

THE HEMOPHILIA BULLETIN

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The last issue of the Bulletin

This year, the Bulletin had its 25th birthday and I had my 70th. It's a good place to stop. The newsletter started in 1981, when I visited the excellent hemophilia centers in Milan, in Bonn and at London's Royal Free Hospital and came home full of wonderful new ideas about DDAVP, porcine factor VIII (FVIII), pasteurized FVIII, and induction of immune tolerance. I sent notes to colleagues who might be interested. The list grew. This issue will go to over 4000 recipients around the world. I'm grateful for the support of the Crippled Children's Guild of Orthopaedic Hospital in the Bulletin's early years, for the bequest of Moses Foster de Masters that carried me through the middle years, and for the support of several pharmaceutical companies in recent years. Thanks also to Debbie Ramires for keeping the address list and to Francisco Hernandez for proof-reading and for envelope-stuffing and mailing. Thank heavens for the Internet, where information now can be disseminated rapidly and cheaply.

World Federation of Hemophilia, Vancouver, May; Scientific & Standardization Committee, Oslo, June

Some topics were covered in both places; notes are blended.

Mark Skinner, president of WFH, charted recent achievements. Emphasis is being placed on diagnosis, rightly, because that's the first step. In the past three years, over 5000 new patients have been diagnosed. I was also delighted to hear that the improvements in hemophilia care brought about in Chile and Uruguay, thanks to Project Access, have been sustained. WFH remains a highly effective and thrifty health-improvement organization.

I attended few of the musculo-skeletal sessions at WFH, having covered them well previously. Brendan Egan of Australia noted decreased bone density in adolescents with hemophilia compared to controls, despite prophylaxis and despite good joints. He pointed to studies showing that non-weight-bearing exercise, bicycling for instance, doesn't build up bone mass well. In one study in normal boys, intensive badminton was correlated with better bone mass than intensive ice-hockey. The greater impact associated with badminton may be needed. Given various reports of lower-than-normal bone density in hemophilia, it would seem constructive to use anti-osteoporosis medications in our less-active patients.

The surgeons discuss problems with common orthopedic operations. Adolfo Llinás of Bogotá listed the many ways in which the hemophilic knee joint differs from the osteoarthritic joint with which most surgeons are familiar. To summarize, the joint has lost a lot of mobility and joint space, the articular ends of the bones have become mis-shapen, the patella is likely to be out of place, the bone may be so osteoporotic that it may need extra support, the muscles are stiff if not contracted. There's extra-ordinary technical and peri-operative difficulty, and sub-optimal results, nonetheless, the patients are very grateful.

The point is, the surgeon experienced with hemophilia is more able to recognize and deal successfully with the special problems of the disorder. That should be obvious. We all know that heart surgery is more successful if done by surgeons, and at hospitals, with lots of experience. Hemophilia needs experienced surgeons (and other specialists) too. Sometimes HMOs disregard the obvious.

Mauricio Silva of Los Angeles reported that radial head excision is highly successful at restoring rotation but not extension and flexion. Pain may not be relieved. A few patients may later need an ulnar nerve transposition. Infection is unlikely. He is often asked about total elbow replacement. There are about 12 cases in the literature, and the infection rate has been about 50%, so he does not yet recommend the operation.

Alok Srivastava of Vellore, India, looked at 115 patients with severe hemophilia. Of these, 15 bled infrequently (five or fewer hemorrhages per year, no more than one joint involved), and the other patients bled often and had multiple joints involved. He looked at polymorphisms of three cytokines previously identified as influencing the severity of rheumatoid arthritis. Two cytokine alleles had a marked association with frequent bleeding. One polymorphism of tissue necrosis factor (TNF) alpha was twice as common in the frequently-bleeding group, conferring a 4.2 increase in relative risk (RR). A polymorphism of tissue growth factor beta conferred a 3.8 increase in RR. For years we have wondered why some patients with severe hemophilia have a milder clinical course. It may not be a matter of clotting factors. Assays of thrombophilic factors haven't provided an explanation. Patients may differ in the immune response to inflammation, influencing the tendency to synovitis, and synovitis provokes more bleeding. Jean-Marie Saint-Remy suggests also looking at the complement system.

Radionucleotide synovectomy for hemophilic synovitis is highly effective, and simple to perform, just an outpatient injection into the joint. Recently, anxiety arose because two children who had had the procedure subsequently developed leukemia. The procedure has been used for many decades, longer in rheumatoid arthritis than in hemophilia, with no previous reports of malignancy. Were these two leukemia cases just a co-incidence? Georges Rivard of Montreal, who first reported using the procedure for hemophilic synovitis in 1971, compared malignancies in 2412 Quebec patients who had had a radionucleotide synovectomy for any disorder with the age-matched general population, using the Quebec and the Canada cancer registries. There was no excess of malignancy: 4.35 cases of leukemia were expected and 5 observed; 9.11 cases of lymphoma were expected and 10 observed; 157.8 primary malignancies were expected and 151 observed. There was no increased malignancy risk in his patients who had had multiple radionucleotide synovectomies. Follow-up studies continue around the world.

Katherine High of Philadelphia reviewed the two factor IX **gene therapy** trials that succeeded well in dogs. In one, the adeno-associated viral (AAV) vector carried factor IX to the liver and resulted in sustained elevations of plasma factor IX of 6, 7 and 8%, respectively, in three dogs, for several years now. Dogs have little immunity to the AAV that was used. The same vector construct was given to humans. Initial rises in plasma factor IX levels were followed by falls, together with increases in liver enzyme levels, due to antibodies to the vector. In further human trials, the same vector construct will be given together with temporary immunosuppression, until the vector is broken down. The factor IX genes survive in the nucleus, predominantly not integrated. In another trial in dogs, an AAV-factor IX vector construct was given by anterograde limb perfusion under tourniquet, targeting myoblasts. The results were good in dogs, so this approach is in reserve for possible human trials later.

Amit Nathwani of London used a different serotype, AAV 8, to which there are fewer pre-existing antibodies. The modified "self-complimentary" or "scAAV8", with two copies of the factor IX gene, allows better expression. The vector is rapidly degraded and cannot transduce human antigen presenting cells. The construct was given to five macaque monkeys through peripheral veins. They have expressed factor IX at levels of 9 to 25% for more than 311 days. Human trials will begin at two centers in the UK and two in the USA, without immunosuppression, in patients without pre-existing immunity to the AAV8 serotype.

David Lillicrap of Canada used blood outgrowth endothelial cells (BOEC) to deliver FVIII genes to FVIII knock-out mice. They expressed one to 8% FVIII in the plasma for up to 175 weeks. FVIII is a larger molecule than factor IX, so more capacious vehicles are needed. BOEC delivered the large VWF gene to dogs with type 3 VWD in Belgium, as just reported in Blood.

Genetic differences may account for at least half of the risk of development of inhibitors. Jan Astermark of Sweden found that certain polymorphisms of interleukin-10 and of tissue necrosis factor alpha are associated with a high risk, about 80%, of inhibitor formation. The genetic influence of the immune system may exceed that conferred by the mutation type. (What are the frequencies of these high-risk polymorphisms in the black African population? Might that explain their high inhibitor risk?)

Marieke van den Berg of Utrecht reviewed again (as reported last summer) the non-genetic influences on inhibitor development, including the negative effects of bursts of intensive treatment early in life. I did not report previously that early prophylaxis appears to be protective.

There are many things we can't do anything about: the mutation, the cytokines, most of the need for early treatment. How should we react as we identify the high-risk patient? Should we start early prophylaxis? In high-risk infants, could we start by a non-intravenous route, perhaps orally, even if resulting plasma levels are low? If early prophylaxis is good in general, is it good or bad in extra-high-risk patients? Future studies of inhibitor development, as in patients on new concentrates, must include all the genetic influences we know about. Results might be biased if a small group happened to contain greater or fewer representatives with unfavorable genes.

Jeanne Lusher of Detroit gave a "final" report on factor IX inhibitors with associated allergic reactions to factor IX on behalf of Indira Warriar. She surveyed USA hemophilia centers in 1997-98 and found a 2.3% prevalence of inhibitors severe hemophilia B. As of May 2006, some 55 patients with factor IX inhibitors had been identified in the USA and 37 elsewhere. A little more than half have associated allergic reactions such as anaphylaxis. A total of 39 patients have been on immune tolerance regimens; 25 with factor IX alone, 14 with factor IX and immune modulation and seven with plasmapheresis and antibody absorption plus factor IX. Five patients achieved tolerance, two with factor IX alone and three with plasmapheresis and factor IX. A nephrotic syndrome developed in a total of 13 patients, all of whom were undergoing immune tolerance regimens. Eleven of the 13 had a history of anaphylaxis. Eleven of the 13 were using factor IX alone for tolerance induction. I wonder whether the "Malmö" approach would be better; it starts with vigorous removal of antibodies followed by factor IX infusion, intravenous gamma globulin and immunosuppression.

The **thrombin generation** test is much discussed. The end-point, the generation of thrombin, is detected by a fluorescent reagent. The test is affected by "the cross-talk of all coagulation factors" as one speaker put it. In other words, it's non-specific. Why the interest? For one thing, it is sensitive to very low levels of FVIII, down to 0.0025 units/ml, a sensitivity similar to the old two-stage FVIII assay. If the test is used to follow a patient being treated with a concentrate, the test has to be standardized to that individual patient's baseline. The test also has been used in an attempt to follow patients treated with bypassing agents, but it was disappointing. It did not predict clinical responsiveness to FEIBA® or to NovoSeven® given to patients with inhibitors in the "FENOC" comparative trial, according to Claude Negrier. As reported previously, a single dose of FEIBA® at a target dose of 85 U/kg was as effective as two doses of NovoSeven® at a target dose of 105 ug/kg per dose, given 2.5 hours apart for hemostasis in joint hemorrhages in hemophilia with inhibitors.

What is **von Willebrand Disease** as of 2006? As you recall, it was classified into three types in 1984, based on multimers: type I (roman numeral), reduced multimers but all sizes present; type II, no large multimers; type III, no multimers (severe VWD). The 1994 definition demanded a mutation on the VWF gene, and defined type 1 (arabic numeral) as a quantitative deficiency, type 2 as qualitative and type 3 as severe. Platelet-type VWD was to be called pseudo-VWD. In 2006, amendments are few, thank heavens, because there isn't another numeral system to use. In recognition of the sparse availability of genotyping, and the complexity of causation of phenotypic type 1 VWD, the requirement that there be a mutation in the VWF gene is dropped. Type 1 is a partial quantitative deficiency of VWF, type 2 a qualitative deficiency, and type 3 a virtually complete deficiency of VWF.

Type 1 VWD is not so purely quantitative as was envisioned in 1994. Nowadays it encompasses conditions with some decrease in the *proportion* of high-molecular-weight (HMW) multimers. It includes some conditions with circulating mutant subunits. It includes some conditions such as the former type IC and the Vicenza variant with more rapid clearance of circulating VWF. It includes conditions with secretion problems, e.g. abnormal VWF subunits that prevent the release of normal VWF from the cell. **So what characterizes type 1? VWF is able to react normally with platelet receptors and to attach to FVIII. What characterizes type 2? VWF does NOT react normally with**

one or the other of these, either with its platelet or with its FVIII receptors. Types 1 are said to be generally capable of satisfactory DDAVP response and types 2 are not, but that's a misleading presumption.

Ian Peake of the UK reviewed the collaborative European study on type 1 VWD, as reported last summer. About a third of index cases (57/150) had a mutation in the VWF gene and had some abnormality in multimer structure by the sensitive tests of Ulrich Budde in Hamburg. Another rough third (51/150) had a mutation and normal multimer structure. Another rough third (41/150) had no VWF gene mutation and normal multimer structure. Results from the Canadian study of type 1 VWD were similar: a VWF gene mutation was found in 63% of index patients. In Canada, mutations were found in 75% of patients with a VWF:Ag level less than 30% and in 58% of patients with higher VWF:Ag levels. No mutation could be found in three index patients with VWF:Ag levels less than 20%, on repeated testing, a mystery.

So, after these fascinating studies of type 1 VWD, do we now have a crisp definition of type 1? Can we diagnose it securely? No, the dancer has not removed all her veils. Francesco Rodeghiero of Italy had the thankless task of trying to quantitate uncertainty. If we have this and that laboratory test and clinical finding, what's the probability of truly having type 1 VWD? I am glad there still is a role for the clinical hematologist to weigh all the information and come up with a diagnosis, or a probable or possible diagnosis of VWD, and a plan.

Dominique Meyer of Paris asks, is type 2 as frequent as type 1? If you look at the patients hard enough? French patients believed to have VWD or hemophilia have been studied with enviable thoroughness including universal genotyping. Among type 2 patients categorized in France, 33% have 2N !!! (perhaps thanks to the availability of the test and to investigation of patients previously supposed to have hemophilia A), 26% have type 2B, 20% type 2M, 15% type 2A, and 3% the Vicenza variant. (In contrast, in the USA, type 2A is the most common subtype diagnosed.) Type 2N was diagnosed in 118 French patients from 100 families, wow! (Have we been under-diagnosing it so much? I don't think it can merely be more prevalent in France.) Type 2B was found in 93 patients from 79 families. She warns that one cannot rely upon the VWF:Ag/ VWF:RCo ratio in type 2B patients as an indicator of type 2, because it is sometimes normal (as in type 1), depending on the mutation. I delight to hear the relationships of phenotypes to specific genotypes.

Christine Lee of London described the multicenter study that is asking, what is the best repertoire of tests for VWD? Aliquots of plasmapheresis plasma from six different patients (with type 2 subtypes) and two normal controls were sent, blinded, to 30 experienced laboratories. Test recommendations have not yet been made, but comments on individual tests were offered. Sensitivity of tests for VWF:Ag is generally good, but, most laboratories do not read low levels of VWF:RCo, the most common cut-off point being 10%. Ratios of VWF:Ag to VWF:RCo are highly unreliable if these levels are low, so, you can't reliably distinguish type 1 versus type 2 with these tests if the VWF deficiency is moderately severe. VWF:CB (collagen binding) tests have lower cut-off points than do VWF:RCo tests. The major variable in CB is the source of collagen. Equine or bovine type I collagen derived from tendons is the best reagent and human type III collagen the worst. Kits to perform CB tests may include any type of collagen, even the worst, so, read the label, or ask. In multimer tests,

it's very easy to miss type 2M. Multimer tests using radioisotope labelling methods are more reliable, alas, than methods using the environmentally-desirable chemi-luminescence or enzymatic labelling methods. VWF:FVIII B, the test for factor VIII binding, was performed adequately in 80% of labs. The test suffers from the lack of a plasma standard or abnormal controls. In general, laboratories that performed all five of the above tests did better at differential diagnosis than those lacking one or more assays, which is no surprise. It was difficult to distinguish type 2A from 2B without RIPA (ristocetin induced platelet aggregation), a useful but unpopular test because it's done on fresh platelet-rich plasma. Bob Montgomery of Milwaukee mimics RIPA with a radio-labelled test for VWF binding to platelets, using thawed platelet-poor patient plasma. A man from Argentina (I didn't get his name) said that he mixes platelet-poor plasma from patients suspected of having 2B VWD with platelet-rich normal plasma, to perform RIPA, and is able to diagnose 2B. I'll bet he's right. It would be easy to test his suggestion. Or, add normal fixed platelets to the subject's platelet-poor plasma to perform RIPA.

Among the pre-analytical variables affecting VWF test results is sample integrity. Emmanuel Favaloro of Australia finds that HMW multimers can be lost when blood is left at 4° C for a few hours before centrifugation. We've all been trained to put blood samples on ice to wait for centrifugation. We may have been wrong. HMW multimers survive better if whole blood is left at room temperature when there will be a short delay before centrifugation. Some subjects are more, some less sensitive to loss of HMW multimers when processing is delayed. Prompt centrifugation and freezing are desirable, of course. Bob Montgomery warns that similar loss of HMW multimers may be seen if plasma is thawed at room temperature instead of at 37° C. Nobody has tested whether refrigerated centrifugation is helpful or harmful.

As I listened to these reports on VWF lab tests, and reports on FVIII assays from WFH, I thought, it's all important. It's important how the sample is obtained, how it's processed, how it's assayed, against what reference and so on. Highly-experienced labs have some problems. Casual labs can be expected to have a lot more problems. Faced with a patient who might have VWD, what is the ordinary clinician to do? It's cost-effective to do all the tests available right at the outset, given that clear-cut results are not always obtained. It's cost-effective to use the best laboratory available, and to solicit the attention of an expert at that laboratory. From Quest Diagnostics, in southern California, one can request a "Von Willebrand comprehensive panel" which includes multimers, VWF:Ag, VWF: activity (a test substituted for ristocetin cofactor), VWF:CB and factor VIII. One can specifically ask for the comments of Mervyn Sahud, who was once a Fellow with me under the tutelage of the late Paul Aggeler. Tell him, or the expert of your choice, something of the patient's clinical presentation, and his race and ABO blood group (which affect FVIII and VWF levels.) An array of tests, including VWF:FVIII B, also is done by the Mayo Clinic laboratories (William Nichols). There's also the Blood Center of Southeastern Wisconsin (Bob Montgomery). VWD genotypes are available from the molecular genetics laboratory of the City of Hope, Duarte, California (Steve Sommer). Genotypes have been proposed to elucidate suspected type 2 VWD. But, it's not always easy to know whether a given patient might have a type 2 variant. I'd like to get genotypes whenever VWD is suspected.

The propeptide of VWF is split off after the molecule enters the circulation. The amount of circulating propeptide is normally similar to

that of VWF:Ag. Excess circulating propeptide suggest excess breakdown of VWF, as with circulating antibodies to VWF or with VWF that's overly vulnerable to proteolysis as in type IC or Vicenza variants.

Rare bleeding disorders were discussed at the Factor VIII and IX Subcommittee. Tony Waegemans reported on the pharmacokinetics of an LFB fibrinogen concentrate in five afibrinogenemic patients; the T1/2 of the activity was 81.5 hours and that of the antigen was 91.6 hours. Uri Seligsohn of Israel has been studying Glanzmann's thrombasthenia and its mutations. The highest prevalence, one in 81,000, is in Jordan, and it's almost as high, one in 143,000, in Israel/Palestinian territories. It's also fairly common in Iran and Tunisia, and less common in Europe. He has identified seven founder mutations, one with an estimated age of 750 years and another of 350-600 years. That's not long in evolutionary terms, but, might heterozygosity convey an advantage? Perhaps in later life, might it protect against thrombosis? If you're a heterozygote, do you also need aspirin to protect against thrombosis?

At WFH, Bruce Evatt, retired now, talked about **hemophilia centers**. In developing countries, the first few steps towards organizing hemophilia care with the establishment of centers, diagnosis of patients and use of local plasma products or small amounts of imported concentrate has a big effect on the well-being of patients and generates a lot of enthusiasm. But it isn't easy to sustain effective programs. In developed countries, "only constant pressure prevents reduction in services". Effective hemophilia programs have been shown to reduce clinic visits and hospitalizations and to reduce life-threatening bleeding and to reduce crippling. Success has led to complacency on the part of patients and their organizations. Hemophilia centers have low visibility in their host institutions.

We see fewer patients in person, but spend proportionately more time on phone calls, e-mail and paper-work. Dr. Evatt's solution for the survival of hemophilia centers is to include thrombophilia and women with bleeding disorders. In the USA, sickle cell disease, which requires intravenous therapy, fairly frequent clinic visits and fairly frequent hospitalization, is often appended to hemophilia clinics. But none of these conditions pay well, and increased volume of low-paying conditions isn't enough.

I've been in hematology over 40 years now and I don't remember a time when the survival of hematology, as an individual specialty, wasn't marginal. Those going into it do so out of passion for the field. Increasingly, they survive by practicing oncology part of the time, sometimes most of the time.

I didn't know whether to laugh or cry, when, during the SSC meeting, one after another multi-center survey or study was proposed. Participating in such collaborative efforts can be interesting, but, the effort may not pay off in ways that support the hematologist or his center. He has to satisfy the administration of his institution (by bringing in surplus "overhead" money through grants, by gaining newsworthy

attention) and his academic division (by publishing, as a named author.) Multi-center efforts usually are not funded, or barely funded. They rely heavily on volunteer efforts from already-busy people.

Sometimes I dream, what if I had Bill Gates' resources and could devote some of them to the field I love? I agree with Don Feinstein, who, at his recent retirement party in Los Angeles, said he'd like to reduce tuition at medical schools so graduates don't feel forced into high-paying specialties or into pharmaceutical company positions to pay off educational debts. I'd like to establish many more endowed academic positions for non-malignant hematology. And I'd like to endow major hemophilia centers (or centers of non-malignant hematology) with adequate funding for lots of physiotherapy (the most-neglected highly-effective therapy for hemophilia), for very good coagulation laboratories, for genetic counseling and testing, for training interested young doctors, and for someone to co-operate in all those SSC, etc, surveys and collaborations that are truly useful but hard to get done.

I have lived in interesting times. I had wonderful mentors when I was young, who helped me pursue my interests: Paul Aggeler, Ralph Wallerstein, Silvija Hoag, Judith Pool in San Francisco, and Samuel Rapaport in Los Angeles. I am glad to have started treating hemophilia in the early 1960's, with whole plasma, so that I appreciated cryoprecipitate and concentrates and DDAVP when they came along. In the 1960's, there wasn't anything we could do for inhibitor patients. I first used a prothrombin complex for a woman with an acquired inhibitor in 1971. She was bleeding externally from every venepuncture site, as well as internally, and I didn't think she'd survive. The infusion of the (activated) prothrombin complex stopped her bleeding like turning off a faucet. Nowadays, elective surgery is performed on patients with inhibitors using modern bypassing agents, especially NovoSeven®. I hear that hemostasis is not always optimal but usually adequate (I have no personal experience). Elective surgery is a great achievement nonetheless, and progress is bound to be made. Many years ago, inhibitor patients might die because surgery was deemed impossible.

The future promises to be exciting. I believe that gene therapy for hemophilia will be achieved in humans soon. It won't be perfect, and there will be complications. Everything has complications! Patients want it desperately, so they'll have to learn the pros and cons from experience. I am fascinated by the identification of cytokine genes that affect bleeding disorders. I'll have to learn more about cytokines to halfway understand the latest revelations. And there is so much more to study! I hope that genotyping will become the standard of practice in the USA as it is in several European countries, most notably France, not only for patients, so we may understand them better, but for involved relatives to enable informed family planning.

The Bulletin has been fun, it has focused my mind. I'm grateful for the interest and moral support of many people. I'll still be around, still sentient for a while, ready to be delighted at the next discovery!

