TRANSCRIPT

ACAAI Symposium

A Closer Look at Hereditary Angioedema: Expert Perspective on Optimal Management

November 10, 2009

Slide 1: A Closer Look at HAE

Dr. Bryan Martin:

Good morning, everyone. I'm Bryan Martin, I'm your moderator today. I'm a professor of clinical medicine and director of the allergy section in the allergy-immunology fellowship at The Ohio State University.

It's my pleasure to welcome you to this continuing medical education symposium, *A Closer Look at Hereditary Angioedema: Expert Perspective on Optimal Management.*

I would like to thank the sponsors of this symposium, The American College of Allergy, Asthma, and Immunology, and Robert Michael Educational Institute LLC. I'd also like to thank ViroPharma Incorporated for providing an educational grant for this program.

I'd like to discuss a little bit about how the program will go today. It will consist of four segments. First, Dr. Bruce Zuraw will discuss the pathophysiology of hereditary angioedema, or HAE. Next, Dr. Michael Frank will discuss the role of human and recombinant C1 inhibitors in treating HAE. Then, Dr. Allen Kaplan will discuss kallikrein inhibitors and bradykinin type II receptor antagonists and alternative treatments for HAE. Lastly, we'll take your questions.

Look to your workbook for the learning objectives for this program and for the full disclosure information for your speakers.

In order to receive CME credit for the program, you must complete the activity evaluation form.

Slide 2: HAE Pathogenesis and Mechanisms

Now we'll move on. At this time, Dr. Bruce Zuraw will begin the first presentation, *The Pathophysiology of Hereditary Angioedema*. Dr. Zuraw is a professor of medicine, chief of the section of allergy and immunology, and the allergy/immunology training program director at the University of California School of Medicine in San Diego, California. Please welcome Dr. Zuraw.

Dr. Bruce Zuraw:

Thank you, and thank you for attending. This is obviously the hard core audience still here on Tuesday—and those are my two daughters on the other side of Florida, so as close as I could come to a picture of Miami.

Slide 3: Disclosure of Conflicts of Interest

And let me just say by way of disclosures that I've had relationships with all of the companies that have been working in the area of hereditary angioedema.

Slide 4: Overview

And with that out of the way, I'd like to mention my learning objectives. Basically, I'm going to cover four areas. First, the discovery of C1 inhibitor deficiency in hereditary angioedema. Second, the identification of the mediator of swelling that causes the angioedema. Third, the mechanism by which patients with hereditary angioedema may become C1 inhibitor deficient. And finally, a few words about the vascular permeability defect in HAE.

So this is really a symposium about the treatment of HAE. And I'm, in essence, the warm-up act to basically give you the underlying information that will allow you to better incorporate the information you'll hear in the subsequent two talks.

Slide 5: Discovery of the Fundamental Defect

So, let's start with the discovery of the fundamental defect in HAE.

Slide 6: Hereditary Angioedema: Osler

While angioedema was recognized since the 16th century, and Quincke in 1882 very nicely described angioedema, it was Sir William Osler in 1888 who really first identified the salient features of hereditary angioedema—specifically, the fact that this was an autosomal dominant disease. It was a disease that was passed down in families with a relatively high penetrance. From that point in 1888, until 1962, there was almost no real progress made in understanding this disease.

Slide 7: Deficiency of Kallikrein Inhibition

And in 1962, Landerman and his coworkers published this paper in the *Journal of Allergy*, identifying that patients with hereditary angioedema had a deficiency of an inhibitor for serum globulin permeability factor and/or plasma kallikrein. Unfortunately, while they identified the absence of this activity, they never identified the protein. So this observation really didn't get the attention that it deserved.

Slide 8: Deficiency of C1-Esterase Inhibition

A year later, however, Virginia Donaldson made what was the seminal observation, which is that the sera of patients with hereditary angioedema lacked a protein, which was called C1 inhibitor.

And that name basically stuck with the protein, and it really focused a lot of attention in the field on complement proteins. I'll discuss the implications of that in a couple of minutes.

Slide 9: C1 Inhibitor

But this is the molecule, this is a picture of the molecule that Virginia Donaldson discovered was relatively lacking in HAE. And this is just two views of the molecule, rotated 90 degrees. And if I could just point out a couple of features—one is this loop up here, which is called a reactive mobile loop, and it actually is very mobile. It waves around in the fluid, and it's where the proteases attack it and cleave it. Once it's cleaved, this mobile loop with the protease gets transferred down into this beta sheath down here and gets buried within the molecule, forming, in essence, a covalent bond between one arm of the reactive mobile loop and the protease.

Slide 10: HAE: Two Genetic Variants

Now, a couple of years after Virginia Donaldson's observation, Fred Rosen in Boston then extended this by showing that patients—that there was a variant form of hereditary angioedema that was characterized actually by normal antigenic levels of C1 inhibitor, shown in this agar electric precipitation plate.

Slide 11: Types of HAE

Then basically this is our modern understanding of the types of hereditary angioedema. The type that Virginia Donaldson initially described, type I, is characterized by low antigenic levels of C1 inhibitor and is thought to be about 85% of the affected patients. The type that Fred Rosen found, this variant or type II form, is relatively less common, about 15% of the patients. C1 inhibitor is normal, but the functional levels of C1 inhibitor are low. So, in the end, both forms are characterized by low levels of C1 inhibitor function. Now, more recently there's a so-called type III HAE, and you notice 85 plus 15 equals 100, so I didn't leave much room for type III. And to be honest with you, I don't think anybody in the world has any idea how common or rare type III HAE is, and I'm not really going to discuss today, beyond telling you that it's characterized by normal C1 inhibitor antigen and function.

Slide 12: Identifying the Mediator of Swelling

So let's move on then, I'll talk about what is the mediator of swelling in HAE.

Slide 13: C1-INH in Proteolytic Pathways

And if we consider which proteases in blood are inhibited by C1 inhibitor, I show that in this figure with these reddish circles—and you can see there's a lot of red circles in these interrelated proteolytic systems.

Slide 14: Function of C1-INH

But fairly early on attention was focused on two different systems. One was the contact system by which high-molecular-weight kininogen is cleaved through the activation of coagulation factor XII and plasma kallikrein to active plasma kallikrein. The kallikrein then cleaves high-molecular-weight kininogen and generates bradykinin. Alternatively, complement component C1, when it's activated, then will activate C4 and C2, and C2, with help from plasmin, was thought to generate a peptide that was called C2 kinin. And these became the two leading mediators.

Slide 15: HAE and C2-Kinin

And in fact, there was direct evidence, as shown in this article in *J Exp Med* [*Journal of Experimental* Medicine] from 1988, that C2 kinin, at least peptides that match what C2 kinin was supposed to be, would increase vascular permeability. However, despite this, there's never been any evidence that sufficient amounts of C2 kinin are generated *in vivo*, and nobody's been able to really show that this truly has relevance to hereditary angioedema.

Slide 16: Activation of Contact System

In contrast, a lot of evidence has accumulated that shows that bradykinin *is* a mediator in HAE, and basically, it is almost certainly *the* mediator of swelling in hereditary angioedema. And the number of *in vitro* and *ex vivo* studies, some of which are summarized here, showed that plasma kallikrein was activated in HAE and that bradykinin was generated.

Slide 17: Activation of Contact System

Furthermore, when we started looking at plasma, patients with attacks of angioedema, we could show that during an attack, high-molecular-weight kininogen was dramatically cleaved and that it started to recover as the attack recovered, and that this happened in every patient we looked at. It was also accompanied by cleavage of C1 inhibitor, which plasma kallikrein was very efficient in being able to do, which would further reduce C1 inhibitor function.

Slide 18: Increased Vascular Permeability

And then an experiment that I find particularly persuasive was done in Al Davis's group, a paper published by Shoemaker et al. They started with an old observation that if you take HAE plasma and incubate it *ex vivo* at 37°C, you generate a factor that increases vascular permeability. Okay.

Their important contribution is they could get the same activity if they used *normal* plasma and depleted it of C1 inhibitor with an antibody. What this allowed them to do is experimentally address the question about the mediator. So they then took C2-deficient plasma, depleted the C1 inhibitor, and if C2 kinin were the cause of swelling in HAE, you should have ablated the vascular permeability factor. But in fact, they still found it. In contrast, when they used high-molecular-weight kininogen–deficient plasma, which contains bradykinin, and now if you lacked the bradykinin moiety and depleted the C1 inhibitor, you got no change in permeability. Then they further showed that this permeability-enhancing factor could be blocked by treatment either with enzymes that destroy bradykinin, or a bradykinin-receptor antagonist. So this was really very directly addressing the question of was bradykinin the mediator.

Slide 19: *In Vivo* Generation of Kinins

Furthermore, after this, we finally got antibodies that could recognize bradykinin with sufficient avidity that we could develop radioimmunoassays. Nussberger et al. showed that if they drew blood from the site of swelling and compared it to blood drawn simultaneously from, let's say, the contralateral arm, that the bradykinin levels were always elevated at the site—close to the site of swelling, compared to contralateral.

Slide 20: C1-INH Null Mice

Then the final bit of evidence, before what you're going to hear in the last talk today, again came from Al Davis's group, where they used a C1 inhibitor knockout mouse. So this is now a mouse that has no C1 inhibitor. If they injected this mouse with a blue dye, the blue dye leaked out because of a persistent vascular permeability defect, and that's shown here in the foot paw. And if they gave this mouse exogenous C1 inhibitor, you can see that the bluing went away, when it was normal. But the key experiment is shown here. If they crossed this mouse and made a double knockout so it was deficient in C1 inhibitor and it was also deficient in the bradykinin receptor, you again had no bluing. So it shows that the vascular permeability defect in HAE or in C1 inhibitor deficiency, is mediated by bradykinin.

Slide 21: Bradykinin Is the Mediator

So I would conclude, and I hope that you'll agree, that *in vitro*, *in vivo*, and animal data all show that bradykinin is the mediator of vascular permeability and is responsible, therefore, for the angioedema in patients with HAE.

Slide 22: Mechanism of C1-INH Deficiency

So, let me move on and talk for a couple of minutes about why do patients with HAE actually have the deficiency of C1 inhibitor.

Slide 23: Variable Synthesis and Secretion

And based on the cloning of C1 inhibitor that was accomplished by multiple groups in the mid-1980s, it became possible to play with the protein a little bit. This is just an experiment we did with transfected cells, where we pulse-labeled them and we could follow the newly synthesized C1 inhibitor protein. If we transfected normal C1 inhibitor, you can see that at the beginning it was within the cell, but then it disappeared from within the cell and appeared outside the cell. And if we looked at a type II mutant protein, a variant form of HAE, it looked very much like the normal protein. But if we looked at type I HAE, shown here, it persisted within the cell for much longer, and it never really appeared in any great extent outside of the cell.

Slide 24: C1-INH Mutations

And it became clear why this was happening—again, based on the ability to use molecular techniques. Now, this is a schematic of the C1 inhibitor gene with its eight exons, and—this is a little bit out of date, I could have many more arrows which show where the point mutations are. There are now more than 150 mutations that have been identified in patients with hereditary angioedema, and they're really scattered throughout the gene.

Slide 25: C1-INH

But I want you to particularly pay attention to the mutations right here in this sort of magenta-looking area because it turns out that the mutations right here are almost always, with only one exception, the ones that cause type II HAE, and all of the others outside this or in the other exons cause type I. And why is that true? Because the type II mutations involve the reactive mobile loop. So what we believe is going on is, in type II, you have your mutation right around this area, the molecule is secreted, but it's no longer able to be cleaved by the protease, so it can't inhibit the protease.

On the other hand, type I mutations involve the structural part of this protein, and we believe that that leads to misfolding of the protein and the inability to secrete it out of the cell.

Slide 26: Free Energy Map of Protein Folding

Let's talk for a minute or two about folding. When we address folding, it's helpful to think in terms of thermal dynamic gradients. This type of gradient, shown by a funnel, would characterize the folding of a small protein, a protein of less than 100 amino acids, tends to fold easily and has a very smooth gradient that it can follow.

Slide 27: Free Energy Map of Protein Folding

On the other hand, a large protein like C1 inhibitor, which is also glycoprotein, which makes it even tougher for it to fold, is thought to have a number of peaks in this gradient, and which—as

the protein travels down this gradient, it can get trapped behind these peaks, and it can't come out as a normally folded protein. So that—it's actually pretty tricky for proteins to fold, and if they're mutant, it makes it that much more difficult.

Slide 28: Quality Control of Glycoproteins

So, in fact, the cell has evolved very sophisticated methods to help proteins fold, and it's based on the fact that a protein like C1 inhibitor, which, as I said, is a glycoprotein, that it controls the removal of glucoses and mannoses to shuttle it around the endoplasmic reticulum and make sure that it's going to the right spot so that when a newly formed protein enters the endoplasmic reticulum, these sugars are added or subtracted. What happens is that, if it folds correctly, it's exported into the secretory pathway and then out of the cell.

On the other hand, if it's not folded properly, the sugars are put back on and then taken off, and eventually you get the mannoses removed, and then it's targeted for cytosolic degradation through retrotranslocation, out of the ER to the proteasome and where it's thought to be destroyed. In some genetic diseases, it forms polymers at this point, and that causes its own set of problems, like an alpha-1 antitrypsin deficiency.

Slide 29: Mutant C1-INH Forms

Just to show you that this is relevant in HAE, we measured the amount of complexes within the cell, based on wild- or normal-type C1 inhibitor, type II mutant C1 inhibitor, or type I, and sure enough, we found that the type I proteins in fact accumulated within the cell, in complexes with endoplasmic reticulum chaperones.

Slide 30: How Bradykinin Induces Angioedema

So, let me basically finish now with a few slides about how bradykinin actually does increase vascular permeability.

Slide 31: Role of VE Cadherin

It appears that the key molecule for controlling vascular permeability is VE-cadherin. VE stands for vascular endothelial, and cadherins are proteins that form both homodimeric bonds as well as heterodimeric bonds. So this would be one cell, this would be another cell, and in its fully assembled form, the fact that these cadherin molecules are associating with each other from one cell and across cells, forms a very tight network that's responsible, in large part, for the ability to prevent the movement of fluids from one side of an endothelial layer to the other side of an endothelial layer.

Slide 32: How Bradykinin Causes Angioedema

What happens if you have a mediator such as bradykinin? Bradykinin activates phospholipases. It's going to generate calcium flux inside the cell, in DAG [diacylglycerol]. This will activate PKC [protein kinase C] and calcium-dependent calmodulin. You're going to phosphorylate beta-catenin that's attached to VE-cadherin. And the end result of this is going to be increased vascular permeability as you lose this VE-cadherin, because when it's phosphorylated, it's basically internalized and destroyed. On the other side of it, the PKC and calmodulin are going to activate myosin light-chain kinase. You're going to get contraction of the actin myosin cytoskeleton, and you'll get cell contraction.

Slide 33: Bradykinin's Effect on Permeability

I show this now in a prettier form on the next slide. So in this case, VE-cadherin is stained in green—and this is in a non-stimulated cell. If you hit the cell with a mediator that acts like bradykinin, this is what happens to the VE-cadherin. You no longer have it outlining the cells. It's largely destroyed and down-regulated, so you've lost the glue that holds these cells together. At the same time, the actin stress fibers, which are hardly seen at all in the non-stimulated cells, become these thick bundles of contracting fibers. So the cell both contracts, the glue around it disappears, and fluid can leak through between the endothelial cells.

Slide 34: HAE and Regulation of Bradykinin

My last slide shows a couple of other factors that I think may be involved in the severity, let's say, of HAE and how some of the drugs work.

So if this is high-molecular-weight kininogen up here, and this is kallikrein acting on it—that will liberate bradykinin. Bradykinin, which is shown here, is destroyed by a series of enzymes called kininases, including angiotensin-converting enzyme or ACE—that you can see here. One of the kininases, carboxypeptidase, removes the carboxy terminal arginine and, as shown here, bradykinin acts on the B2 receptor. If you take away the carboxy terminal arginine, you get des-Arg bradykinin, which acts on a different bradykinin receptor called the B1 receptor.

So we showed, at least in some patients early on, that a polymorphism of the B2 bradykinin receptor appeared to correlate with severity. There's some very recent data suggesting that the B1 receptor may in fact be involved, that this des-Arg bradykinin could affect it, so there's a lot of room for regulation here.

The last bit of data is from France, and it's particularly interesting because it addresses— how do androgens work? That's not really my direct topic, but let me just say a word about this. What these authors showed is if you look at HAE patients not on androgens, this is their C1 inhibitor functional levels. If you put them on androgens, it goes up a little bit, but not that dramatically, but they got better clinically. On the other hand, if you look at the effect of androgens on

aminopeptidase P, which is this kininase here, it has a much more dramatic effect, suggesting that maybe some of the drugs that we have used for this disease are not acting on C1 inhibitor itself, but could be acting on these kininases, which in the end is to say that there may be lots of possibilities for how we approach this disease, novel ways of treating it that we haven't even found yet, and I hope that the future is bright.

Slide 35: Thank You

I thank you for your attention.

Dr. Bryan Martin:

Thank you, Dr. Zuraw.

For those of you who have questions, we'll be happy to answer them after all of the faculty have presented.

Slide 36: Recombinant and Purified C1-INH

So now we'll move on to a review of hereditary angioedema treatments: human and recombinant C1 inhibitors, presented by Dr. Michael Frank. Dr. Frank is the Samuel L. Katz Professor of pediatrics, medicine, and immunology at Duke University School of Medicine in Durham, North Carolina.

Dr. Michael Frank:

Thank you. I want to thank the Robert Michael group and ViroPharma for inviting me.

This is a great day for me, because when we wrote a paper saying that we thought that C1 inhibitor ended attacks of hereditary angioedema and it came out in 1980 in the *New England Journal*—I'll talk about it in a minute—that was 29 years ago. And we've waited patiently for a very long time to see the drugs that are becoming available for treatment of this disease to become available, and the patient group has waited very patiently for a very long time.

Slide 37: Disclosure of Conflicts of Interest

But like Dr. Zuraw, there are five companies developing therapies for hereditary angioedema. I've consulted for all five companies. For one of the companies, Dyax, I chaired the Data and Safety Monitoring Board.

So the objectives of this group, and this is not the one that's in your book, the objectives are to review therapy, and I'm going to say a word about current therapy because I think the way the forum is set up, we haven't spent some time on current therapy. You heard something about androgens, and I want to say just a word about current therapy. I want to review the earlier data

saying that C1 inhibitor is effective— I'm sorry—that C1 inhibitor is effective in prophylaxis of this disease as well as in treatment of acute attacks. I want to review the FDA-approved preparations of C1 inhibitor that have come on the market over the last year and more recently, and I want to discuss the preparation that's currently awaiting FDA decision.

Slide 38: Significance of Serum C1-INH Levels

Now I wanted to start with a point that will help explain why all of the studies that are being done do not show 100% effectiveness of C1 inhibitor.

First of all, what you see from this slide is, we took a group of patients many years ago and divided them into patients with serious disease, just on the basis of their physician's impression, and mild disease, and we looked at their C1 inhibitor level. The targets are patients with type II hereditary angioedema.

And what we found was that there is *no* relationship between the level of C1 inhibitor and the extent of disease. There are people who have this disease, as I'll mention in a minute, that are born with this. The abnormality is present in their cord blood and they have their first attack at 70. So there are factors that take place in hereditary angioedema that we do not understand, and there are several factors, like stress, that make a big difference.

Slide 39: Functions of C1-INH

Now you just heard from Dr. Zuraw that C1 inhibitor is surely responsible for the development of this disease, in that the patients *always*, except for type III, but types I and II patients always have an abnormality of C1 inhibitor. As I said, the *level* of C1 inhibitor doesn't seem to be responsible for determining the extent of disease. And C1 inhibitor affects Hageman factor and its fragments. It affects the clotting system, the kinin-generating system and the fibrinolytic system. So it's a general inhibitor of plasma systems, and it appears that the kinin-generating system is responsible for the attacks of hereditary angioedema, as we just heard.

Slide 40: Long-Term Prophylaxis for HAE

Now long ago it was noted by the Swedish that epsilon aminocaproic acid, a fibrinolysis inhibitor, affects the attack frequency in hereditary angioedema—and this was a totally serendipitous finding. A group of surgeons working with epsilon aminocaproic acid decided to basically treat every patient that came through their clinic with epsilon aminocaproic acid, and they wrote a paper saying we've treated thousands of patients with epsilon aminocaproic acid, just to see what it would do. Now, this is in an age where IRBs [institutional review boards] were not quite as dominant as they are now, and what they found was a few patients with hereditary angioedema seemed to get better. There was a follow-up study that said that hereditary angioedema patients get better, and so that was the first drug that came long. And the Europeans have been using a drug which is a cyclized version of epsilon aminocaproic acid, which looks

like glysine, and this cyclized version also is effective but also is not available in its oral form in the United States.

Slide 41: Long-Term Prophylaxis for HAE

We introduced the use of danazol years ago for the treatment of hereditary angioedema, and danazol has made an enormous difference in the lives of these patients.

Slide 42: Side Effects of Danazol Therapy

But, in fact, it is not completely effective. It is prophylaxis and comes with all of the side effects of androgen therapy. So, most of the patients develop weight gain. And if you look down this list, which I'm not going to spend time on today, it's not the subject of our discussion, you'll see that there's a long list of side effects of androgens that we've all become familiar with, as athletes have taken androgens over the years.

In general, the side effects are mild, and most of the patients faced with having attacks of a life-threatening disease and severe abdominal pain attacks are very happy to take the androgens. But some people can't—and, in fact, some people have side effects that are overwhelming.

Slide 43: An Inherited Disease

Now as you heard, this is an inherited disease, and it doesn't skip generations. It's equal in males and females—turns out to be more serious usually in females, and estrogens make this disease worse. So, in fact, we've really needed something more for the treatment of these patients.

Slide 44: Why We Need New Therapies

We found that impeded androgens are not clinically effective for 48 hours. So if a patient comes into the emergency room with a hereditary angioedema attack, you've got a major problem on your hands.

There are no IM or IV preparations. All the androgens that work are methylated products, which must be given orally. When patients have abdominal attacks of HAE, they're having swelling of the viscera, they're having swelling of their GI tract, and the ability to absorb these kinds of materials is extremely limited. Testosterone itself, which of course can be given as a patch, does not work in hereditary angioedema.

Now, we can't use androgens in children because of the danger of epiphyseal closure, and we can't use them in pregnant women. More than that, my problem has always been that I couldn't use them in women who wanted to become pregnant because you never knew exactly when the pregnancy would start, and it was in the early weeks of pregnancy that they might be the biggest problem. As I mentioned, they have many side effects, which although usually mild, may

preclude their use. The androgens are ineffective in some people, and that also is a problem in a limited number of people, but it's clearly there.

The plasmin inhibitors like EACA [epsilon aminocaproic acid], for unknown reasons, also do not show any effect for 48 hours. They're inconvenient to take. And toxic side effects, which I haven't got time to get into in detail, are really common. They're more difficult to use than the androgens.

Fresh frozen plasma, although widely used, may prove dangerous in some patients.

Now, if you think about it, the original idea of Henry Gewurz and Bob Good was that you're replacing the C1 inhibitor that these patients are deficient in, and therefore you'll end the attack, and that usually happens. But you're also replacing the depleted bradykinin systems, you're replacing high-molecular-weight kininogen and kallikrein and prekallikrein. And what happens is that, in some patients, rarely, those agents cause more bradykinin formation before you get the effect of C1 inhibitor, and we have all seen patients get worse. I know of a patient that had to have a trach fairly recently, when somebody used fresh frozen plasma.

So, the conclusion is that in the United States, treatment is substandard. Why is it substandard in the United States? And that is that our European neighbors have developed therapies that are really more effective.

Slide 45: New Therapies for Acute HAE

Now we have new therapies developing for hereditary angioedema. We have the C1 inhibitor therapies, which I'm going to talk about. Lev, which is now owned by ViroPharma, has a product called Cinryze™. CSL Behring has a product called Berinert®. The FDA approved Cinryze in October 2008 for prophylaxis, and the FDA approved Berinert—it was called Berinert P, now Berinert, just last month for acute therapy. Finally, there's a C1 recombinant preparation, made by Pharming, called Rhucin®.

So, we have three different preparations of C1 inhibitor, as well as some additional preparations made by other companies, which you're going to hear more about from Dr. Kaplan, and all of these medications are looking to be effective. So we really do have a different approach to therapy.

Slide 46: Emerging Therapies for HAE

I'm not going to spend much time on this slide because you just heard a lot from Dr. Zuraw on the mechanism of hereditary angioedema. But you'll see that C1 inhibitor, which we're talking about here, has an effect on factor XIIa, it has an effect on kallikrein, and therefore has an effect on several of the steps that lead to hereditary angioedema attacks.

And as you've heard from Dr. Zuraw, there are other things going on—for example, degradation—that we don't totally understand.

Slide 47: Early Isolation of Plasma C1-INH

Now, as you also heard from Dr. Zuraw, in 1963, Virginia Donaldson described a deficiency of C1 inhibitor in hereditary angioedema patients. It was an important observation. At that time, three different groups set out to purify C1 inhibitor from plasma. The Dutch Red Cross set out to purify it, and that ultimately is the ViroPharma product that we're dealing with now. Behring Pharmaceuticals at that time also set out to purify this. And again, we're going back into the '60s and '70s. And the third group that set out to purify it was The American Red Cross. All of these groups were groups that were preparing IVIG [intravenous immunoglobulin] for preparation and therefore were getting the blood from thousands of donors and had plants set up to separate the various blood products.

Slide 48: What the Studies Have in Common

Now, in the new therapies, all these therapies have certain things in common in the studies that are being done now. They're all placebo-controlled trials with each subject receiving either drug or placebo once. And the prophylaxis trial is a crossover study, and I'll talk about it.

All have a preliminary screening visit in which the diagnosis is confirmed, the patients are found to have low C1 inhibitor, antigenically or functionally, low C4, normal C1q.

All enroll individuals who are early in attacks. The entry criteria vary. Most of the studies have used patients within 4 to 6 hours of the start of an attack. One of the things that you'll see is that we've had a little bit of variation in our success. And because this is a self-limited disease, these attacks usually get worse for about a day and a half and better for a day and a half. That means that if you get patients late in an attack and they're starting to get better, your placebo patients will start to improve, and it will look like your drug is effective.

All suggest that individuals maintain the medications which they've been on chronically. The dose of androgen isn't changed. All suggest that narcotic treatment is not acceptable or deemed a treatment failure. Again, there's been some variation from company to company in narcotic treatment. The type of attack acceptable for treatment protocol varies from study to study. Some have allowed peripheral edema attacks. Some do not. Some allow facial attacks. Some do not. For some studies, the FDA has allowed C1 inhibitor to be used as the rescue medication. There is a long experience with C1 inhibitor.

Slide 49: Early Plasma C1-INH Replacement

Now, we work with The American Red Cross product. They began to make purified experimental batches in 1974 from plasma. We reported in 1980, as you can see, the biochemical effect of the preparation that the Red Cross was making, in eight patients with hereditary

angioedema, and we reported the effectiveness of the preparation in treatment of HAE attacks in five patients.

Now, in 1980, the AIDS epidemic appeared, and suddenly The American Red Cross was not worried about hereditary angioedema. They were worried about HIV in the blood pool, and they stopped making purified C1 inhibitor. So they did not continue their studies.

Slide 50: Effect of C1-INH Infusion

But what we reported was data that looked like this, and we reported it in more patients. We showed that the C1 inhibitor level goes up strikingly, the closed circles, and comes down very quickly. So it's not like IVIG. We showed that the C4 level goes up after the C1 inhibitor level and also comes down fairly quickly, but later than the C1 inhibitor level. Two of the patients that we reported were having attacks at the time that we infused the C1 inhibitor. And in both patients the attacks ended, although in this patient down here, who was having a throat attack, a laryngeal attack, we had to give two infusions to end the attack.

So this was done, and we really thought that the problem was solved at that point.

Slide 51: Purified C1-INH

Well, it wasn't solved. So in 1996, we had an opportunity to use a preparation that was made by a company that no longer is in existence called Immuno. And with Fred Rosen and Tom Waytes we did a study in which we did a double-blind study, prophylaxis, in which we gave patients either drug or placebo in a blind fashion, and we looked at the level of C1 inhibitor and the level of C4. And what we showed was that, in fact, the C4 comes up slowly, as I said before, and can be brought into the normal range, and the C1 inhibitor has a relatively short half-life.

About 60% of these patients who were chosen because they had very serious disease got better and had no more attacks. In about 40% of patients, some attacks persisted, and that's true in all of the studies that are being done.

Slide 52: Length of Time to Response

The outpatient part of the study was done by the late Fred Rosen, who had worked hard on this disease. He had patients who had a variety of attacks, and he looked at the response in 30 minutes. Now, here are patients on C1 inhibitor and placebo. And as you can see, basically none of the patients on placebo started to get better in 30 minutes, although one patient may have started to have a little bit of a response.

On the contrary, even in 30 minutes, in his outpatient part of the study, some of the patients were getting better. In fact, a large number of the patients started to show some improvement. By 240 minutes, many of the patients on placebo were starting to get better, and most of the patients on C1 inhibitor were improving.

Slide 53: Pasteurized Plasma C1-INH

At the same time we started to do these studies, CSL Behring started to do studies, and their product is now called Berinert. It was first licensed as a non-pasteurized product in Germany in 1979—so that's a long time ago—and as a pasteurized product in 1985. Bork's first mention of C1 inhibitor to terminate an attack was in a case report in 1979, although he didn't give biochemical data at that time. So you see that people were getting the same idea at about the same time.

It's been approved either as a licensed product or for compassionate use in Europe since the early 1980s and approved for therapy of acute attacks in October 2009—so last month, it was approved for the treatment of acute attacks. It's been reported on extensively by our German colleagues, Konrad Bork and his colleagues.

Slide 54: Nanofiltered Plasma C1-INH

Now, the Lev product—Lev was a company in the United States that was purchased by ViroPharma, and their product is called Cinryze. It's a derivative of the Dutch Red Cross preparation, and they also made batches very early, as early as 1972. Agostoni—and Cicardi, who was at this meeting—reported that the preparation was effective in the treatment of hereditary angioedema in a case report in 1978 and in a longer report in 1980, again without biochemical data, but saying that the product was effective.

The manufacturing arm of the Dutch Red Cross merged into Sanquin in 2003. In 1989, heat treatment was added, and now there's a nanofiltration step to be sure that there are no viruses in the preparation.

In October 2008, it was approved by the FDA for prophylaxis at 1000 units biweekly.

Now, these are very unusual products. It is very rare for the FDA to be confronted with products that have been in constant use in Europe for 20 years with a safety profile—and there is a safety profile, and these are very safe agents.

Slide 55: Time to Relief: Pasteurized C1-INH

This is one of Bork's papers published in 2005. Now, they tended not to use androgens because they had C1 inhibitor. You can see, first of all, the number of attacks that he was able to find and to collate the data on. This is 1800 attacks of abdominal pain in this case, and, as you can see, in about 30 minutes, many of the patients were starting to respond. Some of their patients responded later, but by 180 minutes, virtually all of the patients had responded.

Slide 56: Trial Design: Nanofiltered C1-INH

Now, the ViroPharma placebo trial was approved a year ago. In fact, this was a trial in which drug or placebo was given for a 12-week period. Patients received either Cinryze or placebo. It was a simple crossover study in which those patients then went on—who were on Cinryze, went on to placebo. Patients on placebo went on to Cinryze. It was completely blind.

Slide 57: Normalized Number of Attacks

And this is the overall data of the trial. Here is the attack frequency in patients before they were on drug or on placebo, and this is the attack frequency on Cinryze, and you can see a bunch of things from this. The first is that the patients in general got strikingly better. The second thing you can see is they often didn't get 100% better, and we'll come back to that.

Here is a patient that got strikingly worse on drug as opposed to placebo.

Slide 58: Patient Event Charts

Now, this is a chart that they prepared, which I think is really very instructive. These little blue and red figures are when the patients were having attacks. This is the patient shown, patient by patient, on placebo, and the patient shown on drug. You can see that most of the red dots and blue dots are on the placebo side. Many of the patients got completely better, like this patient here and this patient here.

Some of the patients did not, and one patient, the one I showed you on the last slide, got strikingly worse. This is a patient who had many life events that suddenly were challenges. One of the interesting things about this disease is that stress makes this disease worse. As we've heard, ACE inhibitors and things of that sort—this patient had a number of life events that made her life miserable at the time she was on drug and got much worse. When the study was over, her life events sort of quieted down, she stayed on Cinryze and continued to be well.

Slide 59: Secondary Endpoints

This simply is the kind of endpoint showing things that favor Cinryze or placebo—basically, the attack frequency, the severity, the number of open label infusions, duration of attacks, days of swelling, all improved on drug, and that's over placebo, and that's why the material was approved for use.

Slide 60: Pasteurized C1-INH vs Placebo

Now, this is the CSL Behring study that was recently published, last month, in the *JACI*, and the basis for the FDA approving this drug for acute therapy only a few weeks ago. And if we look at the bottom part of this slide, which—this is a statistical manipulation of the data, but if you look

at the bottom part of the slide, you can see it perhaps more clearly. First, there is one difference between the CSL Behring study and the study of ViroPharma, in that it was a dose-response study. They tried 10 units per kilo and 100 units per kilo—I'm sorry, 10 units per kilo and *20* units per kilo. The ViroPharma study was 1000 units twice a week, and it could be repeated once a week in their study.

So what they showed was, first of all, this is the response in placebo patients. They got better, as all patients with hereditary angioedema do, over a period of time. Here is the response on 10 units per kilo. In fact, this was not statistically different from the placebo group, so 10 units per kilo was felt to be a failure. Here is 20 units per kilo. In fact, there was a striking difference in terms of response time between these patients and the placebo patients, and it was on this basis that the drug was recently approved.

In none of these studies has side effects been a major problem. And as I mentioned, in Europe, these drugs have been used for 20 years without infection and without much in the way of side effects, and that's really a very important point.

Slide 61: Mean and Median Time to Response

Now, in the Behring study, this is a compilation of one of their data, here is abdominal attacks and facial attacks. This is the mean and median time for response. As you can see, the median time for response, the middle point, patients responded to placebo in this period of hours and in a much shorter period of hours on C1 inhibitor. If you look at the means right above, it's almost 9 hours and 3.5 hours. This is 20 units per kilo in this column and 10 units per kilo and placebo.

If you look at facial attacks, again, big difference, with a really rapid response time at 20 units per kilo compared to placebo. Again, both moderate and severe attacks responded. Again, if you look at the severe attacks, big difference in the response time on 20 units per kilo and on placebo. And it is on the basis of these kinds of data that the drug was approved.

Slide 62: Recombinant Human C1-INH

Now, Pharming has the third product that is coming to the market. It is not approved yet, but it is being tested and reported to work. Pharming has a very clever product. The Pharming product is produced in rabbit milk. The human C1 inhibitor gene is introduced into rabbits under the regulatory control of a bovine casesin promoter. And with that kind of promoter control, it is secreted in the milk so that a pregnant animal can be milked and the human protein isolated from the milk. This is an amazing advance if you think about it. I can't help thinking about people sitting on really tiny little stools, trying to milk rabbits. But I think I'm wrong on that.

Slide 63: C1-INH and C4b/c Time Profiles

Here is some of the Pharming data. Here is the response on placebo and here is the response on the Pharming product. And again, the Pharming product is very, very—is effective, it is glycosolated differently from the normal human C1 inhibitor. You have to use 100 units per kilo

to have an effect. But again, it is reported in a limited number of people so far to be effective. And the data looks very similar to the data from the Behring study and from the ViroPharma study.

Slide 64: Theoretical Problems & Disadvantages

There's no question that C1 inhibitor is good treatment for hereditary angioedema.

Well, the plasma products have a risk of infection. The risk is minor, since no infections have been observed with the current products in 20 years. Both start with the blood of healthy donors and go through potent virus reduction purifications.

At present all of these products are given IV. This represents a disadvantage.

Since the patients are heterozygotes, allergy to the administered product, with the plasma product, is highly unlikely since it's the normal physiologic protein, and it's circulating in the patients. With the recombinant product, the glycosylation differs from the normal C1 inhibitor, and there might be some risk of allergy. I haven't seen it reported. The half-life is short, and I mentioned that. The advantage is it's not a serum product, and theoretically the supply is limitless.

I won't go into the kallikrein inhibitor and bradykinin products because Allen Kaplan is going to do that now. So, thank you very much for your attention. I do think these are wonderful days because the patients, for the first time, have a way of being treated with prophylaxis and acute therapy that speaks to their disease directly.

Thank you.

Dr. Bryan Martin:

Thank you, Dr. Frank.

Slide 65: Kallikrein & Bradykinin Inhibitors

Our final presenter, Dr. Allen Kaplan, will now discuss a review of hereditary angioedema treatments—kallikrein inhibitors and bradykinin type II receptor antagonists. Dr. Kaplan is a clinical professor of medicine at the Medical University of South Carolina in Charleston, South Carolina.

Dr. Allen Kaplan:

Thank you very much. I would like to thank ViroPharma and the Robert Michael Educational Institute for their support in this lecture and this program.

I'm going to focus on therapies that are not yet available in the United States, but one of them perhaps imminently so. These are geared towards targeting the bradykinin-forming cascade directly. One of them is an inhibitor of the enzyme kallikrein, and the other, an antagonist that is active at the bradykinin receptor.

Slide 66: Disclosures of Conflicts of Interest

My disclosure is shown here in terms of research, research support or pharmaceuticals for which I consult and also speakers' bureau.

Slide 67: Overview

And the objective of this talk then is to explain the theoretical advantages of inhibiting kallikrein or having the bradykinin receptor antagonist in the treatment of hereditary angioedema. We'll talk towards the end about clinical trial data for ecallantide, which is the kallikrein inhibitor, and icatibant, the B2 bradykinin receptor antagonist, as therapy for acute angioedema. And we'll perhaps contrast current therapy with new approaches that are on the horizon.

Slide 68: Factor XII-Dependent Bradykinin

I'm going to begin by talking a bit about the bradykinin cascade, but my focus then will be on the enzyme kallikrein and why it's important to try to inhibit it in this disease, as well as some words about bradykinin itself. This is a very simplified diagramatic representation of the plasma kinin-forming cascade. So you all know it begins with factor XII, and you see the little arrow that comes back on itself because the initiating step of factor XII activation is an auto-activation step. So that if you have even a trace of active enzyme present, that in the presence of an appropriate binding surface, it will gradually—and I want to emphasize gradually and slowly—activate more factor XII.

So, one question that you might ask, that we tend to just sort of cursory state and not think about, is, where is the surface and where does bradykinin generation occur? And I'm going to show some data to suggest that the natural surface in our body, where it may be occurring in hereditary angioedema, is in fact the surface of the endothelial cell. Once you activate the enzyme, we all know it initiates blood clotting, which goes through factor XI, but the important part for us is that it will convert pre-kallikrein to the active enzyme kallikrein, which will digest high-molecular-weight form of kininogen and give us the molecule that's causing the swelling in this disease.

So if you think of kallikrein then, one function of kallikrein, is to digest this substrate and make bradykinin. I think everybody knows that, and it's kind of obvious. However, it has a second function in this system, which is equally important, and that is, this surface activation of factor XII is really slow. So, make believe you had a circumstance in which you were discussing bradykinin activation, and you had activated 5% of it, which is sufficient to make a lot of kallikrein. But 5% would leave you with 95% of the factor XII, bound to the surface but not yet activated.

The second function of kallikrein is a feedback function in which it activates factor XII to factor XIIa, 50 times faster than this autocatalytic reaction that is shown here.

Slide 69: Kallikrein Activation of Factor XII

And I show that diagramatically then in this next slide, which kind of looks at the cascade backwards then. So here's the enzyme kallikrein, which is now rapidly activating factor XII to factor XIIa on the surface and then converting the kininogen to release bradykinin.

So when you inhibit kallikrein, you're not only inhibiting the enzyme that makes bradykinin, but you're really inhibiting the initiation of the entire cascade—because more than 90% of factor XII activation becomes kallikrein-dependent by this feedback kind of reverse scheme that I'm showing you here.

Slide 70: Activation of Endothelial Cells

This slide shows the functions of bradykinin in a pictorial way that Dr. Zuraw alluded to, that bradykinin will then bind to the B2 receptor, cause vasodilatation, increase vascular permeability, and cause angioedema. It's interesting that the bradykinin, when it interacts with this receptor, activates the endothelial cell. And you saw the sequence of mapkininases and myocin light-chain kinase that are activated, but it actually causes the release of two other vasoactive factors: nitric oxide and prostaglandin E2. It's possible that they have some augmentation effect on this, but it's clear that if you inhibited either one of them by itself, you would not affect this disease. You've got to block the bradykinin.

Also shown at the bottom is a mechanism by which the fibrinolytic enzyme plasmin is made. There's more than one. But it was alluded to early and epsilon aminocaproic acid is a prior treatment that was effective for prophylaxis. And the fact is that one of the ways you can get plasmin is by bradykinin stimulating the B2 receptor, the endothelial cell secretes tissue plasminogen activator, or TPA, and directly converts plasminogen to plasmin.

Slide 71: Function of C1-INH

This more complicated slide puts the cascade together and shows you all the places that C1 inhibitor inhibits, and it's like every enzyme of the pathway is inhibited, so I'll not go through it. It blocks factor XII, it blocks kallikrein. Here's the kallikrein giving you bradykinin.

But let's follow it down a little bit further. The enzyme kallikrein shown here has another function. Here is the feedback activation of the factor XII that I alluded to earlier. Say 5% autoactivation this way and the other 95%, the kallikrein you produced, activating the factor XII to factor XIIa. Once it does that, there is a second cleavage of the factor XIIa by the kallikrein, which forms a factor XII fragment, molecular weight 30,000 rather than 80,000, and that

fragment enzymatically activates the first component of complement, and it, of course, is inhibited by C1 inhibitor.

You all know that in hereditary angioedema, when the patient is stable, C4 levels are low, and we use it for diagnosis. That's because C1 in the absence of C1 inhibitor is unstable, and some of it is always active. And 95% of the time you can rely on a low C4 to help you diagnose the disease. When you have an attack of swelling, you make this fragment, which is dependent on kallikrein, it further enzymatically activates C1, the C4 level will drop to zero in almost everybody, and then you will digest the other substrate of activated C1, and that's why C2 levels become low during attacks of swelling.

Slide 72: Stained Endothelial Cells

The next point is, I alluded to earlier, and that is this cascade can be activated, is present, and can be activated on endothelial cell surfaces. So here, for example, is a preparation of endothelial cells in which we incubated them with factor XII— I'm sorry, we incubated the cells with normal plasma—and we looked to see if the factor XII is bound or the kininogen, high-molecular weight-kininogen is bound, using an antibody that was peroxidase labeled, which turns it kind of brown, which you can see here. So the factor XII is on the cell and so is the high-molecular-weight kininogen. And if you do the same thing with congenital deficient plasma, we have factor XII-deficient plasma, incubate the plasma with the cells, add the antibody, of course you find nothing because there's no factor XII and likewise no kininogen because it's congenitally absent, which shows you the specificity of the reaction.

Slide 73: Structure of Endothelial Cells

So, when you look then at the endothelial cells, on it is factor XII and kininogen. And the prekallikrein is attached to the kininogen—the prekallikrein does not bind directly. Both of them are bound, require an ion. Zinc ion happens to be it, and the amount of zinc needed happens to be exactly what we have circulating. There are receptors, complex receptors, that bind these factors, which are shown here, and I'll not go through. One is gC1qR, a cytokeratin 1 and a u-PAR. And we have two bimolecular complexes, so the u-PAR/cytokeratin 1 here binds factor XII, and the cytokeratin 1/gC1qR binds kininogen. So, when you activate factor XII on the surface, you convert the prekallikrein to kallikrein, and you see here the double effect of kallikrein, cleaving the kininogen to release bradykinin, which hits the receptor, and the kallikrein feedback activating the factor XII, which is the major activation mechanism in a quantitative sense.

Slide 74: Activation of Contact System

This slide was shown briefly by Dr. Zuraw. What John Curd showed here was that in these little blisters made in HAE patients, the amount of kallikrein, compared to making the same blisters in normal persons, was elevated about 50-fold so that in the absence of C1 inhibitor, this kind of trauma to the skin—which, by the way, activates kallikrein in all of us, too—but it's markedly accentuated in hereditary angioedema.

Slide 75: *Ex Vivo* Generation of Bradykinin

Likewise, if you look at it from the point of view of bradykinin, this is a slide of our data in 1983, where we were looking for the C2 kinin, the alternative kinin that Dr. Zuraw mentioned to you, which, at the time, was an alternative candidate for the swelling. You could look at it by permeability, but you could also look at it by contracting either a guinea pig ileum or a rat uterus. And the original C2 kinin paper did it by contractile activity, even before they looked for permeability. So, here was a contraction to two nanograms of bradykinin at the beginning and end of our assay, and all we did was take HAE plasma, put it in a tube, and did nothing to it. We just let it sit for time. The longer it sat, the more bradykinin was there, and when we looked for C2 kinin, we could not reproduce the original data, it was flat as a pancake, and we found nothing. In fact, this was one of the first papers that suggested that bradykinin was it and not the C2 kinin.

Slide 76: *In Vivo* Generation of Kinins

I show this again, although you saw it with a different point being made, that sure, bradykinin is elevated when you have an attack of HAE. But look at the difference between the arm that's swollen and the arm that isn't. That makes the point, I think, that it starts as a localized process, or at least is markedly accentuated in terms of bradykinin activation at the site of the swelling, even though you can pick it up virtually by monitoring any vein that's accessible in that particular individual.

Slide 77: Effect of C1-INH on Factor XIIa

I'll show you something that will be published that looks at these enzymes. We did this with Lev/ViroPharma, and although my topic is the kallikrein and bradykinin antagonists, this was infusing patients with the C1 inhibitor to replace it and looking at the enzymes in bradykinin to see what happens. You see a baseline for activated factor XII, which is much higher than is present in normal, telling you that the kinin system is activated even at baseline in these patients. I've never seen it normal in any HAE patient. When they have an episode of swelling, it goes up, and as you infuse the C1 inhibitor, it goes down.

Slide 78: C1-INH Effect on Plasma/Kallikrein

If you look at the two enzymes we've mentioned today—kallikrein, the more critical for the swelling, but also plasmin—they go up, they go down. Don't let this fool you. The plasmin looks more dramatic than the kallikrein, but when you have no C1 inhibitor, kallikrein binds to alpha-2 macroglobulin as an alternative, and the synthetic substrate that we use sees kallikrein bound to alpha-2 macroglobulin—it retains about 30% of its activity because alpha-2 macroglobulin binds it sterically but does not kill the active site. Nevertheless, the data was still clinically significant almost immediately after the infusion.

Slide 79: Effect of C1-INH on Bradykinin

And likewise, I'll only show the bradykinin curve—that's this one here, shown in purple, watching it go up and then come down as the attack is aborted.

Slide 80: Targeting the Kinin Cascade

So, there's multiple reasons—it should be perfectly obvious that if bradykinin is the mediator of this swelling, then if you block plasma kallikrein, you not only stop its cleavage of kininogen, but you stop it from activating factor XII and you really slow down the whole cascade. Likewise, it's self-evident, that if bradykinin is the mediator, if you get enough antagonist in there and block it, you would at least abort an attack, although you've done nothing regarding the production of that bradykinin.

Slide 81: Plasma Kallikrein Inhibitor

So we have the first drug, which is a plasma kallikrein inhibitor, ecallantide. It's quite potent, if you look at the KI inhibition constant for this. It's a Kunitz-type serine protease inhibitor. It has 60 amino acids. It is administered subcutaneously with a very short half-life. You could not use a molecule such as this for prophylaxis. But its half-life is long enough to inhibit kallikrein and potentially stop attacks of swelling. There are two phase III double-blind, placebo-controlled studies, EDEMA-3 and -4, for which I'll show you data in the next slide, and it was effective for cutaneous attacks or abdominal attacks or facial attacks. And the two studies showed good efficacy.

I should add that the FDA has been reviewing this, and the word is that within a month or so, they should be hearing a decision as to whether this will or will not be approved. So the result may be imminent.

Slide 82: Effect of Ecallantide on TOS

They used a score system that was called TOS, or Treatment Outcome Score, shown here. They took into consideration the site of the swelling, whether it was mild, moderate, or severe, then looked to see whether there was significant improvement, a little improvement, no improvement, worsening, significant worsening. These changes were given a score. Significant improvement got 100, then 50. If nothing much happened it was a zero, and if you got worse it was negative. So a positive number means it was successful. If you look at the two studies, in terms of the comparison of ecallantide with the placebo that was used, the difference in symptom score or Treatment Outcome Score was highly significant in both of the studies.

Slide 83: Ecallantide Safety

It is generally well tolerated. There were a number of mild events that were similar to placebo, which you see in almost any drugs, when you read the list of potential side effects. There was occasional GI upset, colds, cough, or pharyngitis—which is inconceivable that it was truly due to

it—headache, fatigue, and so forth. There's no known effects on activated PTT, and nobody had any bleeding. There were, however, occasional anaphylactic-like reactions in some patients. It was not clear why. Contaminating proteins in the vehicle by which it is produced has been considered. Drug antibodies have been sought. There is one patient who anaphylacted to it, who anaphylacted a second time with a repeat dose. On the other hand, the latest large trial, which I do not have here, had more patients than the previous, and there was not a single anaphylactic reaction. The incidence is probably so, but relatively low.

There was a precursor to this, a large protease, that was a kallikrein inhibitor called Trasylol®, which was briefly used and available, and virtually everybody anaphylacted to that on the second or third dose. So that took care of that. It was off the market mighty fast.

Slide 84: Icatibant

And the last one is icatibant. This is now a second-generation bradykinin-2 receptor antagonist. It resembles the bradykinin structure but has intercalated within it a number of synthesized amino acids, which limit any agonist effect, but make it a good high potent antagonist. It's again given subcutaneously, it has a short-life, and there are two double-blind, placebo-controlled studies called FAST-1 and -2, respectively.

FAST-1 did not reach clinical significance, which was done in the US, and it did not get through the FDA. FAST-2 had a very good result, comparable to the others that you've heard earlier today. It was done in Europe, they okayed it, and I believe it was August that it became available in Europe for treating acute attacks of swelling.

Slide 85: Efficacy of Icatibant in HAE

And this shows you those data, the data for it. In the US, here's the icatibant and the comparator in terms of an efficacy scale, where it did not reach clinical significance. On the other hand, FAST-2 in Europe, the comparator had little—by the way, the comparator was interesting in the Europe study, it was tranexamic acid, which is an antifibrinolytic agent that was used there for prophylaxis. It still is, particularly in children, where they wanted to avoid the use of androgen. But using that as their control in this study, the icatibant was highly significant in aborting attacks of swelling.

Dwelling for a minute on the FAST-1, which has to be repeated in the United States, we have the data on that—and Mark Riedl sent me this slide, in which he had broken down the data in terms of the site of swelling. And interestingly, this is placebo versus icatibant, it easily reached statistical significance in aborting peripheral angioedema. They used skin pain as a criteria, which I personally think is kind of odd, but it gave a borderline result. And it was not significant in the way they looked at abdominal pain. The curiosity was that the placebo did mighty good in, however they were measuring it, for the abdominal pain, and that led to a *P* value that was not significant.

Slide 86: Comparison of Emerging Therapies

And so the therapies that we have now are a variety of preparations of C1 inhibitor. Dr. Frank mentioned Cinryze, of course, was the first one approved here, and it's being used for prophylaxis. I have to update my slide on the Berinert preparation, which 3 weeks ago was indeed approved for acute, but not for prophylaxis. There is no approval—I don't even know if it's submitted yet—on the recombinant Rhucin that he mentioned from rabbits. But bear in mind, because of the abnormal glycosolation, it has such a short half-life, that it can only be used for acute treatment. I don't think it would be possible to use it for prophylaxis, any more than you could the kallikrein inhibitor or the bradykinin antagonist. So, with the kallikrein inhibitor ecallantide, one has to watch for potential allergic reactions. Other side effects were really minimal, and, I think, negligible. It has a short half-life. It has the advantage of being a subcutaneous injection rather than being given IV. It's given in three 1-mL subcutaneous injections to get enough in to treat acute angioedema. And it's pending approval, I think imminent—oh, well, it's pending a decision, I think imminently so. And icatibant, the reactions to that—that's the bradykinin antagonist—were almost exclusively local reactions of no consequence, very short half-life. No other complications that I know of. Approved in Europe, but has to redo the study in the United States.

And that's the state of affairs of these molecules.

Slide 87: Emerging Therapies for HAE

This slide has all the molecules on it, but shows it in an inhibitory fashion. In other words, here's the, going backwards, here's the bradykinin receptor antagonist, it blocks it here, it has no effect on the cascade. You are hoping, like a natural attack of swelling, which aborts in 3 to 4 days anyway, that if you block the bradykinin and aborted the attack, whatever quiets it down, normally will in fact do so. The kallikrein inhibitor, ecallantide, then acts mainly here in a double-edged way, it blocks the kallikrein cleavage of the kininogen and the feedback activation of factor XII by kallikrein, shown here. And C1 inhibitor, no matter how you give it, blocks everything. So it blocks kallikrein, it blocks the feedback inhibition, and what's different is the first one up here. Blocking activated factor XII or the fragment, the only inhibitor that's effective in human plasma is C1 inhibitor, and you have to replace it to get an effect.

Slide 88: Thank You

Thank you very much.

Dr. Bryan Martin:

Thank you, Dr. Kaplan.

Slide 89: Question-and-Answer Session Part 1

It's now time for the question-and-answer session. If you do have a question, please go to one of the microphones in the room at the aisle—you can see them on either side—and use the microphones to ask the question and we'll take as many questions as we have time for. Question 1?

Audience:

Yes, I'd like to ask as many of the speakers as would care to answer. Given the two currently available C1 inhibitor replacement products, how would you propose incorporating those into treatment of patients with C1 inhibitor deficiency, with some comment regarding even though one is approved for prophylaxis and another for treatment of acute attacks, is there really any reason to believe that they wouldn't be interchangeable? And then, once these other products become available, presuming that they will, how would you then subsequently incorporate those into the treatment as well?

Dr. Michael Frank:

Since I spoke about C1 inhibitor, I'll give that first question a try.

I think the point of the question is quite appropriate and that is that the two C1 inhibitor products are both made from plasma, the purification methods are slightly different, but both have been used in Europe for 20 years and really have been without side effects and without infection. And what's more, if you look at them biochemically, they're really very similar, so that one would think that they're probably interchangeable.

It is true that the Cinryze product was approved first and was approved for prophylaxis. The second product was approved for acute therapy. My guess is that some people will use the Cinryze product for acute therapy and it may be that some people will use the Berinert for prophylaxis. I think that the decision that you make, as to whether you're treating prophylaxis or acute therapy, is going to boil down to the point that I made about patient severity. This is a highly variable disease in terms of severity.

There are a group of patients, who belong to the patient support group, who have attacks once a week. And those attacks can be totally debilitating. I've had a patient some months back who had an abdominal pain attack on no therapy that was so severe that her husband couldn't get her to an emergency room. She basically had to have an ambulance called and taken on a stretcher to the emergency room. They're very severe. So that if you had a patient that was having an attack a week and mostly abdominal pain, I think you would choose a prophylaxis approach to therapy. It would be terrible for the patient if you chose to wait until each attack occurred and then start treatment.

On the other hand, if you had a patient that had a two or three attacks a year, you might choose treating—acute therapy. Certainly having acute therapy available in the emergency room is important.

Now, the patient that I mentioned that had this very severe attack chose to go on prophylaxis even though her attack frequency was not all that high because she basically felt that she couldn't stand continuing to have attacks. So there's a point at which physician–patient interaction and response is going to determine where the crossover is.

Now, none of us know exactly how the kallikrein inhibitor and the bradykinin receptor antagonist are going to work out. It has been mentioned that they can be used subcutaneously, and that is important. Similarly, we don't know exactly how the recombinant product is going to be.

I think one of the issues clearly is going to be cost, and we really have to address that. Insurance companies—there's only one product out there that we know the cost of. I haven't heard even the cost of the Berinert, which was approved just a few weeks ago. So the cost is going to be an issue, and I'm sure that it will be an issue with the insurance companies.

Some patients are very satisfied with androgen prophylaxis and don't want to change, and it surely is going to be the cheapest of the various therapies.

So, there are some variables here that we just don't know the answer to, and I think the physician sitting down with the patient and working out, A, the kind of insurance that they have and, B, how necessary it is to treat them frequently, is going to determine a lot of this.

Dr. Bruce Zuraw:

If I could also comment on this, I agree completely with what Dr. Frank said, but I want to make a plug, so that you're aware that the Hereditary Angioedema Association, which is the patient group working with a group of physicians who are very interested in HAE, have developed a national HAE registry, in which we hope to collect real world data from the treatment efficacy as well as the natural history of the disease. And we're going to try to get the type of information that we can ultimately use to answer the question that was asked.

I agree right now you can't compare the different studies for reasons that Dr. Frank very nicely showed during his talk—they're simply not convertible. So, I think we have to get this data, and we have to learn, what's the most cost-effective treatment for HAE? And that may vary from patient to patient.

One other point I'd make, that I don't think we've discussed today, and again, it may not be possible given the cost structure, but I firmly believe that if you're going to use C1 inhibitor or any of the drugs for acute therapy, there's going to be a real premium in terms of the efficacy on giving it very early in an attack to stop the permeability before a lot of fluid moves into the

tissue. If you have to wait until you say, okay, it's a severe attack we should treat, the efficacy of the treatment is going to be greatly reduced. So I just wanted to throw that out as well.

Dr. Allen Kaplan:

And I would comment that we know the least in terms of comparative experience on these kinin-type molecules, the kallikrein inhibitor or the bradykinin antagonist, because there's not vast experience with it, the way there is with C1 inhibitor even for acute attacks. So there's no simple way of comparing them at this point.

I spoke with Marco Cicardi the other day, he was here, because he has the icatibant for use in Europe, and he didn't have a sense to what degree it was affecting his practice in terms of treating acute attacks of HAE, which he's been using the Berinert preparation for years and years. So time will tell on that.

I think the quick subcutaneous injection has some advantage. You could think of it in terms of this will depend upon the politics of whatever country has these drugs. Perhaps once they get past other safety issues, it could be preloaded in a little syringe, perhaps available for known HAE patients at home, before they're on their way to the emergency room, to get it in really fast. Perhaps avoiding an attack before they need to go, or at least getting ahead of the game, so that they're in better shape and less likely to have a laryngeal edema—excuse me—when they arrive at the emergency room.

We know that IVIG was given intravenously and now can be given subcutaneously. Perhaps some of the C1 inhibitor preparations ultimately could be converted from IV to a subQ administration, if you could get concentrated enough preparation that could be administered in such a fashion.

So I think all of those are things for the future, and we'll just have to wait to see how it plays out.

Dr. Michael Frank:

I'd like to make two additional points. And that is, number one, we actually have done a study with the Cinryze preparation in swine, giving it subcutaneously and seeing how it compares with the blood levels. And in fact they're quite comparable. They're slightly lower, but they're much more stable. So this was a prophylactic study. My guess is since it's got less toxicity, in terms of subcutaneous injection, than IVIG, that people will look into this much more carefully. And I'm told such studies are ongoing.

The other point to be made is that there's some negotiation that's going to have to be done between hospitals, insurance companies, and patients. This is a sufficiently rare disease, that it may be that expensive products are not going to be available in the emergency room, and patients do have to be treated quickly.

As you heard from Dr. Kaplan just now and Dr. Zuraw, the earlier you treat a patient, the more efficacious the treatment seems to be. And one way of dealing with this is for the patient to have the material at home and either give it to themselves or bring it in to have it administered. Whether this can be worked out or not is not totally clear to me at the present time.

Dr. Bryan Martin:

I'll attempt to take questions in the order that they came to the microphone. Question 2?

Audience:

Thank you. I have a 17-year-old female who's currently successfully treated for the last 12 months. She has a port, central port, and we're just about to release her to do it at home. Mom and her are being trained in our clinic.

So—two questions: one, she's 17, and she is technically or theoretically protected. Does that open different options for birth control for her? Could you comment on that?

And two, with the central port, can I administer this quicker and also use the off-label Cinryze for her at home acutely, and would that be 1000?

Dr. Michael Frank:

Let me start on that one. Because estrogens make the disease worse, and because menstruation is sometimes associated with attacks, and because patients, women, often, in my experience, get better during pregnancy and worse after—and the reason I say that is it's not the German experience, interestingly. It's been true in America and been true in Australia, but not true in Germany. That's the reason we started using danazol. Before we used danazol, we did a double-blind trial with progesterone because it goes up during pregnancy and then comes down shortly. And in fact everybody felt better, but their attack frequency did not go down. That means to me that a person, even without these drugs, could use Provera® as a birth control without much concern.

Dr. Bruce Zuraw:

But I would say that I would not use an estrogen-containing birth control method because, as again you heard today, these medicines are partially, largely effective, but not completely effective, and you'll certainly make—or very likely make her worse if she gets an estrogen-containing birth control pill.

In terms of using the port for home, I believe that patients can be trained to do this safely with good antiseptic technique. And although the pharmaceutical company can't say it, I don't think there's any restriction on my saying that I think—and Dr. Frank already said it—either Berinert or Cinryze in my opinion could be used interchangeably for prophylactically or acute, so

whichever one she has, if she's using it prophylactically and she has an acute attack, I would certainly, if she were my patient, encourage her to use it for the acute attack through the port.

Dr. Allen Kaplan:

I would just add, theoretically, when you think about the kinds of things that trigger attacks of swelling in these patients, that although they are only understood partially if at all, but among the things that estrogen does is to raise your factor XII level. And it does that significantly—it was shown by Oscar Ratnoff a number of years ago—that obviously would, if activated, would push you towards making more bradykinin, so it might be one of the estrogen effects.

And although we didn't have time to mention it today, Dr. Frank repeatedly mentioned stress as something that can trigger attacks of HAE. There is a heat-shock protein that we have described that is stress-induced, secreted by endothelial cells, that is at least a factor that should be looked at because it activates the kinin system and bypasses factor XII. In other words, if you had no factor XII there, this secreted protein from endothelial cells will interact with the prekallikrein—kininogen complex, convert the prekallikrein to kallikrein, and make bradykinin without factor XII. So it's the first bypass that's been described in the system, and it could conceivably be triggered by infection or stress.

Dr. Bryan Martin:

Our next question?

Audience:

Yes, I have two questions. The first is a brief one. I wanted to know, what is the comparator that was used in the FAST-1 trial? I'm half-expecting it to be morphine or something because it was used for abdominal pain.

Dr. Bruce Zuraw:

Actually, the comparator was saline. But your question is very shrewd because the FAST-1 study did not censor the patients if they received morphine or narcotic, and basically it simply measured time to beginning of relief, whether or not they got a pain medicine. So, in fact, if you really look at the data carefully, you'll see that many of those placebo responders were responding to narcotic pain medicines, and it's not captured in the data.

Dr. Allen Kaplan:

It really was—and you saw that it was, above and beyond everything else, it was the assessment of abdominal attacks that threw their data into the non-significant region. And I'm sure that was at least part of the problem, and their criteria for assessing abdominal attacks in this disease.

Now, they got away with it in the other study because they used the same criteria and they used tranexamic acid as their comparator.

Dr. Bruce Zuraw:

If you look at the FAST-2 study, what you'll see, though, is that a much higher percentage of the patients had a skin attack compared to abdominal. And I think that's the difference in these studies.

Dr. Allen Kaplan:

Indeed, that's what skewed it, but they have to do it over. Hopefully they learn from the experience, maybe.

Audience:

If I can go into the second part of my question: is there any potential role for any of these new molecules targeting the bradykinin receptor or kallikrein for other forms of angioedema? Such as acquired forms of C1 or, for that matter, idiopathic isolated angioedema?

Dr. Allen Kaplan:

Let me take a crack at that one. I would use it first for ACE inhibitor angioedema. That's the number one cause of angioedema in emergency rooms in the United States. It's different from what we've been talking about, which is overproduction of bradykinin. ACE inhibitor prevents its degradation and presumably accumulates to such a degree that you begin to get swelling that is indistinguishable from the swelling we've been talking about.

I think the easiest one to perhaps understand—it doesn't guarantee that that's the way to go, would be the bradykinin receptor antagonist. If you could get it in fast enough, I think you would abort an ACE inhibitor attack. If you could block kallikrein—because, of course, we're not overproducing, that might be less efficacious—although it would be good to block it. And likewise, raising the C1 inhibitor couldn't hurt either. But the most obvious one in that circumstance would be use of the icatibant.

When you get to the acquired, which is generally harder to treat, any one of the therapies theoretically might be helpful. But it would be hard to get enough in to do it. But theoretically, they would work. But I think it would take—I think it's more difficult to do, and the activation is massive sometimes in the lymphoma patients with massive amounts of immune complex, that are depleting the C1 inhibitor, and it may be tougher to catch up with the C1 inhibitor by simply infusing it. But for an acute attack, perhaps the bradykinin antagonist or the kallikrein inhibitor would be good.

And why don't I stop and let my other colleagues—because they look like they wish to comment on that one and come back for a moment to idiopathic angioedema.

Dr. Michael Frank:

I'd like to say another word about the acquired C1 inhibitor deficiency. Remember, acquired C1 inhibitor deficiency is often associated with a monoclonal antibody to the C1 inhibitor. Patients can have acquired C1 inhibitor deficiency for decades before any sign of malignancy, and I've even seen patients die of old age in their mid-80s, who have had acquired C1 inhibitor deficiency for four decades, without any sign of malignancy at the time that they died.

The response to treatment of acquired C1 inhibitor deficiency, therefore, may depend on the amount of antibody that they're making, its affinity for the C1 inhibitor, and, therefore, there may be quite a bit of difference from patient to patient. I've seen patients respond to danazol. I have a patient now who is responding to danazol with acquired C1 inhibitor deficiency. I've seen patients who have not. I've seen patients respond to purified C1 inhibitor, and I've seen patients in whom infusion of purified C1 inhibitor, infusion of plasma, led to no increase of C1 inhibitor at all except in the 5 minutes following infusion, and no change in the C4. So it depends on the particular patient and the particular characteristics of their disease.

Dr. Allen Kaplan:

With regards to idiopathic angioedema, people may vary in their percentages on this, but in my practice, and I see an awful lot of it, I would say 75% to 80% of them can be treated with high doses of antihistaminicss. Now, if that's true, that eliminates more than three quarters of it, that you would even give a second thought regarding this stuff.

There's a paper by Cicardi that had some of his idiopathic angioedemas that he thought were refractory to antihistamines, but I have to add that we would use doses that are much higher before we would decide that, that seem to have elevated bradykinin levels, and therefore, he was alluding to the possibility that they have some other mechanism ongoing that might involve the kinin system, even though there are no data as to what that might be.

There certainly is a subpopulation of extremely refractory patients, certainly to antihistaminics, where you might give something like this a thought.

The final would be, well, does that mean they have type III? I would emphasize that that is *absolutely not* the case for the most of them. For example, what I would look for in a type III, would be somebody with recurrent angioedema who does not have hives, whose complements are all normal. That's sort of the chemical criteria. But they're usually women; the attacks are strongly estrogen-dependent. They do not, in idiopathic edema, get laryngeal edema. Nobody asphyxiates from idiopathic angioedema. And they don't, as a rule, get abdominal attacks. The worst they have is irritable bowel syndrome. So that's kind of different from the description of, quote, the type III hereditary angioedema.

So, I would say that, you know, there could be 1 in 1000 of those floating around and it would be hard to know without doing the factor XII mutation or whatever evolves as to the eventual outcome of understanding that disease.

So, I would say we're not ready to consider these drugs for that without doing a lot more research. But I would say that 75% to 80% of them at minimum are treated with an antihistamine and therefore, what we've said today is not relevant to that group.

Dr. Bruce Zuraw:

If I could just echo that— I agree completely with that and I think all of us up on this podium and probably many of you are getting lots of calls about how can we diagnose type III? Should we be sequencing the factor XII to look for this mutation? And I really think we need to go back to the basics, and these are going to be rare patients, I believe, that have to be very carefully selected before you're going to try to do very expensive or very involved studies because most of them are going to have idiopathic angioedema that can be treated through the old-fashioned methods.

Slide 90 Question-and-Answer Session Part 2

Dr. Bryan Martin:

Next question?

Audience:

We all know there's the classic presentation, but we all know that there's a spectrum of disease. And I just wanted to ask the panel, maybe Dr. Kaplan has seen this more, but I recently saw a young woman, 22, chronic urticaria, angioedema, described basically a lifelong history of abdominal problems. And she reproducibly had C4 level of about half normal, mildly decreased C1 inhibitor, but normal functional value, normal C1q. And I'm just wondering, these people with partial C4 deficiency, are they more prone to having these problems? Would you expect them to respond to any of these therapies? And this young woman, I thought of danazol, but I really didn't want to think of danazol. And I'm thinking maybe one of these new kallikrein inhibitors or bradykinin inhibitors would be more of the way to go if she has continued problems.

Dr. Bruce Zuraw:

Before Dr. Kaplan answers, I just wonder if you could clarify, maybe just for me. Did you say she has urticaria *and* angioedema?

Audience:

Yeah, she had both. I've read the book, I understand. *[laughs]*

Dr. Allen Kaplan:

You ask a tough question. To make a dogmatic statement about how we all know that urticaria doesn't occur with this disease—if you had a patient with HAE who's allergic to fish and keeps eating fish every day, they will have urticaria. And so you do have to eliminate, perhaps in an oddball case, other causes for urticaria and consider whether that might be a possibility.

If you had a patient with a partial C4 deficiency but everything else was okay, there's no obvious reason why they should have angioedema per se. But it sure makes the diagnosis tougher. And then you really have to rely on your—first, your protein C1 inhibitor determination, which usually is accurate and discriminates the type I from the type II pretty well. But the problem is that the determination of functional C1 inhibitor sometimes is difficult, and sometimes you get a borderline value, where you're not sure what is going on. And Dr. Frank may be able to comment on that because he works with complement proteins all the time. But you could even reach a point where doing a therapeutic trial for a while with one of the agents might skew you one way to go, short of sequencing the C1 inhibitor to see whether there's a genetic mutation—that will unequivocally give you the answer.

Dr. Michael Frank:

So for C1 inhibitor, remember there are two genes, there are two gene alleles, one on each chromosome. For C4 there are four, so that there's a C4A and a C4B. It is really fairly common to have low C4 in the population because one of those alleles or even two could be missing. So about a third of the population in some studies that have been done have had some depression in their C4, so you certainly can't rely on that too much. Therefore, you have to be a physician and use all of the information that you have at hand to ultimately make a diagnosis.

Dr. Bryan Martin:

Next question?

Audience:

Just a comment about asphyxiating from idiopathic angioedema—and this goes to Dr. Kaplan, maybe. There's type III, you know, problems, whatever that is. But there are other people that have massive tongue swelling—they may have hives, but massive tongue swelling to the point of not able to speak, swallow, what have you. So it's crisis and a risk of death, and complements are repeatedly normal. And I know, years ago, we gave people urine containers, and we had them collect urine, and like 6 of 10 were acute samples of high histamine in the urine, we could demonstrate—so consistent at least with activation of mast cells. So, I would take exception

when you say that idiopathic angioedema doesn't cause asphyxiation. I think there's certainly a risk of it there, and I would say that there probably are some deaths from it.

Dr. Allen Kaplan:

The lights are right in my eyes. Is that Paul Greenberger?

Audience:

Yes, it is.

Dr. Allen Kaplan:

Okay, then I think the patient has the subgroup of idiopathic anaphylaxis that *you* described with Roy Patterson years ago.

Audience:

That was my point, because there's something else going on.

Dr. Allen Kaplan:

Yes, but I actually make that distinction, even though they're all rare birds. But I think that that massive swelling, where you choke on your own secretions without having laryngeal edema, is so unusual in, quote, idiopathic angioedema, if you will, that I have accepted the *other* nosology, that this is a variant of idiopathic anaphylaxis and has potentially life-threatening swelling. Rather than say it, that 1 in 10,000 idiopathic angioedema is going to asphyxiate, I label them idiopathic anaphylaxis.

Audience:

Okay, thank you.

Dr. Bryan Martin:

Next question?

Audience:

That gets really close to mine—I have a case with angioedema, abdominal pain, and tongue swelling. And all her studies are normal, functional and quantitative, C1q inhibitor and C4, C2. And I called her idiopathic anaphylaxis. And I just wanted to know, is there overlap between these? And just some comments on that.

Dr. Allen Kaplan:

It truly is semantics because we don't know the path—you're talking about two diseases where you don't know the pathogenesis. It's like talking to a pulmonologist about different kinds of interstitial fibrosis, which they, you know, pulmonary fibrosis, which they all debate but know nothing about any of them.

But in general, in idiopathic angioedema, you can get tongue swelling, you can get pharyngeal swelling, but not laryngeal swelling, and it's usually not obstructing. So, I have patients that have idiopathic angioedema—it includes occasional facial and tongue swelling, and if they have GI problems with that, that may need a separate evaluation. The ones that I have had like that, in fact, had irritable bowel syndrome, and that was the diagnosis that they turned up. But you know, they could have had Crohn's disease or something else going on that we would otherwise miss.

So, I think, although we're talking in generalities, I think it's important to emphasize that even if there are very, very rare exceptions, so that people don't get the wrong impression and focus on the exception to the rule.

Dr. Bruce Zuraw:

But I think it is important to reemphasize that idiopathic anaphylaxis is histamine-mediated, as Dr. Greenberger said. It's *not* bradykinin-mediated. The therapy that he and Dr. Patterson published involved antihistamines and corticosteroids, which have no role to play in the treatment of C1 inhibitor deficiency.

Audience:

I checked triptase and histamine in this patient, and they were both normal.

Dr. Michael Frank:

The other point is that many of the patients with idiopathic anaphylaxis will show a better response to epinephrine than any patient with hereditary angioedema.

This is the problem that we're all facing now. The sort of standard hereditary angioedema is not referred to me, and I don't think it's referred to any of us. The problem is this question of type III, since you have no clear understanding of pathophysiology, it is reported to make things more complicated, that the type III patients are more likely to have facial attacks than the standard hereditary angioedema, types I and II. So, this is again an area where more light is going to have to be shed. As we know something, for example, as more of these patients are treated with these various very specific agents, we may have more understanding.

Audience:

A comment on the irritable bowel syndrome—my patient has such acute episodes of abdominal pain, it would be hard to see them as just irritable bowel syndrome. It's angioedema and the abdominal pain together, very acutely.

Dr. Allen Kaplan:

I've seen people double up with it, but it doesn't last. An HAE-like attack could go two, three days. But I can't help you on it. *[laughs]*

Dr. Michael Frank:

I will tell you that the very first patient that was sent to me, who had a total respiratory obstruction after they were sent to me, I mean before they were sent to me, which was the reason, had been signed out at major medical center as irritable bowel syndrome. And I got presented a case by Tim Craig's group just a couple of weeks ago that was diagnosed as irritable bowel syndrome. So, you've got to worry.

Dr. Allen Kaplan:

It could go either way in the end.

Dr. Bruce Zuraw:

And I'm sure you've done it, but you've repeated the C4 during an acute episode, correct? Okay. The answer was yes.

Dr. Allen Kaplan:

And that would help for the 1 in 20 who has a normal C1 inhibitor—normal C4, I'm sorry.

Dr. Bryan Martin:

Our time is nearly up. We have one more question up. This will be the last question that we take.

Audience:

Well, since nobody's here—so another case, which was rather unusual, but maybe makes a point that I'd like you to comment on is, I saw a 70-ish–year-old woman years ago, and she was seen in the ER for abdominal pain—actually had seen our GI clinic the year before, no diagnosis. Very astute family physician draws up complement studies, they're all abnormal. She gets sent to me, I want to confirm the test results before I start her on treatment. They're all normal.

So, I give her my card and say, well, if this happens again, get them at the time of the event. And again—I didn't have to wait long—about a week later she's in the ER, gets the studies, they're all abnormal again. How often do you—I've never seen it before, I didn't read about it, but I believed it.

Dr. Bruce Zuraw:

We published with Len Altman, a patient from the University of Washington—I presume she ended up having acquired?

Audience:

Yes.

Dr. Bruce Zuraw:

A lady with acquired angioedema—we started following when she started presenting with recurrent angioedema. He got me involved, and we looked at her blood very carefully between attacks, during attacks, always normal. A year or two went by, she started becoming abnormal, showing high-molecular-weight kininogen cleavage and C1 inhibitor cleavage and increase in C4, only during attacks. A few more years went by and she became abnormal at all times, in between attacks as well as during attacks. So, it has actually been seen and described. I have no good idea how frequently we pick these patients up and what the natural evolution, particularly in the acquired patients are. But I know that what you're describing does exist.

Dr. Michael Frank:

In our original case series of patients with true hereditary angioedema, there were three or four, if you look at those tables, there were three or four patients who had normal C4 between events. It's not common, but it happens.

Dr. Allen Kaplan:

And there's an odd family that's been reported years ago, that has a mutant C1 inhibitor that is perfectly fine in inhibiting the kinin system. In other words, it inhibits factor XII, it inhibits kallikrein. They do not swell because their kinin system is intact. But this mutant C1 inhibitor does not inhibit C1, and they walk around with a low C4, an abnormal complement, but no swelling. So it just makes the point that unusual combinations certainly can be found.

Slide 91: Obtaining CME/CE Credit

Dr. Bryan Martin:

Thank you for your attention. Thank you for your questions. Ladies and gentlemen, we hope you found the information useful and helpful in your practice. And this does conclude this session.

And thank you so very much for joining us. Thank you for your attention.

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