

THE HEMOPHILIA BULLETIN

July, second issue of 2004. Carol K. Kasper, Orthopaedic Hospital, 2400 S. Flower St., Los Angeles, CA 90007. ckasper@laoh.ucla.edu
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Scientific and Standardization Committee (ISTH)

Beautiful San Giorgio Island in Venice was the cozy venue for this year's meeting. The spring weather was idyllic: sunny but not too warm. A concert was provided one evening. Nino Neri, MD PhD, of Milan, a hematologist and a dramatic tenor, sang some of the most famous Italian arias, accompanied by pianist Leonardo Marzagalia. When a handsome man with a huge rich voice like Dr. Neri's goes into a profession other than opera singing, there must be a big surplus of musical talent in Italy. Congratulations and thanks to Francesco Rodeghiero for organizing the meeting and to Pier Mannucci for organizing the concert!

Pediatric issues

In a satellite symposium, Mike Makris pointed out **overlooked causes of bleeding**. In children under one year of age, where statistics are available, bruising is ten times as likely to be due to deliberate abusive injury than to a bleeding disorder. Noonan's syndrome (multiple congenital defects) was associated with a prolonged APTT in 29 of 72 cases, with low levels of a variety of individual clotting factors or combinations of factors. Beware of a false-normal APTT in patients with an acute phase reaction: a patient, who had mild hemophilia B and a low factor IX level, had a normal APTT when his FVIII happened to be elevated during stress; the high FVIII "made up" for the low factor IX in the APTT, a phenomenon we've also observed. Dr. Makris again reminded us that in some families with mild hemophilia A, a low factor VIII can be detected only with a two-stage assay. A patient had 55% FVIII by the one-stage and 13% by the two-stage assay. Another patient, first examined when stressed, had 70% FVIII by the one-stage and 24% by the two-stage assay; later, at baseline, he had 24% by the one-stage and 8% by the two-stage assay. Alas, in most of the USA, we're lucky to have a reliable one-stage assay available.. The two-stage ought to be made available at reference laboratories, at least. When all laboratory tests are normal (including those for von Willebrand disease, for platelet storage pool disease, for factor XIII, for alpha-2 anti-plasmin), think of Ehlers-Danlos syndrome.

Augusto Federici reviewed a few **drugs useful to promote coagulation in a variety of situations**, emphasizing controlled trials. Excellent evidence supports the utility of **anti-fibrinolytic drugs**, tranexamic acid and epsilon-amino-caproic acid, EACA, Amicar®. Doses of 10-15 mg of tranexamic acid per kg every 8 hours have a high record of efficacy in menorrhagia, GI bleeding, prostatectomy and dental extractions. Lesser evidence supports the drug's efficacy for hemostasis in thrombocytopenia and for reduction of blood loss in major surgery in persons without bleeding disorders. The next-best-documented general-hemostatic agent is **DDAVP**, which is useful not only in hemophilia A and von

Willebrand disease, but possibly also in factor XI deficiency, in various platelet disorders, in uremia and in cirrhosis (but there are no controlled trials for the latter use.) Conjugated estrogens and erythropoietin are also useful in uremia. **Recombinant VIIa** has been used in a variety of circumstances other than FVIII inhibitor management, but not in controlled trials.

Factor VIII and IX Subcommittee

Kathy High reported on the status of the **hemophilia gene therapy** trials, all of which are now inactive. The trial of factor IX gene delivery by means of the adeno-associated viral vector into the hepatic artery was closed because it could not be financially sustained at the slow pace required by regulation. There's hope, after all, hemophilia gene therapy in dogs WAS successful. Persons with liver disease (chronic hepatitis C) may not be suitable for liver-targeted vectors and persons on anti-retro-viral drugs are not suitable for retroviral vector use. More studies will be carried out on animal models.

Donna DiMichele is leading the effort for a **definition of clinical phenotype**, in particular, in patients with FVIII assays of <1%, in whom prophylaxis is being considered, depending on whether or not they have a truly severe clinical pattern of bleeding. One early proposal is that "severe severe" might be defined as more than three infusions for spontaneous bleeding in the first year of life and more than three joint bleeds in the first three years of life.

Marijke van den Berg is also studying **clinical phenotype**. She analyzed annual use of clotting factor per kg in Dutch patients. Among those with severe hemophilia, patients with less current joint bleeding also had started bleeding into joints later in their lives. Is the age of the first joint bleed a predictor of clinical phenotype? Or, does early joint bleeding cause vulnerability to bleeding, as in target joints? She studied patients who have the same FVIII inversion mutation, comparing a subgroup with frequent bleeding to those with less frequent bleeding. The milder-severe group (n=20) had had the first joint bleed at a mean age of 3.8 years and now had 1.5 joints with arthropathy. The severe-severe group (n=18) had had the first joint bleed at a mean age of 1.3 years and now had 5.4 joints with arthropathy. There was no significant difference in FVIII T ½ in the two groups, but there was a marked difference in thrombin generation in an *in vitro* system: the clinically-milder patients generated much more thrombin, and at a faster rate, than those clinically-more-severe.

The issue of **MRIs of joints** in children was raised both by Victor Blanchette and by Keith Hoots. Damage may be seen much earlier than by traditional Xrays. Because of expense, this modality has not been used much, but that also means that its

utility has not been adequately evaluated. It may tell us a lot about early joint changes and the optimal time for prophylaxis.

Sanj Raut of NIBSC wants to develop a **reference for inhibitor levels**, because there is great variation among results on the same sample tested in different laboratories. NIBSC is wondering whether to use naturally-occurring inhibitors or antibodies obtained from cloned cells, an issue on which I have no opinion. They also wonder what inhibitor levels to use, an issue on which I do have an opinion, namely, use a very low level inhibitor that's a genuine inhibitor but is just barely detectable by the Bethesda-Nijmegen assay. To me, the major issue is, does a given patient have a low-level inhibitor? It's a problem in studies of inhibitor development in previously-treated patients: are investigators quite sure that all such patients didn't start off with low-level inhibitors? None of the major inhibitor tests was designed to be sensitive. They just brought consensus on crude definitions. We need a much more sensitive inhibitor assay, probably with techniques other than mixing experiments. Help, help, dear immunologists!

Pete Lollar described **the factor VIII assay "problem"**. There are multiple assays, multiple standards (plasma vs. concentrate), and too much effort put into "democratic" assignment of unitage (by assaying new standards against fresh normal plasma in various labs in the developed world, which don't really represent the whole world.) He is in favor of using the one-stage assay for evaluating plasmas and concentrates, and using a plasma-derived standard (not a separate, concentrate standard) for assaying concentrates. The one-stage assay predominates in the clinical labs of the world. He suggests that it is assay reagents that need standardizing. I have a lot of sympathy for his point of view. I've seen the struggles to keep the international plasma and concentrate standards from drifting too far apart. It doesn't make sense to demand (expensive) chromogenic assays in clinical labs. I'd love to have chromogenic and two-stage assays, as well as one-stage, in the lab, for investigative purposes, but few labs can do so. I also understand his suggestion that all the assaying of a new standard be done in NIBSC, not in co-operating labs around the world. I took part in such exercises, I admire the attempt to relate the standards to real normal people, but what's normal in experienced labs does not represent the world. I happened to have a high proportion of donors of African descent in my samples, which raised the FVIII level of my local reference pool. It took me years to figure out why we were different! Why not "normalize" on white Britons? As we normalize on Greenwich mean time?

Trevor Barrowcliffe reviewed the **history of concentrate and plasma standards** over more than 30 years. Use of concentrate as a standard against which to measure clotting-factor concentrates was a practical decision: in vials of freeze-dried material, concentrates are more stable than plasma. There was less variability when concentrate standards were used. With most concentrates, assay results are similar in the one-stage and in the chromogenic methods but Method M concentrates (like Hemofil M®, produced with monoclonal-antibody affinity-chromatography) assayed higher in one-stage assays than in chromogenic assays, and, B-domain-deleted recombinant concentrate (ReFacto®)

assayed lower. If the one-stage assay were to be chosen, then reagents, instruments, reference plasmas and, especially, deficient plasmas ought to be standardized.

Steffen Rosen reviewed the **advantages of the chromogenic assay**, which include use of a high dilution of the tested sample so there is less sensitivity to pre-activation by thrombin (which also is true of the two-stage assay). The chromogenic assay focuses on just the part of the coagulation cascade where FVIII acts: the end-point is the formation of activated factor X. Rainer Seitz gave the **regulators' viewpoint** on behalf of EMEA, which demands the chromogenic assay. It is, however, looking at the problems and trying to improve the method.

Peter Turecek described a **new thrombin generation assay for factor VIII** in which the trigger starting coagulation is tissue factor. The assay is performed by an ELISA method in a microtiter plate. The reagents include tissue factor, phospholipids, a fluorogenic thrombin substrate and calcium chloride. Fluorescence is detected in a fluorometer. A major advantage of his method is that it is sensitive at very very low levels of factor VIII (which would be helpful in defining "severe" hemophilia, and in following minimal responses to gene therapy.) When various concentrates were assayed, ReFacto®, again, was "different". Claude Negrier used this assay to measure FVIII in post-op plasma samples from hemophilia patients. He found that accuracy depended on sampling only the top part of the centrifuged plasma that was well-separated from the blood cells.

Flora Peyvandi chairs the working group on **rare bleeding disorders**. These include factor deficiencies with prevalences from one in 500,000 to one in two million. The prevalence is higher wherever consanguineous marriages are commonplace, sometimes causing a prevalence similar to that of hemophilia B. Fresh-frozen plasma often is used, which carries risks of viral transmission and volume overload.

Mark Weinstein presented the **FDA position on orphan drugs**. An orphan disorder is one that affects less than 200,000 persons in the USA and for which there is (supposedly) no commercial incentive for preparation of a therapeutic product. Incentives for pharmaceutical companies to develop appropriate drugs include seven-year market exclusivity, a 50% tax credit on clinical testing, awards grants for clinical testing, waiver of certain fees, help with study design, and other benefits. The FDA demands adequate studies designed to make a valid comparison to a control, in order to provide a quantitative assessment of the drug's effect versus, e.g., no treatment, placebo, various doses, or historical controls. When groups are very small, normal volunteers can be used for pharmacokinetic studies. The FDA will have a workshop next year on orphan drugs.

Zera Tellier of LFB, Sigurd Knaub of ZLB Behring, and Peter Feldman of BPL **described the orphan products** made by their companies. (See the Registry of Clotting Factor Concentrates.) Dr. Feldman helpfully listed some of the problems with orphan drug development and distribution, including their unlicensed status and use only on a named-patient compassionate basis, inability to

advertise, possible liability issues, cost of development and manufacture, and problems and expenses of keeping adequate stocks in-date. He'd like to see a special license category for true orphan drugs, with limited clinical trials, generic validation of methods, and permission to market the products. Pier Mannucci thanked the companies that do make orphan products available and made a special plea for a factor V concentrate, for no concentrate exists that contains that factor. He said that regulatory agency demands on manufacturers still are too difficult. I agree, and, there is no meaningful financial incentive. The issue needs a champion who could devote most of his time to untangling the demands and finding financial sponsorship for sustaining existing orphan concentrates and developing new ones. Some parts of the world with relatively large numbers of persons with rare clotting factor deficiencies, because of consanguineous marriages, e.g. Iran, now provide near-universal health coverage. Maybe some orphans will start to look more appealing.

Von Willebrand Disease Committee

Collagen, for the **collagen binding test (VWF:CB)**, is being debated, again. Tony Hubbard says that type 6 collagen reagent is best at detecting VWF lacking large multimers. There is no WHO concentrate standard for collagen binding, therefore, concentrates are not measured in VWF:CB units, and maybe we should be grateful for now. The debate went on a long time, but there was no conclusion, and, I'm confused.

Evan Sadler reviewed the **classification of VWD**, last officially revised in 1994, and, thank heavens, has not changed it except in one detail. He says that a classification ought to have clinical utility, be simple (with a minimum number of categories), depend on commonly-available blood tests and stress concepts not specific testing procedures. Some specific points were as follows: (1) whereas the 1994 definition stated that VWD was caused by mutations of the VWF gene, that demand will be eliminated. It's un-necessary, un-workable. Mutation analysis does not grow on trees. (2) Type 1 VWD, a partial quantitative deficiency of VWF, won't be further defined until the large studies in Europe and Canada are thoroughly analyzed. The distribution of multimer sizes may be normal or relatively normal. (Nice word, "relatively", it avoids re-classifying a lot of patients.) (3) Type 2 VWD may include some difficult differentiations which should not, for now, command a lot of attention. Although there are two known mechanisms for the lack of large multimers in type 2A (namely, decreased synthesis and increased proteolysis), a subdivided nomenclature is not being advised. It is recognized that the former "type IIC" is recessive, whereas most other type 2 is dominant, but, don't fuss. The former Malmö and New York variants now under the umbrella of type 2B are interesting because of lack of thrombocytopenia and lack of notable symptomatology, but, for now, they are under type 2B. Type 2M will include defects either in binding to platelets OR to connective tissue, thus taking care of a recently-described variant with isolated poor collagen binding. (4) Type 3 VWD may expand to include patients with homozygous or doubly heterozygous type 2 mutations. So we can all breathe easier, the classification is fundamentally the same.

Margareta Blomback, a practical person, is concerned about the **diagnosis of mild VWD** (as is Evan Sadler), versus a mildly-reduced VWF level conferring, perhaps, a slightly higher risk of excessive bleeding. In borderline situations, with few symptoms and a mildly-reduced level, should the word "disease" be used, for it will frighten insurance companies and certain employers. She cited the instance of a young man with borderline findings who wants to be a military officer – a career forbidden to "bleeders". I sympathize, and can suggest only that borderline findings deserve borderline candor. The employer should hear that there is no disease and the surgeon that there may be some increased risk. I've had experience with the issue. During the Vietnam war, young men coming to my lab were eager to have a coagulation disorder diagnosed. The mood shifted after the war, but, if a military draft were to be re-instated, a VWD diagnosis might be popular again.

David Lillicrap described the difficult **distinction between a mutation and a polymorphism**. A polymorphism is an alternative normal version of a gene, a different but normal allele. The "rule of thumb" is that a different allele is just a polymorphism if it affects more than one percent of the population. He offered some examples that bring that casual rule into question, namely, the factor V Leiden allele affects about five percent of the population and the prothrombin 20210 allele affects about three percent of the population. The gene for von Willebrand factor is highly polymorphic with 138 polymorphic nucleotides, of which 81 are in exons, scattered throughout the gene. Of the 81 in exons, 32 alter the amino acid sequence. He identified one change that had been classified as a polymorphism but which results in retention of VWF in cells, that is, it has a dominant-negative effect similar to some "mutations". Other polymorphisms have definite but minor effects on VWF levels. Polymorphisms and mutations overlap. Perhaps all sequence changes should be regarded as mutations. (But then, what's the "normal" sequence in this highly-polymorphic gene?)

Zaverio Ruggeri discussed tests for SIPA, sheer-induced platelet aggregation. VWF is important for platelet adherence when the sheer-rate is high but not when it is low. Ticlopidine, but not aspirin, inhibits SIPA. Antagonists of platelet GP IIb/IIIa totally inhibit SIPA.

Catherine Hayward discussed the utility of PFA-100® analyzers. The sensitivity is very high, the test identifies all patients with known VWD. Many persons, however, have a prolonged closure time (the end-point of the assay) with no other abnormal laboratory test. It's unclear whether these are false-positive CTs or whether our ability to diagnose coagulation defects, especially those of platelets, is inadequate. PFA-100® analysis is relatively expensive, and it requires fresh blood. In Canada, most patients needing coagulation diagnosis are represented in the coag lab by their frozen plasma, shipped in. That situation is not surprising, given Canada's geography. The most widely-used tests are those which can be done on shipped, frozen plasma samples, and the least used tests are those which must be done on fresh blood. I don't think Canada is alone in this practical problem.

Pier Mannucci mused on some **unresolved issues**. It is not clear which measurement of VWF function is the most important determinant of mucosal bleeding, nor is there information on the "hemostatic" levels of VWF:RCO or VWF:CB, that is, how much does a person need in order to clot normally? The management of gastrointestinal bleeding remains problematic, what has to be corrected, what dose is important. (My comment is, GI bleeding is often resistant to therapy in persons with normal coagulation mechanisms; there may not be any dose of any therapeutic agent that can be recommended as "correct".) Dr. Mannucci also questions the prevalence of adequate clinical responses to DDAVP (I think the responses are much better than predicted from levels of VWF and FVIII). He also thinks that the utility of agents used for menorrhagia, including birth-control pills, need better objective evaluation of efficacy.

Giancarlo Castaman reported that among type 1 VWD families in the European study, **DDAVP response** had been studied in some 74 patients. Although peak responses were similar relative to baseline levels, patients with mutations in certain locations (propeptide, D'-D3, D4-CK) had more rapid half-lives of the released VWF. (This distinction is unimportant for management of a simple acute hemorrhage but may be important in surgical operations where sustained elevation of VWF levels may be preferred.)

Stefan Lethagen reported on a comparison of the **biological response to DDAVP versus its clinical efficacy**. An adequate biological response to a standard intravenous dose of 0.3 ug/kg was defined as at least a three-fold increase in levels of FVIII and VWF:RCO, plus peak levels of at least 30%. (Pharmacokinetics also are being studied, with plasma levels of these factors measured over a 24-hour period.) He invites additional centers interested in studying biological response vs. clinical efficacy to join the study through the SSC web site. Pier Mannucci cautions that it will be much more difficult than studies of clinical efficacy in hemophilia, a more homogeneous condition.

Derrick Brown described a study beginning in UK hemophilia centers on **type 1 VWD** with VWF:RCO less than 50%. Patients with confirmed type 1 VWD will have three types of **further tests**, as follows: (1) the vulnerability of VWF to proteolysis will be analyzed, (2) the VWF gene will be sequenced to search for mutations, (3) linkage analysis will be carried out on suitable families and, if non-linkage is documented, genome-wide linkages will be sought and additional candidate genes will be sequenced. (Perhaps there's another gene with a major influence on VWF, and we've been too focused on the one "VWF gene" we thought was the one-and-only gene.) So far, one polymorphism, Y1584C, has been found in 19 of 76 affected persons, and 18 of the 19 are of blood group O. The polymorphism co-segregated with an increased susceptibility to proteolysis of VWF by ADAMSTS13 in all subjects tested so far, averaging 33% higher. VWF from blood group O "normal" persons is about twice as susceptible to proteolysis as that from group AB persons. Both the polymorphism Y1584C, and blood group O, contribute to the phenotype of type 1 VWD but should not be considered to be causes of VWD.

Evan Sadler of the USA wonders what is the "normal" population frequency of the Y1584C polymorphism. David Lillicrap reported that he had screened 200 normal blood donors for that polymorphism and found one heterozygote whose VWF level was 50%. Derrick Brown found four heterozygotes in 200 normal controls. It's important to know the VWF levels of persons with this polymorphism (or mutation or whatever we ought to call it.) If they cluster around 50%, maybe we have an explanation for the high frequency of supposed VWD found on population surveys (such as the oft-quoted one of Francesco Rodeghiero) in which VWD was defined by borderline VWF levels. We have a long ways to go before we can truly define Type 1 VWD.

REQUIEM FOR A LIFE-SAVING PRODUCT

Today I am depressed. I was informed that porcine FVIII concentrate, Hyate-C®, will no longer be produced. You may know that the supply has been meager lately, and you may know that the reason is that it's hard to find pigs who don't carry porcine parvovirus, an endemic virus in pigs. It became just too difficult and costly to find uninfected pigs. The parvovirus is also notoriously hard to viral-inactivate.

And somehow, the presence of that virus has been judged to be important, more important than the lives of patients with inhibitors that were salvaged by the concentrate.

Patients who had been treated with the concentrate that did contain the virus have been tested, and all were seronegative for antibodies to porcine parvovirus. Pig farmers and slaughter-house workers also don't have antibodies. The virus does not appear to "infect" humans, or, provoke an antibody reaction. No-one is known to have been harmed by the presence of this virus in the concentrate. We are being deprived of an option for the management of a very difficult challenge, the bleeding inhibitor patient.

Porcine FVIII had a limited niche. It was useful in patients with little cross-reactivity, but in that niche, it worked just beautifully. I remember all the patients pulled back from the brink by that product, and I'm grateful to have had it available for so many of my most active years.

A recombinant porcine FVIII molecule is in clinical trials. That's the hope of the future.

We still have a few copies of the thick monograph on VWD. Some people have asked for a few extras for their hematology Fellows. Please send me your request by email together with your best mailing address.