207) not correlated with dose. While the average decrement (43 x 10^9/L) has not changed between 1980/1985 and 1986/1991, 18 infusions gave a post infusion count of < 150 and 3 of < 50. There were usually associated with repeated high dosing.

(iv) Only 23% of basal (n=97) inhibitors did not react with pVIII and some show complete reactivity with human VIII. 45% of patients gave increased inhibitor to pVIII after treatment. pVIII cross-reactivity inhibitors rose from 25 to 47% after a single treatment and 53% after multiple transfusion.

[Note comment below that PUPs on one recombinant VIII with inhibitors show cross-reactivity with pVIII, but inhibitors to the other recombinanat product do not inhibit pVIII]

2.3 Standards and Assays (Dr Barrowcliffe)

(i) Calibration of New International Plasma Standard

Results 'hot from the press' on calibration of proposed 3rd IRP (91/666) = P2 against current 2nd IRP (87/718) = P1 and fresh local plasma pools (n>15 donors: still gives 2 fold range) = N, by 15 invited Centres (4 UK, 3 USA) each doing 6 sets of assays. Report has yet to go to participants and stability studies of 91/666 are in hand.

Assay	Method	Labs	P2vsP1	P2vsN**	Ratio*	Proposed Potency
VIII:C	one-stage	12	0.82	0.68	1.21***	
	two-stage	4	0.74	0.87	0.85	
	chromogenic	3	0.92	0.85	1.05	
	all	19	0.80	0.73	1.10***	0.76
	CV (%)		7	18		
VIII:Ag	ELISA	3	0.89	0.90	0.99	
	Laurell (sic)	1	0.94	0.92	1.02	
	all	4	0.90	0.91	1.00	0.90
vWf:Ag	ELISA	8	?	?	?	
	Laurell	6	?	?	1.12	
	all	14	0.96	0.90	1.05***	0.93
vWf:RiCof	Aggregation	6	0.92	0.85	1.08	
	Macroscopic (time)	3	0.90	?	?	
	Macroscopic (titre)	3	0.96	?	?	
	all	12	0.93	0.82	1.13***	0.87

Notes:

* ratio should be 1.0 if iu (P1) corresponds to 1ml normal pool. It does not, suggesting (for instance) activities in P1 may have decayed. Hence Trevor's averaged (for P1 and N calibrators) values for proposed potencies. This would also close the gap between plasma and concentrate units.

** N was assayed fresh for C and RiCof, frozen for antigens.

*** Significantly different from 1.0.

(ii) Proposals for New Concentrate Standards

This was basically a repeat and confirmation of the outcome of the May NIBS meeting:

Both Mega and British working standards need replacement, the latter possibly as an EP standard. For this both an intermediate and an high purity concentrate are likely to be filled and calibrated.

The 4th International Standard also needs replacement. This will probably be the high purity product (88/640), calibrated at the same time and showing 1% decay per month at 37° at that time. However it will be recalibrated, partly due to concerns over experience with the chromogenic assay at the time of the original calibration.

At Dr Kasper's request, Trevor agreed to collect and present views on a working definition of intermediate, high and very high purity and also consider the nomenclature of products (eg ± vWf).

Discussion with Trevor (K Mertens was not present, but may be of value) about our in vivo recovery data/samples confirms we should re-assay by chromogenic assay, but Trevor suggests we do this.

The above moves seem to be moving towards a ~10% devaluation of the concentrate unit and ~10% increase in the plasma unit.

(iii) Reference Assay Method

The chromogenic assay has been put forward to the EP (as a replacement for the two stage assay) who have accepted the recommendation subject to an appropriate calibration exercise which Trevor will organise. No manufacturers present raised any reasons to delay such a move by the subcommittee (Cutter asked for time in Amsterdam) so it is also likely to be adopted by

the committee, although in the absence of K Mertens we had no update on any detail of any specific method (see May NIBS meeting Notes).

2.4 Inhibitor Incidence/Prevalence

(i) Dr Y Sultan has yet to finalise her assessment of low titre inhibitors and will now present this in 1993.

(ii) Dr L Aledort presented current data on inhibitor prevalence (unclear if any PUPs) and discussed a standardised protocol for this (which included viral markers such as HCV, but not ALT. Unclear why and Dr Kasper agreed to take this on). He did not present on prophylactic dosing effects but may do so in Athens (WFH).

Concentrate:	Monoclonal	Monoclonal	Recombinant	Intermediate	Int	Int
Study Design	q 3m	q 6m	q 3m	q 20 exposures	Retro	Retro
Inhibitor Frequency	2/33	7/38	13/48	16/63	0/19	1/16
%	6%	18%	27%	25%	0%	6%

The design discussed involved PUP's sampled pre and every 3 months post exposure with half life studies (0,1,3 ± 6h) pre and after any inhibitor formation (concern was expressed over latter - particularly given requirement for > 3d infusion free period prior to any such study). Local and central assays. HBV immunisation. HIV and HCV serology. Stratification of severe and mild patients. Retrospective entry of trials allowable if they met criteria (separate analysis). Agreed that this design was an ideal, required redrafting and would then be circulated to those present (addresses taken by Dr Kasper). The following points were made:

(i) Mannucci agreed with the ideal but had great concerns over possible lack of compliance and requirement to use washed red cells for any transfusion requirement.

(ii) Aledort pointed out that even low inhibitors are costly as they increase usage and VIII clearance.

(iii) Canada plan to continue using intermediate product (? Cutter or Baxter) for some time.

(iv) Antibodies from patients with inhibitors to one recombinant product cross-react with pig and human VIII, while the other yields antibodies preferentailly reacting with human.

(v) Dr's Kreuz and Brackmann are organising a prospective study in Germany/Switzerland/Austria with a design similar to Aledort's (but no half life studies).

(vi) Dr Sultan stated all French patients (including 37 PUPs) have been treated with VHP-VIII since 1988 and a prospective inhibitor study is being set up.

(vii) Dr Lee made a plea that the parameter to be assessed be the "probability of remaining inhibitor free after X (? 10 or 20) exposure days", since the available studies suggest the most important parameters determining inhibitor formation are exposure days (usually <7) and number of infusions. Not all patients are equal and the use of crude numerators and denominators is misleading.

(viii) A Dutch lady (missed her name) summarised the inhibitor incident with Dutch pasteurised product (CPG):

- From 1st January 1984 to 1st January 1990 13 new inhibitors were seen in 139 Dutch haemophiliacs. Only 2 of these were > 5 BT and tolerance regimes have been instituted in both these young people.

- In June 1990 the Dutch substituted pasteurisation for dry heat for their CPG-VIII product.

- In April 1991 a 14 year old previously treated patient developed a high titre (max > 300 BTu) inhibitor on this product.

- An additional 9 Dutch patients developed inhibitors on this product and Dr Vermylen has reported 5 in Belgian patients on this product (total 15), all previously treated with other products and aged 4 to 50 years.

- In March 1992 CLBA stopped manufacture and recalled the product.

- The presumed 'neoantigen' reactivity of these antibodies, apparently associated with one form of pasteurisation (? and/or contact with silica) is under investigation. The speaker was not a fractionator and was uninformed on process details in the absence of any CBLA staff at the meeting (Dr F Feldman suggested that a unique feature of the Dutch process may be use of sucrose and lysine as stabilisers - Armour, for whom he works, and Behring use different stabilisers).

2.5 Virus Safety

(i) Both Dr Mannucci and Dr Kasper said continued ALT testing will need to continue in PUP studies (despite HCV assay and ALT inherent problems) due to concerns over eg chloroform resistant nonA nonB (nonC) hepatitis.

(ii) Dr Morfini described the experience of B19 seroconversion with some products and proposed a protocol for assessing same in PUPs (Appendix 1) arguing both that B19 is a good model for non-enveloped viruses (such as HAV, BK, JC, adeno, papova and polioma viruses) and that B19 was clinically important (his report of haemolytic anaemia in Am J Haematol 1992 and Dr Lyon's 3 patients with fifth disease - in Lancet), that viraemia was seen in 1 in 24,000 donors and antibody in 40 to 60%. This protocol was not discussed and I am uncertain of its status, and have problems with it eg how it will take account of endogenous B19 infection (I presume by temporal association with infusion).

Product	Virus Inactivation	n	B19 IgM pos	%
Kryobulin	STIM	15	4	26.6
Preconativ	Hydrophobic chrom	2	1	-
Koate HT	Dry Heat	1	1	-
NHS 8Y	Super Dry Heat	11	(? 2)	-
Beriate P	Pasteurisation	13	4	30.8
Emoclot	Solvent Detergent	7	5	71

(iii) Dr Azzi (@ Florence) surveyed serological assays of B19 (in past reliant on source of native virus) and PCR and went on to outline her own in-house and two recently issued commercial kits for B19 IgM detection. These use peptide (Parvoscan from Ferring) or recombinant (? VP1 or VP2) (Alifax) antigen and were compared by her with the current gold standard - Cohen's MACRIA (J Hyg Camb 1988). Certainly the peptide and, probably the protein ELISA, appeared from her data to be noisier and less sensitive,particularly the Ferring ket.

		MACRIA pos	MACRIA neg
ELISA	pos	18	5
	neg	6	38

She is less happy with these assays for IgG antibody.

2.6 Miscellaneous

(i) *Dr's Gitschier and Peake*

Were not present and will present their proposals on naming of VIII and IX gene mutations in New York next year.

(ii) *Lowell Winkelman*

Despite quote in 'Recommendations' says 8Y can be got for half the price of Haemate P.

VIII-SM chromatography step takes ~ 50 hours. One of her main tasks at present is on how to undertake reprocessing of failed batches (this never worked for 8Y).

BPL now using bromobutyl bungs from Helvoet as they retain much less water. Also vacuum testing as eg leaks are fatal for 8Y in terms of water gain.

Can heat treat VIII-SM with minimal (< 5%) activity loss at 80° for 72 h.

Medicines Inspector is giving BPL major headaches on their virus containment facility. In addition the R and D pilot plant was not designed with in process viral inactivation in mind.

The VIII-SM process involves a hold step at the cryo stage. To date freezing has been by dropping into LN_2 which gives nice granules that thaw easily without conditioning and give 100% yield (recycling will probably involve addition of out-of-specification VIII-SM to this thaw). For Health and Safety reasons LN2 is now denied and they are moving to chest freezers (electrical).

Fractionation by BTS region (a problem for the proposed recycling approach) has revealed large differences in yield by regions and suggests freezing at -40°C in cabinets (? Oxford) may give better yields than blast freezing.

Their HP-IX has a CTX and is about to go to clinical trial.

(iii) *Duncan Thomas*

States Terry Snape has been 'moved sideways' by Mr Walker and is no longer in charge of production.

(iv) *Henry Kingdon*

Baxter moving in direction of gene therapy and (rapidly) cell implants for haemophilia - see section 7.

To his knowledge no-one has done a radiolabelled recombinant VIII kinetic study in man (although probably in animals) which I suggested would be relevant (in terms of recovery) to the assay problem.

Baxter have (had) problem with non-producer clone in their monoclonal antibody hybridoma master cell bank (they operate 18 continuous perfusion bioreactors < 6 months - or ? weeks - now their own - they have taken over the original contractor) which would have been easy to clone out and resulted in a 10-fold reduction in staffing costs, but for the regulatory hurdles in relicensing.

(v) *D Aronson*

Says Cutter feel it would be feasible to generate VIII deficient transgenic mice "fairly easily".

(vi) *Dr C Hay (Liverpool)*

Is pursuing with C Ludlam a formal comparison of the effects of Monoclate/VIII-SM and SNBTS HP-VIII on CD4 count in HIV seropositive haemophiliacs.

3. **Factor IX**

(i) Dr Barrowcliffe summarised the discussions at the May NIBS meeting emphasising that:

- pre-clinical assessment of HP-IX products (eg for Frag 1.2) is useful
- IXa content of HP-IX's (PCC unassayable) correlates reasonably with Wessler score
- most manufacturers undertake at least preliminary animal testing, but consensus was that batch testing was not appropriate (in Munich discussion Dr Feldman emphasised that Armour would continue to batch test as outlined at NIBS in May and he will publish this soon; and Dr Kohler described the advantages of his rabbit blood pressure model - which is used to test all batches at ? Biotest)

Based on this Trevor proposed:

- that a IXa standard may need consideration at the next meeting
- a comparison of the available animal models be reported (I have written offering my assistance on this)
- further collaborative studies on existing in vitro tests (NAPTT, FCT) had not elicited any great support from this committee
- that standardisation of Fr 1.2 assays may be appropriate but was already under consideration by the epidemiology/risk factor subcommittee (E Preston emphasised that low heparin anticoagulant for Fr 1.2 should be avoided. Citrate with separation in <90 min is fine)

(ii) Dr Lusher (not present) will start collecting a numerator/denominator registry of use of HP-IX in surgery/trauma and any associated thrombotic episodes according to Dr Kasper, who will circulate a proforma with the minutes.

(iii) Dr Peakes report on gene register will be updated at New York next year.

(iv) Dr Brackmann reported the Bonn experience of thrombotic episodes in haemophilia A and B surgical operations over the last 22 years with admirable brevity.

	A	B
Operation	558	70 (PCC and recently HP-IX)
Thrombosis	0	6 (all PCC)

(v) Kabi HP-IX is now licensed in Sweden. They plant to stop PCC manufacutre.

4. <u>von Willebrand Factor</u>

(i) Dr Exner reported results from 10 laboratories (only UK one was Dr Lawrie) undertaking non-isotopic multimer analysis. Participants were circulated with a panel of dried plasma samples (Firkin vWd type B, type IIB, type IID, normal plasma and normal plasma + 20% cryoprecipitate). These were freeze dried but participants also assayed fresh local plasma. Results were assessed by an expert panel of 5 and also compared to low and high resolution isotopic gels done by Mannucci's laboratory. While it had been hoped that high scoring methods would have common features this was not so. Results ranged from pretty poor to better than the isotopic method, the top four methods being:

(a) Raines (Melbourne - see Thrombosis Research) - vertical, blot on nitrocellulose, alk phosphatase, NTB/BCIP.
(b) Pflugshaupt (Swiss - unpublished) - horizontal, blot on PVDF, peroxidase/avidin/biotin, DAB/Co.
(c) Lawrie (as set up by I MacGregor).
(d) Japanese method (Dr Budde's luminol method also scored well on resolution).

Dr Mannucci emphasised primary screening should be by low resolution gels to allow discrimination of higher multimer content.

(ii) Dr Fricke reported the results of a 13 laboratory study to assess the validity of the WHO plasma standard for calibration of "vWf" concentrates 6 samples of revialled (1ml) concentrates (Alpha VIII, Behring, Haemate P, Lille vWf, BPL 8Y, Miles/Cutter and ?) were assayed by the laboratories of Alpha, Behring, BPL, Lille, CSL, FDA, Hoyer, Miles, Roberts, Ruggeri, Gralnick and Mannucci against the WHO standard (87/718: 0.19 u/ml vWf, 0.84 u/ml RiCof) with the following results:

Assay	n	Method	n	A	B	C	D	E	F	G
vWf:Ag	462	ELISA	5	7.7	32.1	17.4	12.5	15.2	11.3	-
		Laurell	6	6.4	32.5	17.4	12.3	14	10.1	-
		IRMA	1	8.4	44.8	18.7	13.9	17	12.5	-
		Mean	12	7.9	37	18	13	15.9	11.7	0.91
vWf:RiCof	240	Aggr	5	5.3	27.7	21.1	11.7	15.3	6.3 }	0.84
		Macro	2	5.1	26.5	16	9.8	11.9	6.5 }	
Ratio vWf Ag/RiCof			-	1.5	1.4	0.9	1.2	1.1	1.9	1.1
Multimers			-	Int	Int	I/High	I/High	I/High	Low	High

Conclude plasma standard (WHO) is OK and no concentrate has as high multimers as the plasma standard.

(iii) Dr Montgomery reported results of a similar study, assessing in one laboratory the effect of different prediluents (severe vWd plasma, 1% albumin or buffer) on 6 named concentrates available in the USA against a local pooled (n>60) calibrated plasma standard.

Assay	Prediluent	Haemate P	Koate HP	Koate HS	Profilate SD	Kryobulin	AHF-SD
vWf:Ag	vWf-d	300	150	280	520	420	380
(18h Laurell)	Alb	290	150	200	400	480	370
	Buffer	Lower	Lower	Lower	Lower	Lower	Lower
RiCof	vWf-d	319	117	180	300	150	90
	Alb	363	171	190	210	259	110
	Buffer	190	81	100	180	124	40
Ratio *	vWf-d	1.05	0.8	0.5	0.57	0.39	> 0.2
(RiCof/vWf)	Alb	1.29	1.16	0.95	0.58	0.85	> 0.2
	Buffer	0.71	0.76	0.75	0.44	0.2	0.2
Multimers		Int/High	Int	Int	Int	Int	Int

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Montgomery suggested dilution in vWf-d but discussions agreed that above results do not justify this and other high proteins diluents (eg 5% albumin - above is 1%) need assessing. Mannucci says he does use vWf deficient plasma (as did Dr Zimmermann - Sixma uses local immunodepleted plasma). Montgomery implied elevated fibrinogen affects vWf assays.

* (Commercial) plasma standards give ratios of 0.82 (WHO), 1.1 (ARP), 1.11 (Pac Haem) 1.13 (GRF), 1.07 (CAP) and low (also low multimers) Mega concentrate std vs 1.0 (normal pool - by definition).

Discussion revealed support for assessment of botrocein cofactor, collagen binding, etc.

(iv) Professor Mannucci repeated his protocol for assessment of vWf concentrates in vWd (with some proposed amendments) and gave a summary of his recently published product assessments by this protocol (this months Blood).

The protocol assesses biological, not clinical, efficacy:

- assess in severe type III vWd (now extended to DDAVP-unresponsive type I and II)
- crossover design with product of proven efficacy (difficulty with selection of appropriate control - Haemate P or accredited cryoprecipitate are possibles - but preference is now to assess changes pre/post infusion of given product)
- assay VIII, RiCof and multimers in product
- infuse at 40 to 50 u RiCof/Kg and assess VIII and bleeding time (± RiCof) pre, post, 1,3,6 and 24 hours (bleeding time just once in 3-6hour frame and only at 24 hours if still shortened at 3-6 hours). In view of Briet's recent Thromb Haem publication now prefer classical Ivy method over Simplate bleeding time as yields less scarring and shorter times

Product assessment:

- there is an ongoing trial of Alpha factor VIII in the USA
- multimer assessment in various products show:

Low:	Koate, Hemofil
Low/Intermediate:	Monoclate, Hemofil-M
Intermediate:	Kryobulin, Koate HP, Emoclot, Profilate HP, Lille VHP-VIII
Intermediate/High:	Beriate, Alpha VIII, BPL 8Y
High:	Haemate P, Lille VHP-vWf
Normal (as NP):	none of above, cryoprecipitate

In discussion Sixma pointed out that vWf fractions as small as half the molecule are active in adhesion. M Hultin confirmed there has been an outbreak of hepatitis B in the USA due to cross contamination arising from an inadequately cleaned spring loaded device for performing bleeding times which uses disposable stylets.

- Biological assessments to date by Mannucci:

Product		Haemate P		8Y		Lille HP-vWf*		Alpha-FVIII		Cryoprecipitate**	
		VIII	RiCof	VIII	RiCof	VIII	RiCof	VIII	RiCof	VIII	RiCof
FVIII	0	11	< 6	9	< 6	9	< 6	10	< 6	-	-
	1h	86	140	91	139	52	? 130	94	170	-	-
	3h	87	?	91	109	57	114	80	138	-	-
	6h	82	?	86	71	64	72	74	95	-	-
	24h	77	?	74	?	74	23	59	25	-	-
Bl Time C/P/N***		3/5/2		1/4/5		0/0/10		2/3/5		1/2/3	

Notes

Above are presumed to be all severe type III.

*** number of patients giving complete (C: to within normal range), partial (P: by more than assay CV) and no (within assay CV) correction

** cryoprecipitate results are retrospective (Am J Haem 25, 55, 1987)

* Behring HP-vWf said to give similar poor response to Lille HP-vWf (Lille say Savidge (in mixture of vWd types) and Malmo (in type III vWd) obtain good results with Lille HP-vWf. Mme Goudemand in Lille has only 2 type III patients in the region). Mannucci also commented on low initial VIII increment with Lille product.

D Aronson questioned whether trials as above are necessary - would a register not do (difficult for licensing requirements). Mannucci's group are assessing ways of avoiding multimer loss during cryoprecipitation. He suspects calpain and hence leukocyte depletion may help (cellular apheresis plasma should allow assessment of this).

Mannucci posed three questions:

(a) How can multimer loss be avoided?
(b) Is a pure vWf product necessary (possibly not)?
(c) What FVIII kinetics occur after infusion and why?

(v) Dr C Kasper plans a registry of use and outcome for type III vWd patients subject to surgery, GI bleeds, menstrual bleeds and trauma requiring concentrate infusion and will circulate a report form.

(vi) The vWf gene mutation registry (Dr's Sadler and Ginsberg) will be published in Thrombosis and Haemostasis in 4 months.

(vii) Dr C Mazurier reviewed the differential diagnosis of the Normandy variant of vWd (autosomal haemophilia) from mild haemophilia. The best discrimination is given by the poor binding of exogenous factor VIII to test vWf immunocaptured from plasma, after normalisation for the extent of vWf capture (an assay developed by Mazurier but first described by Nishino - in Blood in 1989) and by the minimal response to DDAVP in the Normandy variant (Lopez-Fernandez 1992,

Niessner 1983) usually combined with an autosomal family history. Bleeding time vWf Ag and RiCof are normal, but there are differences in the response to various concentrates infusions.

Screening of French patients suggests this variant is more common than previously recognised with 1 of 40 male "sporadic" haemophiliacs (and two of his relations) and 6 of 20 women previously misdiagnosed (plus two women previously diagnosed as female carrier relations of the male case).

Mazurier identifies 14 established cases in at least 6 families (D Meyer, with whom there is some disagreement, argues there are 6 more families) with the most common mutant being Arg 91 Gln, but Thr 28 Met, Arg 53 Trp, Arg 19 Tryp, His 54 Q also being identified.

(viii) Dr Budde gave a long review of the literature and his own experience on acquired von Willebrand's disease in 4 categories: Immunomediated, myelo proliferative disorders, HUS/TTP and other disorders - these being largely of the type I variant. In discussion he admitted that such deficiencies are rarely associated with bleeding, except in cases involving concomitant aspirin administration or thyroid disorders. Dr Budde has identified 106 such patients using a combination of multimer and vWf degradation product blotting assays, with maybe 10 times that number in the literature (excluding diabetes).

(ix) Standards for plasma vWf assay are discussed in Section 2.3.

(x) For the vWf subcommittee matters (eg concentrate use in surgery, proposals for the reclassification of vWd as discussed at the recent Bari meeting) that should be raised at the next (New York) meeting the chairman, Dr Gralnick, proposed people contact him directly by Fax (1-301-402-1612).

5. Protein C and Protein S

(i) *Dr Bertina*

Made proposals, by analogy with protein C, ATIII and vWf deficiency for the classification of protein S deficiency (which should preferably include family history) as:

Type I (autosomal dominant defect in Protein S alpha gene resulting in parallel decrease in antigen (total and free) and activity)
type II (autosomal defect with normal antigen (total and free) but decreased activity. Relatively rare: Mannucci 1991, Nishioka 1991).

He also suggested an additional provisional third group of WP (working party) - type III associated with normal total antigen but reduced activity and free antigen. This may be due in some cases, to changes in C4B binding protein (which may exist in different heteropolymers of alpha and beta chains - the latter being the relevant S receptor).

A number of groups have (monoclonal) antibodies reactive only with free protein S and the beta chain of C4B binding protein.

(ii) *Dr Reitsma*

Summarised the 142 entries (56 unpublished) to the protein C mutation database as follows:

	Type I	Type II	Compound I/II
Heterozygous	99	8	-
Homozygous	9	1	-
Compound	4	1	some

Of these 93 (51 unpublished) are missense (23 being type II ?), 26 are nonsense and 10 splice mutations.

(iii) Tait (and Walker) described the frequency of C deficiency in Glasgow donors against a background of reported incidences of 1 in 16,000 (Broeckman 1983) and in 1 in 32,000 (Gladan 1988) obtained by extrapolation from patients with thrombosis, and 1 in 200 to 300 (Miletich 1987) from donor assessment. They have found 225 donors with levels below the normal range (68 to 136) by Protac chromogenic assay of 9648 donors. Of these 73/225 (33%) are repeatedly low in this assay and 56/73 have family studies in hand. These are mainly young males (levels are higher in women and rise in both sexes with age), but 8 (7 also have low antigen) show evidence of familial inheritance and evidence of deficiency. This gives an estimate (probably underestimate) of 1 in 1000 heterozygotes (? asymptomatic), in line with Miletich's figure.

They have also assayed protein S antigen in 4,000 donors and are assessing families in 10 (1 in 400) with repeat low levels.

(They are also assessing a new Chromogenic kit for simultaneous assay of tPA and PA1).

(iv) Dr R Allaart gave evidence that the parameter giving best discrimination of protein C carriers is the ratio of C activity (or antigen) to prothrombin antigen, and is undertaking a prospective study of thrombosis in such patients (retrospective anecdotes suggest some cases but significance of these is unclear).

(v) Dr J Griffin briefly described the occurrence of an assay for C4B binding protein beta chain (Blood 15/06/92) and the occurrence of mixed alpha and beta C4BBP heteropolymers.

(vi) Dr Boyer-Newmann summarised a 4-centre (2 Italian) exercise assessing 45 normal, 34 type I, 8 type II and 20 high C4BBP plasmas for protein S by 4 assays (also 21 coumarin and 18 liver disease plasmas).

(a) PTT based with APC, Va, cephalin (sensitive to Gla content)
(b) PTT based with APC and cephalin (overwide normal range)

(c) PT based with Protac
(d) D'Angelo monoclonal capture PTT/Xa/APC/cephalin assay

a,c and d give reasonable diagnosis of deficiency but b not so good.

6. **Platelets**

(i) J MacGregor made a plea for a reference platelet/collagen adhesion assay.

(ii) Mike Barnes and Dr Kunicki provided evidence for specific platelet binding domains (via GPII/IIIa, alpha-2/beta-1 and alpha-5-beta-1 integrins) in two specific domains of the alpha-1-chain of collagen type I and type III respectively.

(iii) Dr S Santoro reviewed evidence of the modulation of alpha-2 beta-1 integrin expression in a variety of cells (eg keratinocytes) during growth, differentiation and transformation. He also described changes in this integrin (and GP IIB/IIIa) during the differentiation of the K562 cell line to megakaryocytes by phorbol ester. Alternative stimulation of this cell line can induce erythroid (by haemin) or monocyte cell types.

(iv) Dr G Jamieson provided further evidence that GP IV (CD 36) is a platelet receptor for collagen (? only initial), the sequestrin ligand of malarially infected red cells and for thrombospondin.

There was disagreement in discussion on the last of these (McGregor for, Santoro against).

(v) Dr N Mercier described serological and peptide scanning evidence that the E5 peptide (location not stated) of GP IV provides the collagen binding domain.

(vi) Dr Valet described a computerised system for analysis by FACS of multiple platelet activation antigens (Appendix II).

7. Miscellaneous

(i) Inge Antonnson (Kabi) states their high purity IX is now licensed in Sweden.

(ii) *Trevor Barrowcliffe*

(a) Asks if he can obtain and report data on changes in VIII and IX across viral inactivation steps (SD or heat treatment) as he has a talk to give on this at the Cannes meeting. I referred him to Bruce Cuthbertson.

(b) A list of current NIBS coagulation standards is attached as Appendix III.

(c) NIBS funding is being revised. WHO (standards) and DoH (batch release) will probably continue, although on a more contractual basis, although testing every batch prior to release will be challanged in Europe. Trevor is not too concerned over Strasbourg's moves towards taking over standardisation as they have, as yet, no laboratories. Blood Transfusion working standards are seen as a further source of funds, although if feasible on a wider basis than just the UK this might attract European funding.

(iii) H Kingdon states that Baxter are now working on gene therapy of haemophilia (eg supply of retroviral vectors) but have great hopes of a shorter term solution for subcutaneous implants made from a plsatic which promotes angiogenesis rather than capsule formation which can be used to avoid problems with allogeneic (but ? not xenogenic) cells. This will initially be trialled in Sweden in diabetics using pancreatic islets (? from Belgium cadavers) but may also be tried eg for CHO cells or fibroblasts making factor VIII. Factor IX models are in hand.

(iv) Steam treated fibrinogen from Immuno (1gm in 50 ml) is used in Italy and German speaking countries for replacement in acquired deficiency (Appendix IV leaflet with R Stewart).

v) In the ""Preductive Haemostatic Variables in Vascular Disease" subcommittee Mannucci presented the poor correlation of Fragment 1.2 levels with INR in patients on coumarin and Eric Preston spoke on the artefactual elevation in Fr 1.2 seen in ATIII deficiency if samples are collected in low heparin anticoagulant (confirming K Bauer's results). Citrate anticoagulant is good but processing within 1 hour is recommended.

(vi) Blood Coagulation and Fibrinolysis is doing well and looking for more proposals for reviews.

(vii) A new Chromogenic simultaneous tPA/PA1 chromogenic assay is under assessment by Isobel Walker in Glasgow and by the Dutch and looks good according to S Rosen. He says the promised new factor VIII kit will be ready soon (samples promised).

(viii) France have apparently, through D Meyer, introduced separate plasma standards for group O and non-group O plasma and confusingly express results for both as percent of standard rather than iu/dl.

(ix) H Kingdon's address given as:

(x) D Aronson is Chairman elect of the subcommittees. D Meyer is Chairman of the ISTH.

Next subcommittee meeting will be in New York: 3rd-9th July 1993 (see Appendix V).

(xi) It is obviously impossible for one person to cover 4 sets of 6 simultaneous sessions so the above report is selective of the meeting. Particularly I failed to catch updates on animal models or tissue factor inhibitor.