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U.S. FOOD AND DRUG ADMINISTRATION  
PLASMA PROTEIN THERAPEUTICS ASSOCIATION (PPTA)  
DEPARTMENT OF HEALTH AND HUMAN SERVICES

PUBLIC WORKSHOP: RISK MITIGATION STRATEGIES TO ADDRESS  
PROCOAGULANT ACTIVITY IN IMMUNE GLOBULIN PRODUCTS

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## C O N T E N T S

<u>AGENDA ITEM</u>	<u>PAGE</u>
INTRODUCTION	6
SESSION I	
Pathogenesis and epidemiology of IG product-related TEs	16
Adverse Event Reports to FDA for Immunoglobulins and TEEs	17
Retrospective Claims-Based Cohort Study of Immune Globulin Administration and Occurrence of TEEs	26
Risk Mitigation Strategies to Address Potential Procoagulant Activity in Immune Globulin Products	31
Analysis of Spontaneously Reported Thromboembolic Events (TEE) for Patients Under IVIG Treatment, 2005 - 2010	39
Mechanisms of Thrombosis	48
Findings from Two Mathematical Models of Thrombus Formation Under Flow: FXI and FXIa, When do they Matter?	64
Laboratory Investigations Of Intravenous Immunoglobulin (IVIG) Products Associated With Thromboembolic Events	102
OCTAGAM, Intravenous Immunoglobulin Associated With Thrombotic Events	115
NIBSC Investigation on Thrombogenicity Tests For IVIG	122
Factor XIa and Thrombosis	141
Panel Discussion	172
IGIV and Thromboembolism	173
Potential Causes of Thrombotic Events in IGIV Treatment	181
SESSION II	
Partitioning and activation of clotting factors by manufacturing processes	201

Risk Reduction and Manufacturing	201
Manufacture of Immunoglobulin Therapies - Relationship to Thrombogenicity	213
Preliminary Product Testing and Methods Development - Manufacturer Presentations	232
Minimizing Procoagulant Impurities in IGIV Products - Baxter's Approach	233
Characterization of proteolytical Activity in IgG - Vivaglobin	244
IVIGs & TAEs Procoagulant Activity Elimination	258
Procoagulant Activity Trend Analysis During the Manufacturing of IVIG	271
Manufacturer Experience: Product and Intermediate Testing On Procoagulant Activity	283
Toward a Low Thrombogenic IVIG	296
Panel Discussion	311

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## P R O C E E D I N G S

### INTRODUCTION

DR. MIDTHUN: -- (in progress) -- measure procoagulant activity to consider standards development and to consider all of this as we try to address how we might mitigate the risk of immune globulate-associated thrombosis. And certainly the question is, you know, how can we use this new knowledge to enhance product safety.

I thank you all for coming today. We certainly look forward to presentations. And now I'd like to invite Mary Gustafson to the podium to also make some introductory remarks. Thank you so much.

(Applause.)

MS. GUSTAFSON: Thank you, Dr. Midthun. On behalf of the Plasma Protein Therapeutics Association, I echo Dr. Midthun's welcome. Last fall, when FDA let us know that they were considering a workshop to explore the reasons for the events, our global board of directors immediately and enthusiastically supported our cosponsorship of the workshop.

Our member companies, independently and collectively through the association, have worked to prepare information to share with you today and tomorrow. We look forward to working with global regulators, with others in the industry, with patients and the clinicians who treat them, and academic research and other interested parties and look forward to a very fruitful and productive workshop. Thank you.

(Applause.)

DR. SCOTT: The climate today is poor. This will just be a brief introduction to the goals and the structure of the workshop. It's not fancy and you'll be seeing some very elegant data and information later on. But I think it's important to understand and to remember why we're here and to go through a brief history of what brought us to this point today.

Thrombotic adverse events were first reported in the literature of 1986. And these are serious events. The most common ones are myocardial infarction, stroke, deep venous thrombosis, and pulmonary embolism. But there are miscellaneous other large arterial and venous events that have been described. As Dr. Midthun mentioned, we



recommended precautionary labeling for IGIV products since October 2003, and that website still exists.

The causes have been uncertain. And popular theories have included -- and most of these have some evidence to back them up at least in vitro -- the presence of coagulation factor contaminants in the product, hyperviscosity, vasospasm, and platelet activation or aggregation. And I think we'll hear about a few more possibilities here today.

We undertook a retrospective analysis for thrombotic adverse events occurring between 1998 and 2005. And you'll be hearing an update on that today. This wasn't published, but we noted that there were about 30 thrombotic events reported annually to FDA spontaneously. And that comprised 18 percent of serious adverse events during that time period.

The arterial events outnumbered venous events and they were reported for all products that were on the market in that time frame -- an important point that I'm sure many speakers will come back to later. They were associated, when compared with non-thrombotic adverse events with increased age, increased weight, cardiac risk factors, and

DVT risk factors.

This comes as no surprise to many of you. And the timing relative to infusion was striking for arterial events, in that 44 percent of the events occurred during infusion and 82 percent within the first 24 hours. Historically, there've been observations that Factor XI(a) co-purifies with immune globulin.

And also back around that time, Dr. Alving and Dr. Finlayson from FDA reported that in vitro procoagulant activity in IG products seemed to be mediated mainly by Factor XI(a). Other contaminants that seemed to co-purify sometimes were prekallikrein activator and kallikrein itself.

Dr. Wolberg, who is here today, published in 2000 a paper about IGIV procoagulant activity, and likewise found that it appeared to be mediated largely by Factor XI, Factor XI(a). However, until recently, tests of clotting factors and immune globulin products were not validated or routinely used for these products. And the presence of other procoagulant contaminants in these products had not been excluded rigorously.

In addition, there weren't any studies available

of thrombotic-event-associated immune globulin product lot. So we didn't have some of that direct evidence that it would be good to have, that implicated the clotting factors. This all changed in 2010 when a manufacturer reported thrombotic adverse events in two product lots to us.

We and the manufacturer investigated those lots and implicated Factor XI(a) as the most likely procoagulant contaminant using thrombin generation tests, Factor XI(a) assays, and also a variety of strategies to rule in or rule out the presence of other procoagulant activities or factors.

As a result of these reports and testing that revealed an unexpected increase in Factor XI(a), the firm voluntarily withdrew the product from the U.S. market. And I'm sure you'll hear also about actions in other countries with respect to this product.

So since that time -- that was in August and September of 2010 -- the FDA shared the thrombin generation test in Factor XI(a) test protocols that we used, with manufacturers and regulatory authorities who very promptly undertook development of their own methods using our

protocol or other protocols and began product and intermediates testing.

This was all voluntary and we very much appreciate the amount of work that's been done between that time and now. You'll be hearing about it in the second session. NIBSC began to develop standards in collaboration with other regulatory authorities and manufacturers.

So the goals of this workshop, which is very timely considering the amount of data that's been accumulated between last fall and the present, is to come to a consensus about the most likely causes of thrombotic adverse events in IG product recipients, and importantly to identify promising test methods that can be validated and that are likely to predict in vivo thrombogenic potential of IG products.

We also expect a robust discussion of risk-mitigation manufacturing strategies that may be used, and to provide information on standards development that can be used by all. And there'll be a report or an update of the ongoing testing protocols. So I want to introduce the structure of the workshop which I think of as past, present, and future.

The first session really will focus on the pathogenesis and epidemiology of the product-related thrombotic events. And this will include pharmacovigilance data, scientific background about pathogenesis of large vessel thrombotic events, lab investigations of products associated with thrombotic events performed by regulatory agencies and NIBSC.

We'll hear a talk on the thrombogenicity of Factor XI in vivo. And we have some excellent coagulation academic experts to talk about coagulation in Factor XI and observations in patients with IGIV-associated thrombotic events. The discussion will focus on identifying the most likely biochemical causes of thrombotic events in these product recipients listed in order if we can, and also not to neglect other possible causes of these events.

I think that many of you realize that not all IGIVs -- well, I'll just say that it's certainly clear that thrombotic events can occur in the absence of increased coagulant activity in the product. And so this suggests there probably are other causes of these events.

The second session will focus on manufacturers' presentations and what they found out and what they've

learned about partitioning and activation of clotting factors by manufacturing processes. We'll hear about preliminary product testing and methods development.

And the panel discussion will focus on the manufacturing processes and how they relate to procoagulant activity in products, challenges to validating these tests, approaches to investigating new products or products with major manufacturing changes, and the question of what practical information do we need to help establish upper limits of test results or cutoff values.

And the third session will be essentially focused on the test for thrombogenic potential of IG products with respect to methods and validation feasibility; perspectives on global tests for coagulation factors in these products; and the technical challenges, methods comparability, and standards development for thrombin generation tests; Factor XI(a) assays and application of these or related tests to predict in vivo thrombogenicity; and animal models to predict thrombogenicity that might support some of these other tests.

And the third session has, I would say, a fairly meaty discussion period. And I hope we have a lot of

coffee so that everybody can participate actively, because it will be at the end of the workshop. But what global tests might be most useful for IG products with respect to the range of relevant procoagulant proteins that are detected and their validation potential.

And what specific test, for example, for Factor XI(a) might be most feasible and useful to estimate the thrombotic potential of these products. What reagents and methods development are still needed for these tests, where are the gaps. What animal models are available and relevant to support these tests. And what additional clinical information would be useful to support test relevance.

In the fourth session we'll hear brief summaries from the session cochairs, and Dr. Epstein will deliver concluding remarks. The outcomes is long, and really they relate back to the questions.

We're looking for a consensus about most likely biochemical causes of thrombotic events; identification of tests that will have predictive value and validation potential; an improved understanding of procoagulant partitioning with IG; presentation of standards under

development; and reports of progress towards product characterization; identification of technical challenges -- and I think it's important for people to share their work, and it looks as if we're going to see a lot of that based on our preview of the talks -- and discussion of data that would be useful for estimating relevant cutoff levels.

After the workshop, we expect continued standards development. But naturally, this kind of a workshop leads to the question of whether product testing should be undertaken and if so, which tests are most informative, under what circumstances would you want to perform these tests, and how should limits be established to enhance product safety.

So I want to thank the -- out generous cosponsors -- PPTA and HHS; the Workshop Steering Committee who slogged through this agenda several times; our many speakers from diverse walks of life, if you will, who have come here to provide information; our colleagues in regulatory agencies, NIBSC; and especially my cochair, Dr. Mikhail Ovanesov, without whom I seriously wonder if we'd even be having this workshop.

Thank you very much and we'll now begin with the



first session.

(Applause)

SESSION 1: PATHOGENESIS AND EPIDEMIOLOGY OF IG  
PRODUCT-RELATED TES

DR. GAILANI: Good morning. Can everybody hear me? This is Dave Gailani from Vanderbilt University. My co-chair for this first session is Dr. Mikhail Ovanesov from the FDA. And session again is Pathogenesis and epidemiology of IG product- related thrombotic events. And the first talks we're going to hear about are on IGIV associated Thrombotic Adverse Events and Pharmacovigilance. And Scott Winiecki --

ADVERSE EVENT REPORTS TO FDA FOR  
IMMUNOGLOBULINS AND TEEs

DR. WINIECKI: Good morning. I'm Scott Winiecki from FDA CBER Office of Biostatistics and Epidemiology.

And today I'm going to talk about adverse event reports to the FDA for immunoglobulins and thrombotic events.

Our objective was to characterize thrombotic adverse event reports for the immune globulin products. And we wanted to identify trends in reports across products and other factors. So we looked at things like thromboembolic events as a proportion of all adverse events reported to the FDA byproduct, demographics of the patients, their indication for therapy, the type and site of the event, risk factors for each patient and the time to onset between immune globulin administration and the thrombotic event.

We assembled a case series from the FDA Adverse Event Reporting System, otherwise known as AERS, and our criteria was that each case had to have at least one adverse event term in the embolic and thrombotic events Standard MedDRA Query or SMQ. The report must be received by the FDA between January 1, 2006 and December 31, 2010 and there must be an immune globulin product as the suspect product in the report. We then excluded any duplicate reports and cases in which the thrombotic event preceded the administration of the immune globulin product.

There are some advantages to this sort of passive surveillance. One is that it's a national sample, so it's not limited by geography, by insurance plan. It also provides clinical information about the patients, so we can get an idea of things like medical history, demographics and any other medications the patient might be on that could have contributed to the event. We have product information such as the date of administration, the route, the lot number and it's complementary to the HealthCore data.

Mikhail Menis will be doing the next talk. He's going to be giving the active surveillance side of this and I think they both contribute to telling this complete story. There are some limitations to this data. Of course, there is underreporting; we don't know truly how many events have occurred. We also don't have a denominator, we don't know how many doses have been given.

So we obviously can only work with the data that's reported to us. These cases are not medically confirmed, we're wholly dependent on the information of the reporter, be that a health care professional, the patient or industry representative, primary investigator for a study perhaps, nor are these verified by the FDA. There are certainly

missing data, sometimes something as basic as gender or weight or age of the patient is missing. And to put in a plug for reporting lot numbers, we have only lot numbers for about one-third of our cases and this is very useful to us to be in the track of if there is any pattern with the particular lot that might be an issue.

So anybody who might be entering or reporting one of these things if you can have the lot number that would be terrific. Of course another limitation is stimulating reporting. Anytime there is publicity or regulatory action or labeling change, we tend to get more reports about that particular product because it's in the eyes or the mind of health care providers or patients. There's potentially biased reporting with time to onset. If an event happens immediately after the administration of a product whether it's related or not, it's probably more likely to be reported.

And, of course, we're unable to assess causality, all we can say here is there is administration of a product, an event occurs and those are related in time not necessarily a cause and effect.

Looking at our data, we came up with 209 unique

thromboembolic events reported after all the immune globulin products. Here it is broken down by year from 2006 to 2010. Of course, it varies somewhat by year, perhaps increased somewhat in 2010 with respect to the other years.

Looking at thromboembolic events as a proportion of all the immune globulin adverse events, we found that 7.3 percent of reports -- 7.3 percent of all adverse event reports for immune globulin products were thromboembolic events. All products in substantial distribution had at least one report in our series. Byproduct thromboembolic events ranged from between 4 percent to 25.8 percent of all adverse event reports and most products were less than 5 percent, that is to say less than 5 percent of all adverse event reports were thromboembolic reports.

Looking at the demographics of these patients who had thromboembolic events after immune globulin administration, average age was 54.1 years with a median of 58.6. The range spanned pretty much the entire life span from 82 days to 88 years. And again out of the 209 cases, we had data for 191. Looking at gender, female to male ratio of 0.8:1, and again we had the data for 197. Weight was an average of 78.3 kilos with a median of 80 and, of

course, again spanning the life span we had some small ones in there with pediatric patients of 6 kilos up to a max of 136.4 kilos. And you can see where the missing data comes in. We had that only for 81 of 209.

Indication for therapy were all numbers and by percent. The highest group was neurologic patients, things like CIDP, myasthenia gravis; then primary immune deficiency patients; a miscellaneous category which included things like transplant patients and other less common off-label indications; secondary immune deficiency in mostly patients with leukemia and lymphoma; ITP; rheumatologic patients such as polymyositis. Sometimes the immune deficiency wasn't specified, so we put those in a separate category and two Kawasakis. For type of event, arterial type events were more common than venous events. Some patients, however, did have both arterial and venous events. So you can see that 58 percent had arterial, 36 venous. And in some cases we just had something like clot or thrombosis, so we put those under type not specified.

Risk factors were known for 83 out of 128 patients. Most common risk factors included male gender, hypertension, hyperlipidemia and coronary disease. And most

patients with arterial events had several risk factors, on average 2.7. Here are the risk factors listed by percent. So in addition to the ones previously mentioned diabetes, hypercoagulative state, several patients had had a previous thromboembolic event.

Risk factors for the venous patients were known for 50 out of 82. Most common were use of oral contraceptives, a previous DVT or an in-dwelling catheter. Here you can see those percentages and again I think this speaks to how ill some of these patients are, how debilitated they might be. We had six with in-dwelling catheters, a number who were immobile. For the site of event for arterial events, stroke and MI were the most common. For venous events, DVT and PE were most common. For the arterial patients when other category which included things like thrombosis of an AV fistula or an artery going to a transplanted organ and some patients had multiple sites such as an MI and a thrombosis of the splenic artery.

For the venous patients under multiple sites we put things where if patient had a DVT and a PE or if they had say a DVT and a sinus thrombosis. Most arterial events, as Dr. Scott alluded to in her data, occurred either during

the infusion or in the first 24 hours after infusion, 61.4 percent occurring in those first 24 hours. And we had timing data for 101 of our 128 arterial events. Venous events were most commonly two or more days after the infusion, so 74.6 percent of our patients fell into that category and we had timing for 59 of our 82 events. Again here's the time to onset with the arterial patients having earlier onset in the first 24 hours, the venous patients tending to have events two or more days afterwards.

In summary, thromboembolic events were present across the immune globulin class. For most immune globulin products, our reports of thromboembolic events represent less than 5 percent of all adverse event reports, arterial events more common than venous events. Patients with arterial events tended to have commonly multiple risk factors and arterial events have a shorter time to onset.

I just like to acknowledge a number of people who aided in this talk, both in helping me put it together and in providing constructive criticism. I'd be happy to take any questions.

SPEAKER: (Off mike).

DR. WINIECKI: We have very limited data on that



which I don't have a lot to provide with because in most of those reports you don't have that information. So we've very sketchy information and again we're dependent on what's submitted in passive surveillance data. So it certainly could play a role in some of these events. We simply don't know in most cases.

SPEAKER: (Off mike).

DR. WINIECKI: Yes.

SPEAKER: (Off mike).

DR. WINIECKI: We had no newborns in the case series, our youngest patient was 82 days. There were some pediatric patients, but certainly not a large or substantial percentage, but no newborns.

SPEAKER: I just wondered if you have any data on the venous thrombosis patients in terms of prior DVTs or whether or not they had any general hypercoagulative states (off mike).

DR. WINIECKI: None of the patients had a prior PE, 7 out of the 82 had previous DVT. Yes.

SPEAKER: (Off mike).

DR. WINIECKI: The question was are these all spontaneous reports or do some -- are they solicited or come

from clinical trials. The vast majority are spontaneous reports. We occasionally do receive a report from a clinical investigator which are submitted like any of the other reports, but the vast majority are spontaneously reported.

SPEAKER: Other questions.

SPEAKER: (Off mike).

DR. WINIECKI: No, subcutaneous is contained in this data. Yes, it is contained in this data. Yes.

SPEAKER: (Off mike).

DR. WINIECKI: I think you made a good point, Dr. Golding, that while most of the arterial patients had multiple risk factors, some of them were relatively young healthy people that you would not expect people -- the typical person to have an MI. Certainly there were some lots which had multiple cases, but when you look at the data as a whole I think one of the important points here is that this involved multiple lots, virtually every product on the market. So while it may concentrate under certain lots or certain products, it does seem to be something that is a class effect across all products.

DR. GAILANI: There are no further questions. We

can move on to the next speaker.

DR. WINIECKI: Thank you.

DR. GAILANI: Thank you very much. And when we do have questions, I ask you to please use the microphones that are in the two side aisles. I think some people are having difficulty hearing some of the questions that are asked.

Apparently we were also recording this session and some of the questions are not coming across very well. So if you can just use the microphones.

RETROSPECTIVE CLAIMS-BASED COHORT STUDY OF  
IMMUNE GLOBULIN ADMINISTRATION AND OCCURRENCE OF TEEs

DR. MENIS: Hi. My name is Mikhail Menis and today I'll be presenting retrospective claims based cohort study of immunoglobulin administration and occurrence of TEEs. And as we know, thromboembolic adverse events are labeled adverse events for immunoglobulin products. And on September 2010 due to increase in reported TEEs, Octapharma has initiated a voluntary withdrawal of all Octagam lots. At the same time period, existing OBE/HealthCore exploratory claims data project on IG

safety in commercially insured population suggested that a possible association of multiple immunoglobulin products with TEEs. So, therefore, CBER/OBE has conducted the retrospective claims based study to assess TEEs after IG product as well as most potential risk factors.

Now we have conducted the retrospective cohort study using HealthCore's Integrated Research Database, a health care claims database with about 36 million active participants from 13 states. We have identified approximately 12,000 individuals exposed to immunoglobulin products from about January 1, 2008 through September 30, 2010.

Exposures were based on HCPCS procedure codes used to identify eight immunoglobulin products and TEE outcomes were based on ICD-9-CM diagnosis codes. And here's a list of ICD-9-CM diagnosis codes used to identify TEE outcome with bolded ones identified in our study.

Now this is a descriptive, we conducted a descriptive on adjusted analysis. It was a same day TEE rates per 1000 persons exposed overall by age, gender and IG products. Our primary analysis assessed -- our primary logistic regression analysis assessed same day TEEs

after any IG product dose while persons exposed were adjusting for age, gender, Elixhauser Comorbidity Index, prior TEEs, hypercoagulative state and number of IG doses prior to TEE. Our secondary progression analysis assessed same day TEEs after the first dose. And the first dose was defined as the first IG administration recorded from January 2008 through September 20, 2010 with a two months clean period and a continuous enrolment to make sure that this is the first dose.

Here's our unadjusted same day TEE rates per 1000 persons exposed overall by age and gender. So as you can see the overall TEE rate per 1000 persons exposed was 10.35. And as you can also see, the TEE rate increases with increasing age and among females under 44 the TEE rate is higher than among males under 44. Now this table shows unadjusted same day TEE rates per 1000 persons exposed by immunoglobulin product. And as you can see TEE rates varied by specific products with the highest TEE rates for products D, F and G and the lowest TEE rate for single product A.

Now this is the results of our logistic regression analysis with odds ratios for TEEs on the same

day by immunoglobulin products. When compared to product A, product G was the only one statistically significant with the point estimate of 356 and a PE value of 0.0031. Now there are other -- there are some other products with a borderline significance such as product B and product F. Other statistically significant risk factors included age 45 and over, prior TE or having had prior TEE and hypercoagulative state of diseases.

This logistic regression results show odds ratios for TEEs after the first dose and on the same day. And again when compared to product A, product G remains statistically significant with a higher point estimate and lower PE value. Other statistically significant risk factors included prior TEE and hypercoagulative state. In this analysis, age 45 and older was borderline significant. Now here's our limitation slide and of course occurrence of TEEs after IGs does not mean association is causal. The number of TEE events after IG administration is small and thus risk estimate have wide confidence intervals. Misclassification is possible because validity of the outcome exposure codes are known and may be different depending on the codes. We were not able to adjust for all

potential confounders such as rate of administration. Also full three year analysis and the medical record review are planned in the future.

In conclusion the overall adjusted same day TEE rate identified as 10.35 per 1000 persons exposed or approximately 1 percent of IG product users. And the TEE rates for seven single IG product groups ranged from 7.41 to 20.45 per 1000 persons exposed or 0.7 percent to 2 percent. The logistic regression model as demonstrated increased odds of TEEs among individuals exposed to product G as compared to product A with the increased odds that persisted in the first dose analysis. However, there were some other products with borderline significance. Other important risk factors identified were prior TEEs, hypercoagulative states and diseases as well as age 45 and older.

And thank you so much and I would like to acknowledge our FDA and HealthCore team.

(Applause)

SPEAKER: (Off mike)

DR. MENIS: The only thing I can say, I believe, that they used it a little bit for a different purpose. I believe they used it for intra-operative versus it's a

little bit different purpose. So you know, we will --

SPEAKER: (Off mike.)

DR. MENIS: Not yet. So the medical record review will be conducted. Okay, thank you so much.

SPEAKER: One more question. Sir, could you please use the microphone? They're recording and we are not picking up everybody's voice. Thank you.

SPEAKER: (Off mike.)

DR. MENIS: Okay, thank you. Thank you so much.

DR. GAILANI: Thank you very much. And we will move on now to the next speaker who will be Dr. Steffen Gross of Paul-Ehrlich-Institut.

RISK MITIGATION STRATEGIES TO ADDRESS POTENTIAL  
PROCOAGULANT ACTIVITY IN IMMUNE GLOBULIN PRODUCTS

DR. GROSS: Okay, good morning. Thank you very much. Actually this is a presentation made by Markus Funk, Brigitte Keller who are really responsible in our institute for the pharmacovigilance. But I was asked to give this overview and this presentation is just to give you an historical overview about the TEE rates which were, let's



say, observed in Europe. And in Europe we have seen the first signals in July or August in 2010 and this was because eight cases of TEEs were, let's say, reported and these TEEs were associated with just one batch and four of these eight cases were actually observed in one hospital. Because this was a clustering of, let's say, TEE cases which strongly indicated that there might be something wrong especially with this batch.

And this, let's say, this TEE rate is up to 80 fold higher than the rate of TEEs if you compare to the product information. From the regulatory point of view which actions have we taken, we suspended some Octa authorization for Octagam 5 percent in September 2010. This was also done by the Swedish agency and we recalled all batches of Octagam 5 percent in September.

This was followed by the suspension of some marketing authorization of the contenders and also the recall of these batches. Then we initiated an Article 107 procedure according to the Directive 2001/83 EC. This was triggered by the Paul-Ehrlich-Institut and the opinion came out in September 2010 where the commission decision was to suspend Octagam 5 percent and also Octagam 10 percent.

Following these procedures I think one of the seven procedure which is fully, let's say, pharmacovigilance topic or issue we triggered an Article 31 procedure and the aim of the Article 31 procedure is to investigate the chemical root cause and also the technical root cause. And this Article 31 procedure is still ongoing. Just as the evening the standing committee phase started where the draft European Commission decision will, let's say, propose the lifting of the suspension.

Quite recently in March 2011 first signals were also observed from subcutaneous immunoglobulin Vivaglobin and in-house research by the company revealed that several lots contained certain levels of procoagulant activity.

Subsequently we, let's say, triggered an urgent safety restriction to change the SPC in the similar wording like for IVIGs and also the package leaflet. Furthermore we asked to, let's say, to -- this is the view that the Health care Professional Communication. And again in order to investigate biochemical or the technical root causes, we triggered an Article 36 procedure which -- now I have to trust our lawyers -- is more or less the same as Article 31 procedure, but it was chosen because Article 31 procedure is

applicable for products which are, let's say, authorized via mutual recognition procedure. And this is a special case for Europe of course.

If we compare, let's say, our pharmacovigilance department initiated an extensive survey of all the TEE which were reported for several immunoglobulins and just as CBER gives you a summary of the results of this survey and for the IG O in this case, it's indeed Octagam, but 1 TEE as per 458 kilogram observed between 2004 and 2010 and this reporting rate increased in 2010. It's the reason for the increase supporting but also it means that the reporting, let's say, rate increase because it was known that there might be TEEs associated with the administration of Octagam 5 percent, 5 or 10 percent.

For all the other immunoglobulins which are given intravenously, the reporting TEEs per kilogram IG distributed is known for IG Gs, but if you look down at IG F which is subcutaneous immunoglobulin then you can see that we have a similar rate of TEEs if you look at the kilogram immunoglobulin distributed.

And we compare these two products and you can see this well known increase in TEEs for Octagam 5 percent, but

we also observed an increase in TEE cases for Vivaglobin. And this increase happened between the years 2008 and 2010. Which, let's say, root causes might trigger these TEEs of course has direct factors which might be higher viscosity, which might be high doses or rapid infusion. It was already pointed out that we have just few information about the dose and the infusion in the reports. But for similarity of, let's say, the TEEs required of these two immunoglobulins and coagulation factor contaminants were quite likely to be one of the root causes.

Of course all the patient factors might play a role as age, as co-morbidity, co-medication or even underlying disease but if we go into the, let's say, deeper into these disease factors then from our data or from our survey we cannot see a really dramatic influence of age of the patients but what we can see for Octagam 5 percent and also Vivaglobin is that the risk factors for thrombosis which are documented in patients with TEEs is much less. So it seems to be the case that patients which have a lower risk to develop TEEs are also affected by these immunoglobulins.

If we look, let's say, for patients which might

have other disorders we do not see a really big difference but also obvious again is -- this was also mentioned previously -- that arterial TEEs are much higher if you compare to venous TEEs. And I guess in the next session we will have a nice overview about the mechanism of arterial and venous TEEs. If you look at the onset of TEEs which is shorter than 24 hours post administration period then there might be an increase for Octagam 5 percent but if you compare to other immunoglobulins on the market it might still be quite similar.

In summary, all the increased reporting rates of TEEs what we have seen in Europe were -- for these two immunoglobulins were reported via spontaneous reporting. The signals which were observed are quite consistent with levels of procoagulant activity of certain batches. This is for both immunoglobulin case. We did not see -- let's say we do not see yet such signals for other immune products in the market and for Octagam patients without risk factors for thrombosis concern and also at a higher number of arterial thrombosis.

What we would like to do in the future is that we will reevaluate the data more precisely and we will

come back to the manufacturers to ask for, let's say, for the exposure data, we'll ask for a certain amount which were distributed or administrated because it isn't always the same. And of course we also try to get more information and to link the batches which were associated with TEEs of several products with, let's say, coagulation tests such as the TGA or other TEE I guess we will come to these things during the workshop in more detail. Thank you very much.

(Applause)

DR. GAILANI: Are there any questions.

SPEAKER: Maybe not a question but a comment, maybe Stefan you could clarify whether this German data pharmacovigilance data or European data and if these are German data to what extent these figures could also correlate with the European pharmacovigilance system.

DR. GROSS: According to our pharmacovigilance people this data came from the Eudra pharmacovigilance database, which is the European data.

SPEAKER: I have a question about the last slide that you had up. Could you parse the data -- (off mike) you have risk factors and then you have (off mike). Can

you -- there's an overlap between them. So you have 16 of 21 for example risk factors and 4 with arterial TEE. So what number out of the 16 for example -- were the 4 all out of the 16 or were there patients without risk factors. Can you split them between those with risk factors and those without?

DR. GROSS: I'm afraid I cannot answer your question because I'm not so deep into the details, I'm sorry.

SPEAKER: I notice that your TEE reporting rate for the two products that you compared increased markedly after 2008. Do you think that that's a reporting bias or is there different indications that the products are being used for or is there any --

DR. GROSS: For Octagam, it is definitely biased because the reporting rate increased because hospitals were sensitized for the TEEs and say I guess all the physicians, they reported much more from much more cases from which they thought there might be TEEs. But this increase from 2008 on I guess we come back to this topic. If we're talking about manufacturing processes because what we have seen is that, let's say, the flexibility within the

manufacture process is increasing and this might be one of the main reasons. But then I guess we'll come back to this in the next session.

DR. GAILANI: Other questions? Thank you very much. Our final speaker for this first group is Kenneth Jacobs from Talecris Biotherapeutics.

ANALYSIS OF SPONTANEOUSLY REPORTED THROMBOEMBOLIC EVENTS  
FOR PATIENTS UNDER IVIG TREATMENT, 2005 - 2010

DR. JACOBS: Good morning. My name is Kenneth Jacobs from Talecris Biotherapeutics and I'm going to present the pharmacovigilance data collected by companies that are members of the PPTA that occurred over a five-year period from 2005 through 2010. (Tape interruption) -- submitted data to this presentation. Those companies were Baxter, Kedrion, CSL, Talecris Biotherapeutics and Biotest.

Why are we here today? Thromboembolic events presenting known risk associated with the administration of IGIV that can have serious and life-threatening consequences. An increasing number of the reported events



from one manufacturer of product since 2008 resulted in marketing withdrawals of this product in August and September of 2010. A variety of hypotheses exist on factors contributed to the market withdrawals and also on reasons for contributing to IGIV associated thromboembolic events.

To gain further insight, the PPTA formed a Pharmacovigilance Task Force to investigate thromboembolic events caused by IGIV by examining pooled pharmacovigilance data from companies. The protocol is what I'm going to discuss next. The pharmacovigilance task force developed a protocol to evaluate spontaneous reports by IGIV associated thromboembolic events with the following objectives.

We examined the reporting frequencies of IGIV associated thromboembolic events from 2005 through 2010. We analyzed the demographics, comorbidities, event types, latencies to event onset, IGIV dosing as well as indications. Generated a new -- we looked to generate new hypothesis of possible and/or support existing hypothesis related to IGIV associated thromboembolism. Our basic approach is we use standard MedDRA queries for embolic and

thrombotic events to identify cases of interest and we use company provided data for IGIV distributed to derive TEE reporting frequencies per million grams.

Limitations to this process; spontaneous event reporting cannot verify causality as we previously heard. Rather it serves as an essential tool to identify risk such as an unexpected frequency or nature of events that allows for a comparison of AE rates over time. When we look at the data set from the pooled data from the companies that I previously mentioned, we identified a total of 74 cases from the standard MedDRA query for embolic and thrombotic events. Of those 74, 58 of those cases were medically confirmed, meaning that a health professional provided sufficient information to confirm the diagnoses. Other case reports could have been from consumers, they were not medically confirmed, and that number was 16. Of the total 74, 73 of the cases were classified as serious in the pharmacovigilance systems of the companies.

This slide basically looks at the demographics and whether or not the first dose of IGIV for patients with all are medically confirmed were associated with

thromboembolic events. I think the most important information on this slide is that if you look at the all category, it really doesn't -- it's very much similar to the medically confirmed category with an average age of 58 years. Seems to be about 50-50 male females. But as we all know with pharmacovigilance, we get sparse data, so 65 percent of the case reports had insufficient information to determine whether the thromboembolic event was associated with the first dose of IGIV.

What indications are associated with thromboembolic events? We include here indications for which they -- there were reports of two more indications. You know, if you noticed that the primary immune deficiencies are -- account for about 22 percent, but when you look at neurological indications such as CIDP, Myasthenia gravis, multifocal neuropathy and some neurological events that are associated in the other category, you'll look and you'll see about 31 percent of the associated TEE thromboembolic events reported or associated with their neurological indications as a total group; 24.3 percent were unspecified meaning the information that was provided, there was no indication for

those case reports.

This slide basically looks at the average dose of the -- for cases for which we have information of IGIV for all patients with thromboembolic events by indication and administration. So in the first column, we basically have the number of case reports that we -- that are in the data per indication and then the number of cases in the second column where there's dose information provided. You can see the average dose for those indications and we -- if you took the entire average, all indications included, it looked to be about 44 grams. There is no -- we really couldn't give you information about, you know, how many days that was administered over, administration rates et cetera.

The next slide basically is an introduction looking at the timing of onset of IGIV for thromboembolic event onset for all patients and remember there was a an N (phonetic) of 74 earlier. It appears that 76 percent of the cases occurred after infusion. For us our data only indicated 12 percent occurred during the administration of IGIV.

This slide looks at the thromboembolic events

after infusion, sort of denotes them or categorizes them as arterial, venous and other. For the companies we looked at, two have really four broad categories for arterial and venous. For arterial it was -- we looked at MI and stroke. For DVTs, we looked at deep vein thrombosis as well as pulmonary embolism. So the other category could be reports of thrombosis where the report did not indicate whether it was arterial or venous. In addition there could be something like phlebitis which we thought were perhaps superficial and weren't included.

And what we notice here is that most of the events that we have collected occurred in that greater-than-72 hour window and a majority of those were venous. If you look at the 24-hour window, the majority of those cases appeared to be in the arterial category which to me would be expected as -- an expected rate not for that, but just because I think arterial events you'll present to your physician a little quicker and therefore you'll see it diagnosed in that 24-hour window a little as opposed to being later for venous events.

Next slide looks at the comorbidities for all patients with thromboembolic events. It appeared that 65

percent of the patients in our data had underlying comorbidities; 30 percent of those we had insufficient information to determine if there was underlying comorbidity or not. The occurrence of the comorbidities in all patients, if you look at the data here, approximately 29, the top ones with the underlying cardiovascular disease representing about 30 percent of the cases, diabetes, hypolipoproteinemia, cancer and neoplasms are rounded out. If you look at the bottom of the slide, basically it appears that about 13 patients had more than one underlying comorbidity.

So again as we stated earlier we looked at the - - as a denominator we looked at the millions of grams distributed, and this is basically a trend line looking at the thromboembolic events that we've reported in comparison to Octagam, and you can see that there is really no spikes in the data for the occurrence or reporting frequencies I should say for thromboembolic events occurring from 2005 through 2010 collectively from the members of companies that I stated earlier.

Conclusions; in terms of demographics indications comorbidities there were no distinct

differences between the medically confirmed and the other case reports. The majority of IGIV associated thromboembolic events are reported with comorbidities that they themselves are also risk factors for thromboembolic events. Thus it may be hypothesized that the majority of IGIV-associated thromboembolic events are at least multifactorial. Spontaneous reporting rates for IGIV-associated thromboembolic events of the companies contributing to this analysis remained low and stable and appeared not to increase over the past 5 years.

Existing paths of surveillance, i.e., spontaneous event reporting are functioning well and providing meaningful tool to identify signals, either new -- either new or modifications to previous ones in a temporal manner. Thank you very much.

(Applause)

DR. GAILANI: Any questions for this speaker?

SPEAKER: Do you look for co-medication, or infection associated with the --

DR. JACOBS: I'm sorry, I didn't understand you.

SPEAKER: Do you look for co-medication any practical application because this patient received a lot

of other medicines?

DR. JACOBS: Can somebody help me with that?

SPEAKER: Co-medication.

DR. JACOBS: Any concomitant medications? Did we look at that --

SPEAKER: (Off mike.)

SPEAKER: And co-infection?

SPEAKER: Same thing. No distribution of infection (off mike.)

SPEAKER: Thank you.

DR. GAILANI: Other questions for this speaker?

DR. JACOBS: Thank you.

DR. GAILANI: Thank you very much.

MR. OVANESOV: Thank you. Our second session is dedicated to the pathogenesis of arterial and venous thrombosis, and Dr. Maureane Hoffman is a renowned expert -- the first speaker is Dr. Maureane Hoffman, she is a renowned expert in the regulation of blood coagulation, but she's also a physician and she will describe the actual clinical picture of thrombotic events.

The second speaker for this section, Dr. Karin Leiderman will describe the use of mathematical modeling



approach to explain the possible thrombogenicity of Factor XI (a). Thank you.

#### MECHANISMS OF THROMBOSIS

DR. HOFFMAN: Thank you so much. Well, I don't have any new data. We've dabbled in this about 15 years ago, but I'd like to try to highlight some of the aspects of thrombosis that I think are relevant to our discussion of what causes thrombosis in patients infused intravenously with immunoglobulin products. So I know some of this is going to sound incredibly simple, but I want us to all start with the same basis, the same background. And I think we have to remember that thrombosis is a blood clot in an artery or vein of a living creature.

And that means that blood is flowing so it doesn't -- hemostasis is different process in which the whole goal is to stop bleeding. It's adaptive and thrombosis blocks off blood flow or blood return. And that means the environment in which it occurs is different from hemostasis and different from our laboratory tests.

And therefore the mechanisms may be different and we have to keep -- we don't want to assume that coagulation in a test tube or coagulation that stops bleeding is identical to thrombosis.

So what causes thrombosis? Well, Virchow who is a pathologist -- I'm a pathologist so I have to mention that and show you some pictures -- addressed this quite some time ago. Now, the concept of Virchow's Triad has adapted over the years as our understanding has changed and he still gets all the credit. Nonetheless, some of this is true if -- even if the viewpoint has changed a little bit over many years, and that is that altered blood flow is important, vascular injury and inflammation are important and a systemic hypercoagulable state can be important in promoting thrombosis.

And we'll see that all those things apply to the discussion at hand. So let's see -- so probably all the time we are having injuries to our vessels, and the platelets come along and cover over the site of the injury and if all goes well a little bit of fibrin forms and a layer or two of platelets are deposited and that's about the end of the story. So we are all subject to

intravascular coagulation. Oh no, we don't want to do that again.

But if all goes well, it doesn't block off the vessel. And I think that all of us young or old have some underlying background level of partial thrombosis I guess you can say going on all the time. And one of the points I want to get through to you probably everybody suspects in the back of their mind is that when we add some additional risk factors, we may turn this completely inapparent mural thrombosis into an event that causes us trouble. Okay, now, let's see if I can get this under control.

All right, so one of the things we talk about and all the speakers so far mentioned is the idea of hypercoagulability and we usually think about that as levels of the proteins, the pro and anticoagulants in the blood. But in fact hypercoagulability has multiple components and is probably -- and is incredibly complex and probably each of you could give a different definition of what hypercoagulability is. But -- the proteins are important, but the blood cells, platelets, as well as leukocytes are important, and endothelial cells.

All have roles in hypercoagulability and some of these are easy to measure and some of them are not. And as we've also heard, hypercoagulability is multifactorial. So it's what you're born with, the levels of your coagulation proteins, how reactive your platelets are. But lifestyle and environmental factors play critical roles and some of this goes into what we talk about as comorbidity and risk factors, but not all of it's very easy for us to sort out. So this is different from hemorrhagic disorders and I'm not going to spend time talking about that, but I got into this from the bleeding side and thought, oh, it's simply you have hemophilia and you bleed. Well, of course thrombosis is much more complicated than that.

And the point I want to make here is that risk factors don't just add up, they multiply. So if we say the risk of thrombosis in a normal person of any given age and sex is 1, taking oral contraceptives increases that risk say to 4, a genetic predisposition like Factor V Leiden which is a mutation in the Factor V which makes it much harder to inactivate, that would raise your risk to about five to sevenfold. But if you've got both of them -

- see if I've got a little error over here -- well, anyway, the fourth line down there, you see if you've got both of those things, the risk doesn't add up, it multiplies. So what that means is we may add some risk factor that we think is very small, but it can have a big effect if -- especially if you've got a lot of other underlying risk factors.

Other risk factors include inflammation, and since we're moving fast, I'm going to keep on moving, but inflammation is important as you all recognize. Now, let's talk about arterial versus venous flavors of thrombosis. So again has been pointed out by the other speakers, there are different risk factors for arterial and venous thrombosis. Arterial thrombosis most importantly is associated with atherosclerotic vascular disease and with platelet deposition. So in arterial sites, platelets adhere through Von Willebrand factors, sites of high shear, and those sites are often at sites of atherosclerotic plaque deposition. And atherosclerosis starts young. And as we're talking about risk factors, children have beautiful, pristine arteries, but as you move up that age scale, as plaque deposits the risk

increases. It doesn't go from no-risk to some risk when you increase in age, but rather there's a progressive increase and of course this is influenced by other risk factors, diet, exercise, obesity. And so here's one of my pathology slides and since I don't seem to have a pointer, take -- where the arrow is is a site where a plaque has ruptured, has broken.

And down in the 3:00 to 6:00 o'clock range is -- oh, my technical consultant is here. So this is the atherosclerotic plaque, pretty small, hasn't changed flow that much, but it's ruptured. And as we look here, we've got bleeding into the plaque and that's initiated a big thrombus. So if you're somebody with lazy platelets, maybe you're on aspirin, and you don't have a lot of hypercoagulable risk factors, your plaque rupture might just stop with a clot there, whereas if we've -- you've got more active platelets, higher levels of one of your coagulation factors, maybe a little extra Factor XI (a) floating around, that could make the difference between just depositing a few platelets there and clotting off the whole thing.

So this is -- most people -- this is the aorta.

Most people do not have clot off their aortas almost no matter what, but my point here is that a lot of the material in this clot is this cream or yellowish color and that's platelets, a lot of platelets and thrombin in arterial thrombi. Now venous thrombosis is different. Of course it's common in the lower extremities, but it can also happen at other sites. And different symptoms and of course what we really worry about is developing a fatal pulmonary embolism.

And venous thrombosis is associated with stasis, so patient immobility or something that decreases flow in the veins that you're going to develop a clot in, and it's also associated what we -- with what we've classically called hypercoagulability, increased levels of clotting factors, Factor V Leiden, anti-thrombin deficiency, and so on, as well as a number of acquired disorders, a lot of these lifestyle things and also antiphospholipid antibodies. And so of course this is what we don't want to happen. That's -- those are big pulmonary emboli and obviously an individual that didn't survive the process.

And the point I'd like to make here is that venous thrombosis forms obviously in flowing blood and

it's laid down in layers, and this happens over an extended period of time, not just a few minutes or even probably an hour, but days to weeks or even months. So thromboembolism is multifactorial and venous and arterial thrombosis probably have different mechanisms.

So how do we study thrombosis? I'd like to argue that mostly clinical observation has given us the most useful information. Human thrombosis is spontaneous in the sense that we can't usually identify why it happens at a particular time and in a particular location in the vasculature, and I would argue that there are no realistic animal models. We have models where we cause abrupt or acute injury to the vessel or we tie off two ends of a vein, but those don't really mimic what happens in humans, and we have to take with great care any results that we get from these highly artificial animal models.

So I'm not going to talk about all possibilities, but the two leading contenders for what might provoke thrombosis or probably increase blood viscosity and Factor XI or XI (a) contamination. Now, we backed into this because we were looking for a control IgG preparation to use in studies of lupus anticoagulants and



every preparation we used have procoagulant activity. And that's about the last we've done with it. But we did find that Factor XI (a) could produce a lot or lead to production of a lot of thrombin in a cell-based model of coagulation that we've developed and discouraged us from our studies of the lupus anticoagulants.

I'd like to point out what other people have -- just about every other speaker has shown and that is the timeframe -- oh, and my labels came out, in -- so the wrong color -- but anyway, so this is also a graph, a re-graph of the data in this publication, but is consistent with the FDA publication showing that arterial events more often happen early, that is during infusion and within a day or few days after infusion, whereas the blue venous events happen later.

And I think that maybe not surprisingly means that the two tend to have different mechanisms. And so while our group got focused on XI (a), I suspect that XI (a) is more involved in these early events, and possibly viscosity changes are more involved in the late events. But I've absolutely no data to support that. And you've heard that the characteristics of the patients who develop

arterial and venous thrombosis are different, so I'm not going to obsess about that since I'm probably on the road to taking more time than I'm allotted anyway.

So here's my last slide; can we draw any conclusions? I don't really think we can and as was pointed out a lot of the data that we have is hypothesis generated. And now we really need to -- and maybe some of the later speakers have already applied really scientific approaches to trying to determine what the cause really is rather than just correlations. But I think it's clear that patients that have existing risk factors are at higher risk for thrombosis when you do something else to them like increase their blood viscosity or give them increased levels of procoagulant factors.

I would argue that viscosity is -- seems more likely to be associated with venous events and procoagulants most -- especially XI (a) although there may be others that we haven't studied well, seem to be more likely associated with rapid onset arterial thrombosis. Now, these are hard things to study in humans and so I've gotten some interesting information from my mathematician colleague who'll tell us a little bit about that in the

next talk. Thank you.

(Applause)

MR. OVANESOV: Thank you. I think we have time for several questions.

SPEAKER: Given that we -- you know, we know that it's multifactorial, we don't know exactly -- well, we know theoretically how it might happen, do you think that it's a good idea for this patient to be given prophylactic doses of anticoagulant?

DR. HOFFMAN: Well, I'm not sure I can truly answer the question, but I think we first of all have to carefully assess what an individual's risk of thrombosis is. And there are some of these patients that really seem from the start to be at much higher risk of thrombosis when you do something else to them than others. And so I think based on data from demographic associations, there's probably a subset of patients that would be -- that would benefit from prophylactic anticoagulation. I think the other thing that this brings up is that it's going to be very difficult I imagine to set some cut-off for procoagulant activity in these products, because whether you're at risk for thrombosis when you get them is going

to depend on all the other things, you know, in your makeup, all your other risk factors. And it may be that a reasonable strategy is to put an anticoagulant in the products which won't change the viscosity issues, but then might at least mitigate, since that's word for the day, some of the risks associated with them.

SPEAKER: Do you think that the later clinical onset of venous disease is a reflection of the pathogenesis of venous thromboembolism rather than the time of onset of initiation of the thrombotic event? That these events that occur later rather than in the first day is just different in terms of the pathogenesis of arterial versus venous issues, and that the original injury, whatever happened, is occurring at the same time?

DR. HOFFMAN: Yeah, so again I'm not sure I can answer that. I think there are probably two major contributors to why venous thrombosis happens more slowly. The first one is that that's the way venous thrombosis progresses, I believe. Now, you have -- I think you have to have some inciting factor in the patient. So there's something about that patient that makes them prone to venous thrombosis.

And then when you add another risk factor such as increase the viscosity, or may be cause damage to the vein at the site of infusion or something like that, then you initiate a process as you say that by its nature is a slower process. But I also think that one of the reasons I think that say XI (a) or another procoagulant would be more prone to cause arterial thrombosis is that it won't get to circulate very long before it gets inhibited. So whatever it's going to do, it has to be protected on a platelet or it has to do it fast, whereas the increased viscosity is going to go on for a longer period of time, and I think that that meshes more with altering the flow at venous sites.

SPEAKER: You mentioned about the venous thrombosis. From what you've said and other information it seems to me that it's hardly likely or likely at least that a lot of these go undiagnosed, and the question that I would consider is should we be looking -- using more sophisticated techniques during studies to look for venous thrombosis such as Doppler techniques and ultrasound?

DR. HOFFMAN: I agree completely, and I think that the venous events are probably under-diagnosed for

two reasons, one exactly what you said, well, if it doesn't kill you or something like that then you -- you're not as likely to bring it to your doctor's attention. And of course they happen later and so -- and venous thrombosis is not that uncommon. So it may not be linked with the IVIG being an inciting factor. So I think especially now when everyone's -- well, our consciousness is raised about the thrombotic complications of immunoglobulins, that if someone wanted to do a study on this instead of just passive monitoring, that one of the things that would be very reasonable to look at would be Doppler investigation of patients that get IVIG, because it's not invasive, it's not going to hurt the patient. And I bet you it will show up a lot more events than we really imagined.

SPEAKER: Are you aware of a good epidemiological database for the epidemiology of thrombosis, because I think some of the events that are reported on just background and to make a good judgment would be extremely helpful to have potentially background incidents rates by sub-groups?

DR. HOFFMAN: Yeah, so in the -- I don't know

what the incidence of thrombosis is in the patients that are being treated with IVIG or what it would be if they weren't treated. And I think that's right, that's important information and I don't -- maybe somebody has that data, but I don't. So you don't know how much thrombosis is being increased by your treatment.

SPEAKER: Yeah, and then in that regard just to refer an earlier speaker talking HealthCore Database analysis, right, so thromboembolic events in that analysis were roughly 10 in every 1,000 patients, right? Our own analysis is that there's roughly 1 in every 50,000 applications. So I think if you're going to start talking about controlled clinical studies, you have to recognize that, you know, it's a very difficult thing to study those very low numbers.

MR. OVANESOV: I think we should also consider some natural mechanisms of action of IGIV as a contributor to what we are discussing today. What comes into my mind is the change of the cytokine network upon administration of IGIV. IL-6 is increased, TNF-alpha is increased just to name a few after administration of IGIV with the consequence of up-regulation of tissue factor, down-

regulation of thrombomodulin, impairment of the APC anticoagulant pathway. Also it might well be that natural occurring alter antibodies against platelets, surface receptors, agonistic antibodies could play a role in arterial thrombotic events.

DR. HOFFMAN: Yeah, I agree. So it's possible that some events are unavoidable because the mechanism of the drug is responsible for increasing the risk of those events.

MR. OVANESOV: I think we have time for last question.

SPEAKER: Yeah, one question. The two other major complications that are seen in IVIG are renal insufficiency and aseptic meningitis. And I wonder if any of the people that have reviewed these cases have linked these two together or you have any idea that thromboembolism is causing subtle renal disease or causing CNS problems?

DR. HOFFMAN: I have no idea. You'll have to ask someone else. I have no data, let's put it that way.

SPEAKER: Can you (off mike) --

DR. HOFFMAN: Do you want to come up here?



SPEAKER: In our 1998 to 2005 look at the cases that I reviewed and other -- one other person reviewed, every single record, it was -- I don't think we saw any cases of aseptic meningitis linked to this adverse event. There were some patients with renal dysfunction. Typically that was diabetic, and there were a few other causes, but it seemed to pre-exist the occurrence of the event in question. So we didn't see anything striking at least.

MR. OVANESOV: Thank you very much for this very interesting presentation. Our next speaker is Dr. Karin Leiderman from Duke University.

FINDINGS FROM TWO MATHEMATICAL MODELS OF THROMBUS  
FORMATION UNDER FLOW: FXI AND FXIa, WHEN DO THEY MATTER?

MS. LEIDERMAN: Hi, can everyone hear me? Thank you for including me in this presentation. It's been very interesting so far. I am in the mathematics department at Duke University and was introduced to Maureane Hoffman earlier this year and she asked me if I would be willing to look at the effects of adding activated Factor XI into

my mathematical model of thrombus formation. And this was a very interesting idea, so I'm going to tell you a little bit about those results today.

So the title is "Findings from Two Mathematical Models of Thrombus Formation Under Flow: FXI and FXIa, When Do They Matter?" Okay, so because of the time constraints, I won't go into too much detail about the mathematics and the model. I'll just give you a brief overview.

So mathematical modeling, how do we do this? Well, we first think of a scientific question that we're trying to answer and try to decide if we can develop a mathematical model that can help us answer those questions. So for instance if we would like to know how much thrombin is formed in a particular region due to a certain area of exposed subendothelium under a flow situation, can we develop a model that can help us answer that question? Well, this is something that we've been working on, me together with Aaron Fogelson at the University of Utah for quite a while.

So in this particular instance we first need to list all the important players involved and here that

would be clotting factors, platelets, and the exposed subendothelium. So you can see in this little schematic cartoon that this would be a vessel and perhaps this is some region of exposed subendothelium and there's this region here where coagulation reactions may be occurring and there are things that are being transported in and transported out by flow and also by diffusion.

So the first thing we do is we list the important players as I mentioned. Then we have to be able to describe how these things move and interact with one another and how they're transported and we can do this with -- using reasonable mathematical equations. Once we've developed those equations, then we have to solve them and analyze the results in a mathematical context and then hopefully bring them back to a scientific context so that we can make some predictions about what we found.

Okay, so I'm going to tell you about two models today and the first one I'm calling the non-spatial model, and we use this model to investigate the impact of Factor XI on the clotting system. And when I say non-spatial, I just mean here we're talking about quite a small injury, okay? So in this case, we would imagine that we have say

a patch of exposed subendothelium that might be 10 by 10 microns, okay, so it's fairly small. So what we do here is we assume that we have these reactions on above this exposed patch of subendothelium and what we do is we imagine that inside this small region that all of these clotting factors and platelets and things, since it's a small injury, we imagine that it's homogenous. So everything is very well mixed, and if it's well mixed then we can just track these concentrations in time. We don't need to track their spatial variations, because we imagine that it's homogenous in this small region.

So we can -- so how we do this is we track these using ordinary differential equations. We can track the populations and how they change in time. All right, so that's within this reaction zone. But then we have to think about how things are moving into this reaction zone and how things are being transported out of this reaction zone. So we're talking about modeling this under flow, this is what happens in the body. So we assume here that the advective and diffusive transport in and out of this reaction zone just occurs at a rate proportional to the difference in the species of concentrations in the bulk

and in the reaction zone. This is a very simplistic way to represent this transport and I'll talk about a more complicated way in the next model.

But ultimately what -- the results that we get out of a model like this is we can track the concentration of something within this reactions zone in time. Okay, so this is time and this would be thrombin concentration that's being produced in this reaction zone, and there's three curves here, so this would be may be from three different situations. In this case it happens to be three different flow rates.

Okay, so this is the first model, so let me just get right to the results, since I don't have a lot of time, investigating the impact of Factor XI. So what I'll show you here is one plot. So I looked at two cases with this model. So the first case is with a lower wall shear rate. And when there's a -- you can just think of this as a low-flow situation. And in this case it's not that there's a constant concentration of platelets. So the platelets were distributed fairly uniformly across the vessel wall.

So what does this mean in context of this

particular model? Well, since we're looking at just a small region near the subendothelium we can just represent the bulk concentration of platelets, in this case 250,000 platelets per micrometer, we can just represent that concentration near our injury. So that's what we're assuming here in this situation. So let's take a look at the graphs.

So this is -- what we're looking at here is thrombin concentration after 10 minutes and after 20 minutes of the subendothelium being exposed to the blood. So the two different cases of the solid is with Factor XI in the system and the dashed lines are without Factor XI in the system. So what can we see here? Well, 10 minutes if we compare with and without Factor XI we don't really see a whole lot of difference.

Oh, I forgot to mention that down here this is tissue factor density exposed. So this is one of the things that we're varying. We can vary how much tissue factor is actually embedded in the subendothelium and exposed to the blood. Okay, so what we see here is after 10 minutes we don't see a whole lot of difference between having Factor XI in the system and not having Factor XI in

the system.

But as we bump up to 20 minutes, we actually see that for a certain range of tissue factor, fairly low range, between three and say seven, we can see that there's a pretty significant difference. This is on a log scale. There's a pretty significant difference in the thrombin concentration in that reaction zone after 20 minutes. So why do we only see this after 20 minutes? Well, at low tissue factor and low wall shearing the thrombin is actually being created fairly slowly because there's not a whole lot of tissue factor. So it takes some time for the thrombin to build up and in this case Factor XI is activated by thrombin. So Factor XI actually follows the initial thrombin production. So if the thrombin is building up slowly with low tissue factor, then that Factor XI (a) is being activated after that thrombin is produced and then it has the effect of creating even more thrombin through the formation of more IX (a) and vastly more X (a)s.

Okay, so this was the first result. So now let's think about the higher wall shear rate case. So at a higher wall shear rate, it's known that the platelet

distribution across a vessel is actually non-uniform. So as the flow rate increases there is a higher concentration of platelets near the wall of the vessel. This has been seen in vivo and in vitro studies. This is called a near wall platelet excess. So in this particular model since we're just looking at a small injury what this amounts to is just changing that local concentration of platelets near the wall. So what kind of effect does this have on the system? Well, what it does is if there's a higher concentration of platelets, then you're actually paving over the subendothelium more quickly.

And what that does is it decreases the activity at the subendothelium namely the activation of Factor IX by the tissue Factor VII (a) complex. So after 10 minutes over all the ranges of tissue factor, you can see that again there isn't a whole lot of difference between having Factor XI and not having Factor XI. But once you get up to 20 minutes we see something different than what we saw before. Now at a high range of tissue factor, we see that adding Factor XI into the system makes a very large impact in the thrombin concentration that we see in the reaction zone. Okay, so again why is this happening after 20



minutes, but not after 10?

Well, let's think about what I just mentioned about paving over the subendothelium. So if you pave over the subendothelium and you only have a small amount of tissue factor exposed, you're not going to get much thrombin production at all. But if you have enough tissue factor to sort of handle all the higher concentration of platelets, you're still going to make this slow production of thrombin before it's completely covered over by all those platelets. So in fact you need to have the higher tissue factor to get this system going.

And then once it gets going, the Factor XI sort of takes over and adds this extra burst of thrombin at the end. So the first two points are basically what I just mentioned, but -- so without Factor XI the higher platelet density near the vessel wall paves over the subendothelium and the IX (a) production at the subendothelium is quickly shut down. With Factor XI even though much of the subendothelium activity is shut down, when the tissue factor is relatively high, thrombin being slowly created activates some XI thus creating an alternative pathway for IX (a) production and this leads to much higher thrombin

production.

So essentially the XI (a) is creating this reactive surface for more IX (a) to be created as the thrombin is built up rather than just having to have the IX (a) being created only at the subendothelial region. All right, so from these results we predict that the sensitivity of thrombin production to the plasma concentration of Factor XI depends strongly on both the level of tissue factor exposed by injury, but mostly the interesting thing we found was that this really depends on platelet concentration or platelet count, which might not be something people have thought of before.

All right, so now let me just briefly tell you about the next model. So in this one I'll just briefly tell you about the spatial model. So using the spatial model we're going to investigate the addition of plasma Factor XI (a). So now we're looking at something a little bit different. So if we're adding the Factor XI (a), then we don't need to wait for that thrombin to be produced because the XI (a) is already activated. Okay, so let's just talk about what we see here in this slide. So this is just a simulation from one of the runs of the spatial

model. What you see here is a build up of -- these are actually -- this is a concentration of activated and bound platelets and these white arrows represent the flow. So what we can do with this model is track the concentration of all these species I mentioned before, but we can now track this -- them in space and in time.

So the nice thing about this is we have the ability to model larger injuries which just amounts to a larger region of exposed subendothelium and we can explicitly model the growth of this thrombus and how it's coupled together with the local flow dynamics. So as I mentioned we started doing this because Dr. Hoffman asked if I could see what would happen if I added levels of Factor XI (a) into the system what would I see? So what I did is I just took seven different values or seven different concentrations of Factor XI (a) to see at what point I would see anything happen, what -- when is it going to matter.

So what I'm showing you here is just an increase in the thrombin production as a function of added Factor XI (a) into the upstream concentration of the plasma. So this is a constant concentration that's added into the

system. So over here this is the high tissue factor case after 10 minutes and 20 minutes and here this is the low tissue factor case after 10 minutes and 20 minutes. So in each one of these cases what I'm reporting here is the total amount of thrombin that's produced. I'm not just talking about the concentration now, I'm talking about the total amount of thrombin produced. And what I'm doing is I'm just comparing it to the case when there was no added Factor XI (a) into this system at all. So these are normalized. So when this is at 1, this basically means that there was -- I'm just comparing it the base case with no added Factor XI (a).

So what you see is for a high tissue factor level exposed -- okay, and then on that -- on the X-axis here, this is the added amount of Factor XI (a). So this is 10 to minus 2 all the way up to I believe it's 10 to the 4. This would be like 10 nanomolar. Down here it's 0.01 picomolar of added Factor XI (a) into the system. So in all the cases at right about 1 picomolar is where the behavior seems to start to change as far as thrombin production goes. But the other interesting thing to notice here is that at high tissue factor, even adding 10

nanomolar of added -- of Factor XI (a) we've only increased our total thrombin production by about three-halves, and after 20 minutes it hasn't really increased much over three-halves.

But in the low tissue factor case, we can see that after 10 minutes compared to the base case with that low tissue factor, we've increased three-and-a-half times our total thrombin production. And then -- so that's a significant amount. So what this says is that when there's just a small amount of tissue factor exposed, if you add in Factor XI (a) into the system, it actually makes much more of a difference relative to what would happen if there were no Factor XI (a) within the low tissue factor case.

So I just wanted to show you some spatial plots of the actual thrombus that forms in these cases. So what I'm showing here at the bottom is this is with no Factor XI at all in the system, okay, and you can see that you get this thrombus that forms, but it pretty much stops after some time. And this goes up through 20 minutes. Having Factor XI in the system, but no added XI (a) you get a fairly large thrombus that forms. In this

particular case we're talking about we have a near wall excess and this is a fairly high flow rate. If we bump this up to 1 picomolar, again these two cases don't look all that different, although if we bump this up to 10 nanomolar, we can see that we get a much larger thrombus that forms.

And then the last thing I wanted to mention was just we can also -- what -- another thing we can do with this model is of course look at spatial distributions of all of the different clotting factors in time and in space as they form. So I just wanted to note again in these same four cases, there's no Factor XI, no Factor XI (a), 1 picomolar of added XI (a) and 10 nanomolar of XI (a).

This -- what we're looking at is the spatial distribution of the tenase complex. So this is the activated Factor VIII and activated Factor IX complex on bound platelets. So in this case you don't have a whole lot that is created, but it's enough to get the system going. And then as you start to increase by adding Factor XI you can see that after 20 minutes we get a more substantial amount of this tenase complex. Again it doesn't look a whole lot different when you jump up to 1

picomolar, but by the time you get to 10 nanomolar, we can see a huge difference in the amount of tenase complex that's able to form from adding this extra Factor XI (a).

So the major points I just want to make, just -- let's just recall that Factor XI is dependent on the initial thrombin generation in order to make more IX (a). So XI (a) gets activated by the thrombin, has to be activated by thrombin first in order to go ahead and activate the IX. But if XI (a) is already activated when it's added into the system, it does not depend on the thrombin and it can activate the IX right away. So the two points I just wanted to make here is that what we found is that the amount of impact of Factor XI on thrombin production is associated with platelets. Specifically what we found is that it's platelet concentration, but also levels of exposed tissue factor. And secondly certain amounts of added factor -- added plasma Factor XI (a) when only small amounts of tissue factor is exposed and they significantly enhance the procoagulant activity from what it was when there was no Factor XI (a) added in the system.

Okay, and with that I'll just acknowledge

everyone in North Carolina and my funding in, because I didn't have a whole lot of time to go to the mathematics, I left you with these references of models and I'm happy to take questions or talk more about this with anyone later.

(Applause)

MR. OVANESOV: Thank you very much. We are almost on time and we have time for one question.

SPEAKER: Yes, have you done this modeling taking into consideration inhibitors of XI (a)?

MS. LEIDERMAN: Yes, actually the chemical inhibitors, I've looked at a few different and it turns out that the inhibition just by flow itself is actually a higher amount of inhibition than the chemical inhibitors. So the fact that the flow can take things away actually makes more of an effect than the chemical inhibitors in this particular system. I haven't -- we haven't looked too much at that in the small model, but it's the same thing if you look just at the rates that the -- if you just look mathematically at the rates of inhibition by the chemicals versus being transported away by flow, you can see that the flow rates are higher.



SPEAKER: Thank you.

SPEAKER: By chemical inhibitors, are you talking about the naturally circulating inhibitors, or you're talking about the added inhibitors?

MS. LEIDERMAN: I'm talking about the natural --

SPEAKER: Circulating --

MS. LEIDERMAN: -- circulating ones. So that there's a number of them. Can I remember their actual names, right now? No, C1 --

SPEAKER: C1 esterase inhibitor.

MS. LEIDERMAN: -- esterase, there we go.

SPEAKER: So you -- those are all accounted for in your models?

MS. LEIDERMAN: Yes, I had a list of four or five and I looked at all the different rates, and I compared them all, and I actually added them in and they didn't make much of an effect at all.

SPEAKER: If you go back four slides --

MS. LEIDERMAN: Which --

SPEAKER: The one before this, this one. So I'm struck by this slide in that I don't take account into the no Factor XI graph because people have naturally

circulating levels of Factor XI, so I don't think that's particularly representative and then when I compare the other three I have to say they don't look remarkably different. Your comments?

MS. LEIDERMAN: Yes, so they don't look remarkably different, but I will mention one thing that there are boundary conditions in this spatial model in which this is actually the -- this is the edge of the boundary, and what I should have actually done here is run it longer with an extended region out here and I think we would have seen a little more upstream growth in this top one, and it probably would have grown a little bit bigger.

These are fairly new results, but I mean, what you can see just from looking at the beginning is that this guy if I slow it down here, you can see that the top one is growing a lot faster just from the beginning and --

SPEAKER: Well, I'll point out that 10 nanomolars is lot, lot more than 1 picomolar.

MS. LEIDERMAN: Yes, yes, I know. I -- what I wanted to do was pick the range from the graphs from before where we see it sort of starting to change rapidly and just look at the extreme case, so.

SPEAKER: One more question, I'm sorry, but --

MS. LEIDERMAN: That's fine.

SPEAKER: Have you validated any of this with any in vivo work?

MS. LEIDERMAN: With in vivo work?

SPEAKER: Sure, by monitoring the rate of growth of an induced --

MS. LEIDERMAN: Well, I don't know of a lot of data out there as far as monitoring this. I mean, what I have seen is some of the studies that have been done by DeFeuris (phonetic) looking at the in vivo data, but what has been validated is the -- the non-spatial model has been validated as far as looking at the threshold behavior of the amount of exposed tissue factor to thrombin production. So that has been validated, but --

SPEAKER: In vivo you've been -- you've validated?

MS. LEIDERMAN: Not in vivo, those were in vitro studies done by the Diamond Lab in Pennsylvania.

SPEAKER: Thank you.

SPEAKER: Yeah, my question goes in a similar direction. If you look at this slide or the other one

that we looked at repeatedly we always -- I always have --

MS. LEIDERMAN: This one again?

SPEAKER: Yeah, this one again.

MS. LEIDERMAN: Okay.

SPEAKER: It's a beautiful slide because when we look at it we see that you can add Factor XI (a) at a certain amount. In this case, it's 1 picomolar in your model and apparently it doesn't have an effect on thrombus formation. So my question is do you think your model is predictive to give us some estimate of acceptable threshold levels of (inaudible)? What other limitations are there, mathematic model?

MS. LEIDERMAN: I think that what the mathematical model is doing is sort of pointing out new things that we could look at, specifically the platelet count and injury size, things like that. I'm not going to claim that it's necessarily predictive for what's happening in vivo. I mean, we've set up this model to basically be able to be -- represent it in a lab in vitro. So we've set up the particular flow rates and things like that so that it could be something that could be replicated in a laboratory setting hopefully so that it

can be tested. I don't know anybody who can test this kind of thing under flow, so if anyone knows anybody who can do that I would love to talk with them.

But I'm not going to claim that it's going to be a certain level or not, but I think that what we can see is that the different levels of Factor XI (a) certain are changed over the range of concentrations in which we add, so.

SPEAKER: May I ask a quick question? You said that you didn't see a significant effect or you saw a smaller effect of serine protease inhibitors in solution inhibiting XI (a) compared to the flow itself. Does the model take into account that XI (a) may anchor itself to the components of the quat or is the XI (a) free in solution in this?

MS. LEIDERMAN: Oh no, it binds. I mean, it's activated most -- it's activated while bound to the platelet surface. So once -- as this thing is building up, we're tracking not just concentrations of things moving around in plasma, but also things that are bound to the platelet. So we don't consider that -- one thing I don't consider though is Factor XI being inhibited if it's

bound to the platelet, because I believe from what I've read anyway that these inhibitors affect XI in plasma, but not when they are bound to the platelet surface. I could be wrong but. So I consider that when if XI is bound to the platelet, it's not inhibited by those, only in plasma.

SPEAKER: I see.

MS. LEIDERMAN: It could change if I were to include the inhibition if it was bound to the platelets and if you have information on that, I'd love to hear about it.

SPEAKER: Okay. Thank you.

MR. OVANESOV: Thank you. This concludes session number -- section 2, and we have time for a short break, 10 minute break and please come back at 10:15.

(Recess)

DR. GAILANI: Okay, in the interest of keeping on time here, we're going to start with our next series of talks which are going to be on laboratory investigations of products associated with thromboembolic events. And our first speaker is going to be Dr. Mikhail Ovanesov from the FDA.

Mikhail?

MR. OVANESOV: Great. I'm really excited to talk here today because this will be the first time that we make our data public. We did share our data with other regulatory agencies and some companies in a timely manner but today will be the first time that we present the full story in one place and it so happened that our lab was the first regulatory lab that applied thrombin generation test to analysis of immunoglobulin products and also it so happened that we've been the first to find factor XIa in those implicated lots.

But we became involved in the thrombin generation test almost by accident. We don't do load release test and neither we do product monitoring. Rather our lab is doing basic research on the regulation of blood coagulation and at the -- about a year ago, we had a project on the regulation of clot formation by factor XIa. And I will quickly go through the biology of the problem because it is important for the thrombogenic investigation.

So blood coagulation in a sense is a very simple system, a cascade. It can be activated by two pathways either extrinsic pathway or conduct pathway when factor XII binds to any -- several artificial surfaces and is

activated into factor XIIa. Kallikrein and PK (phonetic) are also generated as well as high-molecular weight kininogen is activated. However people who are deficient in PK, HK and factor XII do not bleed.

Therefore it is currently believed that the conduct path for blood coagulation is either artificial or at the very least is not involved in normal homeostasis in vivo. Factor XI on the other hand, is important for homeostasis because factor XI-deficiency causes bleeding tendency although inconsistent and mild. In the tissue factor pathway, the only way how factor XI can be activated is through a slow feedback loop through -- from thrombin.

Therefore, contribution of factor XI is secondary and can be seen only at lower tissue factor concentrations. In this experiment, thrombin generation was studied in factor XI-deficient plasma supplemented with increasing concentrations of tissue factor in the presence or absence of factor XI, in the presence and conduct pathway inhibitor. And you can see that at high concentration of tissue factor, there is no difference in thrombin generation while low concentration of tissue factor, factor XI makes a big difference.



So when we received several lots implicated in thrombotic events, we decided to use the same experimental system. This slide shows data obtained on four lots of one product, two lots shown in red were implicated in several thrombotic events. The slide shows data obtained on four lots of one product two lots shown in red were implicated in several thrombotic events. These lots are blinded as lots a and d, and lot c was implicated in non-thrombin adverse events. Lot b was a control lot. That was the first experiment we did and it answered a lot of questions.

First of all, we found that implicated lots have high procoagulant activity. Secondly, this activity was dose-dependent. As you can see, each sample was tested either undiluted or diluted four times with buffer and we also found that procoagulant activity is not sensitive to the concentration of tissue factor used for activation.

Therefore procoagulant substance found in implicated lots can promote or induce coagulation in factor XI-deficient plasma. In order to understand the difference between tissue factor dependent and independent activities, we use the second coagulation test. This is videomicroscopy of clot formation and in this test,

recalcified plasma is placed in a small micro-chamber and then plasma comes in contact with tissue factor bearing surface that we call activator. It's a plasmin with tissue factor nebulized to it.

And then we can see how clot is growing on the surface of tissue factor and monitor this process using light scatter and videomicroscopy. On this slide, on each video file, you will see two types of clots. One clot on the surface of tissue factor bearing surface is growing from this tissue factor bearing surface. It's tissue factor dependent. Another type of clot in the volume of micro-chamber and it's tissue factor independent.

So again, implicated lots a and d caused excessive procoagulation all over the chamber. This coagulation was tissue factor independent and also these lots led to the increase in the rate at which clots grew in size. You can see it's here for example. These clots, they grow faster than in the control sample. Now from this experiment, we understood that implicated lots are highly procoagulant and we decided to compare how these lots differ from other products on the market.

I want to tell you that this experiment was done

in August of 2010 and I presented to you to show how our investigation progressed at that time. Since then, we identified several other products with potential high-procoagulant activity and I will speak about them later. In any case, at that time it looked like implicated lots are very high compared to other products on the market and even to other product -- other lots of the same product.

So we investigated the nature of this activity using a single coagulation factor-deficient plasma. We encountered -- we found two types of responses. For example, in PK-deficient plasma, implicated lots were higher than non-implicated and lot c was higher than lot b. In contrast, in factor X-deficient plasma, there was no difference between implicated and non-implicated lots. Also actually there was no thrombin generation at all. This is background.

Similar results were obtained in multiple other plasmas. No inhibition of procoagulant signal was found in normal pool plasma XII, PK XI, VII and PAI1-deficient plasma and procoagulant activity was gone in the IX, VIII, X and V deficient plasma. This slide summarizes the results of experiments on single factor-deficient plasmas.

First of all, deficiency of PK XII, XI and VII didn't block the activity and this means that enzymes of the conduct pathway are unlikely to contribute much to the procoagulant activity of implicated lots.

Then deficiency of X and V block the activity. This means that factor X and thrombin -- activated factor X and thrombin are unlikely to contribute much. Same with factor IX and VIII and now we can see that factor XI and VIIa are unlikely to contribute to the activity as well. This leaves only factor XIa and retrospectively this is not surprising because factor XIa is involved in multiple procoagulant pathways within the intrinsic coagulation cascade and extrinsic pathway as well. It is interesting that two of the coagulation reactions of -- that involve factor XIa were described only very recently.

Now in the next experiment, we tried to compare those response in the thrombin generation test to purified factor XIa or implicated lot that was diluted in the non-implicated lot and those responses were essentially identical. Now next thing we did, we spiked factor XIa into five different products, non-implicated lots or lots of the same product that was non-implicated and we received

essentially the same result.

This means that different lots differ in their activity because factor XIa, they have different activities for factor XIa in those and not because factor XIa is somehow inhibited in some products and not inhibited in others. Now in the next experiment, we tried to inhibit factor XIa-like activity in the implicated lot. CTI, which is inhibitor of factor XIIa didn't block the activity in the thrombin generation test in factor XI-deficient plasma.

Kallistop which is a inhibitor of kallikrein didn't inhibit the activity either. However C1 (phonetic) inhibitor did block the activity partially. When we repeated similar experiment without addition of lipids, monoclonal antibody against factor XIa was able to block the activity completely as well as Alpha 1-antitrypsin and Alpha 2-antiplasmin. Collectively these data show us that those inhibitors that can block activity of factor XIa whether these inhibitors are specific or not, are able to block the activity of implicated lot, not completely.

Those inhibitors that do not block XIa activity, they are not able to block the activity of implicated lot. Now the inhibition wasn't complete in the presence of

lipids and I would say that this is typical for inhibition of factor XIa. It's really hard to inhibit the activity of XIa completely either with one inhibitor or several inhibitors combined. And we tried different concentrations of inhibitors in different times of incubation.

We also did a second experiment in which we used a different experimental system. We used a fluorogenic, chromogenic substrate called SN13a. This substrate is very sensitive to factor XIa and I will speak about the substrate later. However the substrate can also be cleaved with factor XIIa, PKA and kallikrein. Now when we used this reporting system, we found that Kallistop can actually block only partially the activity of some of implicated lots.

This means that some of the implicated lots or products can also be contaminated with kallikrein. Whether this kallikrein has procoagulant activity is not clear from these experiments, of course. In the next experiment, we compare the factor XIa-like activity measured using this SN13a substrate to the thrombin generation test activity and we found really good correlation for the four lots that we had in our hands at that time.

When we measured the antigen of factor XI and we know that our ELISA is sensitive to both factor XI zymogen and factor XIa enzyme, we found that the level of factor XI in lot c was higher than that in the implicated lot a, which means that factor XI antigen does not predict procoagulant activity. Now in the recent months, we found four products with potentially measurable procoagulant activity and I'm presenting the data on approximately 170 lots here.

In each case, there was a good correlation between thrombin generation peak measured in factor XI-deficient plasma and factor XIa-like activity measured using a SN13a substrate. However in some cases we had clear outliers. Some lots had very high factor XIa-like activity compared to the thrombin generation. We suspect that this is because there was another contaminant that can be present in those lots and factor XIa substrate SN13a is not very specific.

In the next experiment, we tried different enzymes that are related to the blood coagulation cascade and virtually all of them are able to cleave the substrate. Some enzymes are more -- the substrate is more sensitive to

some enzymes. For example, XIa is indeed the most preferred enzyme for the substrate. However, almost all enzymes were able to cleave it. And this provided us with a unique opportunity to investigate the nature of the procoagulant activity of implicated lots even further.

The boundary of the grey area on this graph is provided by the activity found in non-implicated sample b and implicated sample d. This means that if for example, all the procoagulant activity that is found in the implicated lot is provided by plasmin, for example, then we should expect to see between 2 nanomoles and 20 nanomoles of plasmin in the product. We did this kind of analysis for the enzymes and found the ranges of enzyme concentrations which are relevant for immunoglobulin product in question.

And we tested this concentration in the thrombin generation test and we found that only factor XIa, factor Xa and factor IXa are able to induce procoagulant response within the relevant range of each enzyme. Of course, if you use a very high concentration of enzyme, for example, kallikrein here, you can induce procoagulant response. However this response would be in some sense irrelevant for



the immunoglobulin product because it's way above the activity measured using non-specific substrate and also one cannot really exclude the contamination of Kallikrein with Factor XIa.

Now, in 170 lots of 4 products that we tested so far and we tested actually 300 lots of many products, but only 4 products are shown here, we had about 40 lots that had been implicated in thrombotic adverse events. Some of these lots were implicated in more than one event, but all of them are shown here. And you can see that again there is a very good correlation between thrombin generation peak and Factor XIa-like activity.

However -- and I think this is the most important part of my presentation, you can see that activity alone does not predict the event. Not all of the highest activity lots were implicated of the thrombotic events, and not all of the lots that were implicated in thrombotic events had high activity. However, on the other hand when we pooled all the implicated lots together, we found that the absolute majority of implicated lots had in fact high activity as measured with the thrombin generation test.

And high means that the activity was equal to the

activity -- to the half of the activity that we found in the implicated lot D; half activity and above and it covers most of the implicated lots. I need to warn you about the limitations of this kind of analysis. Only small fraction of non-implicated lots was tested by us.

Data on lot-specific thrombotic clusters that is cases when multiple thrombotic events were reported for only one lot were not analyzed by us separately on this graph, and also when we pooled together implicated lots from different products, we essentially disregarded any differences in doses and other differences in patient populations that are receiving different products.

So in conclusion, we found that Factor XIa is the primary procoagulant contaminant in immunoglobulin products. Other relatively non-procoagulant contaminants, that is Kallikrein and Factor XI, are also found in implicated lots. Products have different Factor XIa activities which is likely due to variations in the manufacturing processes. Many of the thrombotic adverse event implicated lots have high levels of Factor XIa-like activity and thrombin generation test measured activities.

And -- however procoagulant activity alone does

not predict -- if this particular lot will be listed as the one with reported thrombotic events, so we think that host factors are likely important as well. The majority of this test, of the lot testing has been done by Samuel Woodle in my lab. And this project was started by Alexey Shibeko who also did many inhibitory experiments and many titration experiments.

The sample -- we received the samples from products and those samples were forwarded by Christine Anderson and we received products from Mei-ying Yu Lab, and all communication with companies and selection of lots was done by Dr. Dorothy Scott with full support of our office, directors. And we -- I wish to thank manufacturers who provided samples for testing and we kept all the samples blinded for this reasons. Thank you very much for listening to my presentation.

(Applause)

SPEAKER: Questions, please.

SPEAKER: One problem usually working with low molecular weight substrates is just relative specificity for selected or specific enzyme. And that what you showed then, we published some data about that substrate which has

about the same activity for thrombin and XIa. But even -- and you showed -- you tested a few enzymes.

But the problem is that working with biologic systems very often, at least in other hands, there is amidolytic activity protelysing (phonetic) small substrates but no procoagulant activity of -- in that system. That happens as well. So that might be lack of correlation with thrombotic events and XIa activity or so-called XIa activity or substrate proteolytic activity, let's call it, that could be because of contaminants.

Now another question, maybe I'm wrong, but your conclusion that it is XIa is mostly on eliminating other possibilities, not direct evidence that it is XIa activity. For example, I guess you mentioned that -- you have an antibody to XIa. Is it really specific for XIa?

MR. OVANESOV: Yes. Yes.

SPEAKER: That's not -- okay.

MR. OVANESOV: Yeah.

SPEAKER: Thank you.

MR. OVANESOV: We try to do these kind of experiments.

SPEAKER: You have a question here?

SPEAKER: Yes. So we've correlated Factor XIa with arterial thrombosis. I was wondering if you have any upcoming studies on increased viscosity and its correlation to venous thrombosis?

MR. OVANESOV: This investigation was focused on in vitro assays only. And in fact what I'm showing here is in some sense a byproduct of the assay development that we've been performing when we've been characterizing different products on market. So we hadn't done experiments on animals but you will see several presentations later today and maybe tomorrow that show experiments on animals.

SPEAKER: We have time. One more question.

SPEAKER: Just so I understand, a question; if you eliminate all Factor XI from these lots, you would still see thrombotic events occurring and what is the frequency of that in those lots that have no factor?

MR. OVANESOV: Okay. I'm -- I -- it's a very interesting question. I am struggling with answering it because it -- I don't want to reveal product related information. But let's put it this way. It looks like that the products that have very low procoagulant activity

in our in vitro assays also have very probability to -- or very low rate of thrombotic events.

Now, when you have a product that is produced using different manufacturing methods, the ones that lead to different demands of Factor XIa-measured activity in the lots, you would find that lots that have low Factor XIa activity produced using second method, they have lower rate of thrombotic events compared to the lots produced using the manufacturing method that generates high XIa activity in the lots. That's the extent of my answer.

(Laughter)

SPEAKER: I think we need to move on to the next talk. Thank you very much.

MR. OVANESOV: Thank you.

(Applause)

LABORATORY INVESTIGATIONS OF INTRAVENOUS IMMUNOGLOBULIN  
(IVIG) PRODUCTS ASSOCIATED WITH THROMBOEMBOLIC EVENTS

SPEAKER: Our next speaker is Johannes Dodt from the Paul-Ehrlich Institut.

MR. DODT: Sorry for the delay. Good morning,

ladies and gentlemen. I'd like to share some results of our investigations into the batches involved in thromboembolic events and in others which were not involved. And the work was done by the people that sit on the first slide and I have the pleasure to present the data this morning.

The Paul-Ehrlich Institut is doing batch release on the IVIGs and within this procedure, there were no objections found with any of the batches involved in thromboembolic events. And when this issue came up, additional samples were requested and tested for additional parameters like physico-chemical parameters, anticomplementary activity, Fc function, sterility, pyrogens, but nothing suspicious was found.

At that time we got involved. I'm from the Department of Hematology and Transfusion Medicine and we did an extended testing including global tests for coagulation factors and we wanted to identify the amidolytic activity or activities observed in these concentrates and we did immunochemical assays.

So first of all, I'd like to present some data on the global assays. We use the modified Thrombin Generation

Assay for this purpose as well as the non-activated partial thromboplastin time which is a pharmacopoeial assay required for prothrombin complex concentrates and Factor IX concentrates according to the European Pharmacopoeia. This work was published by my colleagues and I have the honor to present the data.

First of all, the system in which they analyzed the batches was build-up by plasma, substrate plasma where the plasma was -- or the blood was collected in CTI so to exclude any activation of the contact phase activation system. And you see here when you trigger this plasma with different amounts of Factor XIa, you see a nice dependence of the thrombin peak from the time.

But after having -- after a certain threshold, let me see, of about 0.5 picomolar Factor XIa, you receive the same amount of thrombin and only the time to peak differs in the system. When you plot the data, time to peak against Factor XIa concentration, you get a nice correlation. Linear response -- those response curve ranging from 0.1 picomolar to about 1,000 picomolar. And you see there is a very, very good correlation.

We have used this system to analyze different



batches involved in thromboembolic events, but also batches from the same manufacturer where no adverse events were reported and we analyzed also batches from other manufacturers. You see in blue the control. There's no activation or thrombin generation in the control. The system was not triggered with tissue factor.

It was only triggered by the addition of calcium and phospholipids in the sample. And to see that IVIG batches from other manufacturers hardly give any response in this TGA system and with batches from the manufacturer implicated, you see a wide range of time to peak batches. Some -- and you see batches, I've put in this slide only one, which has a time to peak of about 10 minutes or below. And there are others ranging from 10 to 30 minutes.

Using the non-activated thromboplastin -- partial thromboplastin time, you see here some lots we investigated. In red, the line 150 seconds is a threshold given by the European Pharmacopoeia for the prothrombin complex and the Factor IX concentrates. Batches which would give a lower time to clot times would fail this assay.

You see here batches of the manufacturers -- of

the manufacturer where batches were involved in TEEs, but in blue, batches from other manufacturers. And the batches with red arrows are those which were involved or where we have reported cases of adverse events. One batch with a red star that was also analyzed, but this batch was not on the market, you see that all the batches involved in thromboembolic events would fail this assay.

We did a correlation between the time to clot and the time to peak of the TGA and you see that the -- both tests would recognize suspicious batches. On the other hand, the NAPTT has a disadvantage that it has only a narrow range whereas the TGA has a wider range. And you can get a intermediate result or results for intermediate batches as well. The limit of the NAPTT is the clot time of the control that usually is about 250 to 300 seconds.

So the next question was the identification of amidolytic activities and we applied for this assays for specific correlation factors. We especially focused on prekallikrein activator, kallikrein and factor XIa.

The PKA assay, the prekallikrein activator assay is also mandatory for IVIGs. And this result may not be seen in correlation to the thromboembolic events, but I

would like to show the data. When you do the assay you have a blank, which means yet that you do not include PKRs reagent for the prekallikrein activator.

And you see here that the batches or some of the batches reduce the blank activity and that is very -- not funny -- it is just an observation that batches involved of this manufacturer reduce the PKA activity and this is just an observation. I cannot tell you whether that means anything to the thromboembolic events.

This is not seen with, let me say, the batches number 4, 5, 6 where you hardly can see any blank activity or PKA activity, and 20, 21 you have very low activities regarding kallikrein. But I have to admit this is a chromogenic substrate assay and they are not very specific.

Next we wanted to identify amidolytic activity and we took one of the batches involved in the TEEs. This is batch number 10 on our slides. We use the chromogenic substrate which is, let me say, specific for kallikrein, but also another substrate which has a broader range of specificity according to the manufacturer that also detects to a tPA, kallikrein and so on.

And you see here, we applied several inhibitors and made a dose dependant evaluation of the activity against these substrates. And you can see that one good inhibitor, plasma inhibitor, the C1 inhibitor, regardless whether you -- with both of the substrates 83, for example, is not a good inhibitor at all and alpha-1 proteinase inhibitor does also not inhibit activities in the range applied.

On the other hand, please look to the yellow -- to the blue circles. You see here's activities we found with PPACK II. This has a substitution of toluene versus phenylalanine with respect to PPACK I. And you see here that was substrate S-2288. We cannot inhibit all the activity with PPACK II.

We then wanted to find out which possible proteases -- protease could be involved in this and therefore we used several proteases like kallikrein, XIa, plasmin, Xa, 2PA. And the batches involved as well as factor VII-activating protease we call that HABP for historical reasons.

And with -- you see here the activities we observed with the two substrates, and again the PPACK II

cannot inhibit the activity of IVIG batch number 10 as we saw before, but also factor XIa is not inhibited at all with this substrate.

Then we went on and yeah we went back to the chromogenic activities we identified with, you know, within PKA assay. You see here we made an ELISA for the prekallikrein antigen and we used a similar approach to detect the kallikrein activity. We did immunocapture of the kallikrein and then we measured the activity with a chromogenic substrate in this case, S-2302.

And you see here that we found upto 60 nanomolar antigen, prekallikrein antigen but as well up to 40 nanomolar kallikrein activity mostly of batches involved of the highest content of activity we observed in batches involved in TEEs.

But, for example, look also to the batch number 21. We have a high prekallikrein antigen present, but we have only -- or we have no kallikrein activity in this batch. Okay, we did the same with factor XI. Here we looked for the factor XI antigen by an ELISA and we did also an immunocapture of factor XIa, of factor XI and factor XIa and we detected the activity by S-2288

substrate which is obviously clipped by factor XIa.

And we did as well factor XIa clotting activity which you can see here in red. The batches indicated with a star were all analyzed in this factor XI PTT. However, where you can't see any red bar there isn't any activity found. So you can see here that we found up to 14 nanomolar factor XI antigen. Most of the data show, let's say, 4 to 8 nanomolar and on the right hand scale you see the nanomolar factor XIa and the blue which relates to the blue and red boxes are columns and we found here up to two to three nanomolar factor XIa but most of the batches involved in TEEs have around one to two -- one and a half nanomolar.

We made a correlation of the NAPTT clotting time with the other tests we applied. And as the most significant correlation we found is the XIa. Chromogenic activity, there were no significant correlation to the XI antigen to the prekallikrein antigen and the kallikrein activity. And another significant correlation we found to the time to peak in the TGA.

So our conclusions from these investigations are as follows. We have used a modified TGA that is able to

detect factor XIa in a range from 0.0 to 1000 picomolar. The read out parameter in this assay as we use it is time to peak. That is shortened to below 10 minutes for batches associated with TEEs. Batches from other manufacturers exhibit time to peak values above 30 minutes and the TGA is able to detect, let me say, suspicious blots whether they are related to thrombogenic events that is not the question.

Blots associated with thromboembolic events fail also the release criterion of NAPTT assay calling to the European pharmacopoeia. As I told you this test is mandatory for PCCs and factor IX concentrates. And NAPTT and TGA both identify the same batches as suspicious.

We can -- have seen that TGA separates three clusters of IVIG batches. That means those with -- associated with TEEs are below 10 minutes time to peak and others within let me say 10 to 20 -- 30 minutes. And those which were not suspicious have time to peaks above 30 minutes.

There is a good correlation of NAPTT and time to peak of the TGA. And another conclusion is -- another finding is that the PKA test shows background activity. I

cannot explain why this background activity is inhibited in one of -- in some of the batches as you have seen. That is a different question, but that could mean that the prekallikrein activator assay as it is described in the pharmacopoeia is not applicable for these immunoglobulins.

Factor XIa and kallikrein we identified as amidolytic activities in IVIG batches. According to our investigations factor XIa can be determined in the presence of PPACK II by its amidolytic activity versus substrate S-2288. But you should always keep in mind these substrates are not specific.

And there is good correlation of NAPTT and factor XIa activity. And our conclusion is that based on the data it is very likely that factor XIa is a thrombotic agent in these IVIG batches.

Thank you for your attention.

(Applause)

SPEAKER: We have time for one or two questions.

SPEAKER: Talking about activity in TGA you ever see thrombin generation if you have IXa in your system. It is quite efficient activator of coagulation system leading to thrombin generation.



On the other hand there are no good substrates or synthetic inhibitors, small low-molecular weight inhibitors for IXa. So your discrepancy between IXa inhibition by PPACK 2 and a lack of inhibition in the product could be related to the presence of IXa but not XIa

MR. DODT: Regarding the first part of your question, I think IXa would give a response in this assay. I have not done the experiments myself, but what I know from my colleague is that it would recognize IXa.

And regarding the second part of whether we can -- that means can we exclude IXa. I think we have made our assays specific because we have immunocapture before we add the substrate. The -- I think that is specification step for this assay and the antibodies do not recognize IX. So I -- in this case can exclude IXa activity.

SPEAKER: Thank you.

SPEAKER: I Just wanted to ask, what concentration did you test the products at -- for both NAPTT and the TGA assay?

MR. DODT: We diluted all -- you know, we had 5

and 10 percent IVIGs in our study and we diluted all to 5 percent.

SPEAKER: And then you tested neat?

MR. DODT: We tested neat and 1 to 10, I believe.

SPEAKER: And what is -- the percentage --

MR. DODT: The data are from the neat.

SPEAKER: From the neat?

MR. DODT: Yes.

SPEAKER: Can I ask a question? Do you know the IgM concentration into two lots where PPACK could not quench the signal?

MR. DODT: No.

SPEAKER: Right. I think we should probably --

SPEAKER: One quick one.

SPEAKER: What's the half life of your thrombogenic substances? So it wouldn't explain venous thrombosis would it?

MR. DODT: Could you please repeat the question?

SPEAKER: Yes, what is the half life after the infusion of factor XIa and how could it explain venous thrombosis?

MR. DODT: That question I cannot answer. Is there anybody who could answer that?

SPEAKER: This is not published by anybody yet.

SPEAKER: Okay. Thank you very much. We will move on to our next talk; Dr. Laurent Fleury will be speaking.

OCTAGAM, INTRAVENOUS IMMUNOGLOBULIN ASSOCIATED  
WITH THROMBOTIC EVENTS

MR. FLEURY: Okay. Thank you. First of all, I want to thank the organizer to invite me for this conference. It's a great honor for me to present the work that has been done in the laboratory of AFSSAPS. AFSSAPS is the French official medicine control laboratory, the French OMCL. That just mean that we participate to the batch release of blood product as (inaudible) and vaccine.

So this first slide here just to show you that we have -- for us the problem is specifically for Octagam. On the first table here, the number of TEE from the French

pharmacovigilance. On the second table, the quantity of all the IVIGs on the French market which were sold the last three years and on the last one here, you can see the right -- that mean the number of kilo which is supposed to give one TEE.

So when we realize this, we decided to perform tests on the Octagam and all the IVIGs available on the French market. So we choose ten batches qualified as TGA plus by the OCTAPHARMA, 10 or 11 batches qualified as TGA negative by OCTAPHARMA. And we find 8 batches involved in TEE. We could see -- we will see on the next slide that we scroll this TEE. So we have three batches that scroll TEE+++ and five with only one plus. And we took three batches of all the other IVIGs on the -- available on the French market.

So we have 29 batches of Octagam and 20 batches of other IVIGs. So we tested for the prekallikrein activator for an NAPTT, the factor IXa and factor XIa. Especially for XIa, we use the substrate S2366. And we know this one is not specific. This substrate could be cleaved, for example, by the kallikrein. And we use the TGA.

For this one, we use the CAT, the Calibrated Automated Thrombogram and the Thrombinoscop software. This is supplied by STAGO. And there's an important point here. We never use plasma deficient for factor XIa. We only use normal plasma. You have here the concentration of tissue factor and phospholipid, and all the final concentration of our IVIGs was 1 percent before we test it.

So the first thing we did here is to calibrate the TGA against factor XIa, and you could see here a good correlation for all the parameters of TGA. The time to peak, the lag time, the peak height, even the ETP which is the area under the curve. And for the velocity, the velocity you can calculate it by the formula. Here this is the peak height divided by time to peak minus lag time. And you could use this parameter also. So good correlation when we use standard XIa.

So very quickly, we have the question about the batches involved in thromboembolic event. And we decided to score this risk of those batches and here, you can see, for example, for the sample E here, this one is involved in eight thromboembolic event. So we decided to score this one as three plus and we calculate like this score for each

batches we have involved in the thromboembolic event.

So now here, on the right -- on the left, you have the 10 batches called TGA negative by OCTAPHARMA after the 11 batches called TGA positive by OCTAPHARMA and after you have the 8 batches involved in thromboembolic event. In blue, this is a five score TEE+ and in red the TEE+++. We don't have TEE++. And after -- you have all the other IVIGs available on the French market.

So for us here, it's clear that there is a difference between Octagam between these batches, this lot of Octagam and all other IVIGs available on the French market, especially for the PKA for XIa and IXa equivalent. For the NAPTT, it's not so evident for us. I think when I see the data from the pie, I think we have to work on our NAPTT test.

Okay, here the same sample in same order just for the result of the TGA. And here, same thing first. Sorry, I just forget something here. Here for the sample here, those one here, we have a signal for XIa and for IXa, and we don't believe this signal is -- in fact is, could be XIa or IXa. Just because when we dilute the sample, the signal increase and here you see, we don't find the signal, it

does not correlate to the TGA.

So now, when we try to correlate between factor XIa and the TGA, we don't find any correlation. So I really think our TGA -- something wrong with our TGA maybe. Here we try to do the same thing as our colleague from the pie and you see it is not so evident for us that the -- we could discriminate -- it's not possible for us to discriminate all the IVIGs by this method.

Here, just because we could for one batches here called TEE+++, we could find all the plasma pool used to product these batches, and we just have a look if maybe there's something wrong in all the plasma pool for factor XI in here. So there's no evidence for this.

Okay, and my first conclusion. There is a significant difference in several quality attributes for Octagam versus other IVIGs available on the French market. We have a good correlation with TGA and factor XIa when we compare to the standard. But when we correlate TGA and factor -- all the equivalent activity in the immunoglobulin, we lose this correlation decrease. However, XI TGA is systematically observed in TEE implicated batches especially when the TEE score with three

plus.

So when we say this, we decided to monitor to follow the quality of the new Octagam batches obtained by the improved manufacturing process and to compare this new Octagam with the Octagam TGA and the OCTAPHARMA TGA and the OCTAPHARMA AFSSAPS, and to compare for the -- all parameter. And first here, you could see the two method.

We have a good correlation between the two method for the four main parameter of TGA. And here when you -- the data in blue, this AFSSAPS data in orange, the OCTAPHARMA data, you could see the data dissention (phonetic) but they are not just possible. So we come back in this one after. We decided to calculate our own cutoff. Here, you have the OCTAPHARMA specification with a cutoff 11 minutes for lag time, 80 minutes for time to peak and 350 minimum for the peak. And we decided to propose our own cutoff on the limit and we took the mean of the 18 batches of the new Octagam and we add to standard deviation.

So this gives here the cutoff you could see in the blue line. The blue line is AFSSAPS cutoff and the orange line, the OCTAPHARMA cutoff. And you could see here



that the batches here, batches which is the most involving TEE are always exclude with the cutoff. Here there is one point, which is just on the cutoff for the OCTAPHARMA method, and with AFSSAPS, we always exclude this point.

Okay, so what about other IVIGs? We just here -- I just put back the same data you I showed you before. Here at the beginning, you have the batches involved in thromboembolic event. After you have the -- all the other IVIGs available in the French market and after the 18 -- the 19 batches of new OCTAPHARMA Octagam. You could see here that the cutoff exact for one here, for -- okay, for one immunoglobulin, for lag time and time to peak, all the other could be released with this cutoff.

And for the peak here, it's another one which is just a limit. So based on the TGA, the new Octagam appeared to be on the same range of the other IVIGs which are non-implicated so far in TEE and which are available on the French market. So -- but we don't know actually what product could be test with this -- shall we -- we use this test only for Octagam, for all the IVIGs and what about other immunoglobulin, subcutaneous immunoglobulin, for example.

And if another question is if the TGA method for monitoring thromboembolic potential is shall we use it in a routine test or just for the manufacturer in a quality control after an important change and what parameter we have to follow. Okay, so maybe there is a collaborative study and maybe we will have some answer to this issue on the afternoon. So I just want to thank all the people who participate to this study in the laboratory of AFSSAPS. Thanks for your attention.

(Applause)

SPEAKER: It's time for questions. Okay, if there are no questions, then we can move on. Thank you very much.

MR. FLEURY: Thank you.

SPEAKER: And our next talk is by Dr. Elaine Gray from NIBSC.

#### NIBSC INVESTIGATION ON THROMBOGENICITY TESTS FOR IVIG

MS. GRAY: Thanks. Thank you. I guess to start off, I'll just say that I think in the U.K. we were very fortunate and like our European colleagues and now our U.S.

colleagues, we didn't actually have any TEE event implicated with the IVIG we use although we have two batches which have been withdrawn. So it was actually quite difficult for me to look at the product and find out what we could do with it.

So we started off by really trying to set up some assay systems to look at the baseline level of different IVIG products that's licensed for use in the U.K. And we tested these products on factor II, factor X, XI, VII and also the inhibitors. And they all seem -- appears to be below the level of detection. However some of the products were found to have high kallikrein activity. Now, we also find that some of these products also have high factor XI clotting activity.

So we started looking at different homogeneity tests and we know the NAPTT is an established test for factor IX concentration. Indeed, it's also being used -- also used for factor IX concentrates. We look at a number of different substrate plasma, the normal plasma, XI-deficient plasma, XII-deficient plasma. And we essentially follow the EP assay method.

And here's examples of the results we get. The

top panel shows the effect of the Product Z in normal plasma, XI-deficient plasma, two different type of XI-deficient plasma, one freeze-dried and the other one is fresh frozen plasma and the XII-deficient plasma. And in the normal plasma tested from neat down to 1 in 10, as you can see that, that's a dosage-dependent shortening of the clotting time in normal plasma.

In the XI-deficient plasma, we found that there's also shortening of the blank time and in the XII-deficient plasma, there's also shortening of the XII-deficient plasma clotting time. Now, by comparison to the other IVIG product, we find that some of the products -- so Product B and Product E also was able to shorten the NAPTT at a 1 in 2 dilution. However, if you look at all these products at 1 in 10 dilution, we found them to be -- to actually pass what we think is the criteria for the factor IX concentrate.

So I think that's important we think about testing of these products, exactly what concentration we should be testing these products at and then when we talk about the pass/fail criteria, exactly which concentration we're thinking about. For the XIa assay, we decided we

should set up a functional assay that should be specific for XIa. So we did this by basing the assay method to the conversion of IX to IXa by XIa, and then subsequently the IXa generation is based on the generation of Xa caused by the IXa in the assay itself.

The -- we validate this assay in that in the absence of XIa. We do not generate any Xa at all. So this assay is dependent, totally dependent on XIa, the presence of XIa. Again, in this particular assay, we find that we have a varying amount of XIa that's in all these different products with Product Z that having about approximately 10 times more XIa than the other products.

So, we then went on to look at thrombin generation tests mainly because that was what most the other laboratory was looking at. We have quite a bit of experience in looking at this particular test method mainly for its use as an initial diagnostic for looking at different type of hypercoagulability and also for bleeding disorders. And under the International Society on Thrombosis and Homeostasis, there has been a working party for quite a few years looking at the standardization of this assay.

We know that there's three commercial assay method, two fluorogenic, one chromogenic substrate-based. However, all these methods intrinsically different. They have different concentration of critical reagents and also different way of quantifying the readouts. Currently, we have no formal standardization. There are high intra-lab and inter-lab variability and I will show you some of that data in a couple of -- next couple of slides.

We know that the results of one kit cannot be compared with result from another kit because there's no way of comparing the data. Optimization of the trigger/stimulus necessary for different application is absolutely essential. So this is the result from one of the collaborative study that I've run for the SSC, and here's looking at result from CAT which is the one that -- the method that was presented by the French authority earlier on, CAT.

And here, we're looking at peak thrombin and the results is from 45 labs. The sample A, B and C are three different plasma samples and the absolute value is the peak thrombin in nanomolar. And you can see that for -- with these three plasma samples, the percentage CV for the peak

thrombin vary from 24 percent down to 17 percent. So this is a really quite high variability. However, we did try in some way -- try to see if we can actually help to lower this inter-lab variability by normalizing the results against reference plasma.

And here we use A, B, C and also another plasma E as a reference material and then normalizing the results against that. We define that we have a marked decrease in variability when the peak thrombin is recalculated as a ratio to a reference plasma. So for example if you look at the last column against plasma E, sample A, the inter-lab CV have been lower from 24 percent down to about 9 percent. So by doing it this way, we can somehow get inter-lab agreement or better inter-lab agreement.

This slide shows the results from technothrombin, just the TGA method performed by Octapharma. This is again peak thrombin and here we got result from 27 lab and this is again showing the percentage CVs. The absolute value of peak thrombin from the 27 lab for these three sample vary from 41 percent to 56 percent. So this is very high inter-lab variability. When we try to normalize that against a reference plasma, again, we pick the last column

against the reference plasma E. Yes, we did have a low -- a decrease in inter-lab variability.

However, we could say that that's still really not very acceptable with this level of variability. So with these things in mind, we set up the assay. We actually decided to go with the CAT method and we use a number of different plasma just to test out the actual assay variability, and we use phospholipids. And here are some results. We looked at the effect of purified XIa in XI-deficient plasma. The top panel, we look at the effect of tissue factor in this particular assay.

As you can see, that with varying concentration of XIa, we have -- we don't appear to have a very good dose response in the lag time. So this is looking at the first panel. Actually it's very difficult to point. So anyway -- so you can see that the dose response for the lag time in the presence of tissue factor in the top panel is not a big -- not a good dynamic range. However, we did see a dose response in terms to peak thrombin being generated.

It goes -- it increases from 250 at 0.3 nanogram up to 320 nanomolar of thrombin when we're adding 20 nanogram of XIa. Now when we actually take out the tissue



factor and just use phospholipids alone in this particular assay, we did actually get a very good dose response with the lag time here looking at the lower panel there. And it seems to be that the conclusion for us is that the tissue factor decreases sensitivity of the lag time to XIa and the phospholipids alone increases sensitivity of lag time to XIa.

So looking at the product itself, we have here -- we have the Product Z in XI-deficient plasma. Again, the top panel shows the -- what kinds of response you get in the presence of tissue factor and phospholipids. Again the lag time, we find that there is some dose response, but the dynamic range again is not great. There's good dose response in the peak thrombin being generated.

However, again, taking out the tissue factor, shown as the bottom panel, increases our sensitivity to the procoagulant activity in terms of the shortening of their lag time.

So what we conclude on this is that the presence of tissue factor minimize the concentration effects on the lag time. When we look at the effect of this particular product on factor VII and factor IX-deficient plasma, it

was clear to us that we did not get any response at all when we put the -- this product into factor IX-deficient plasma. However, we were able to get a response in VII-deficient plasma.

Our conclusion from this is that this product was able to generate thrombin in VII-deficient, but not in factor IX-deficient plasma. This suggest a procoagulant component in Product Z generates thrombin by the contact/intrinsic pathway above the level of factor IX. So, what about the thrombin generation potential of other IVIG product? Here we looked at this product again in normal plasma this time. As you can see that the Product Z shortened the lag time at both dilution.

There's 1 in 5, and 1 in 20 dilution, except for -- again except the Product Z, all the product -- all the other product actually show some inhibitory effect on the lag time. And this batch of Product Z are more procoagulant than other products shown by shortening of the lag time and increase in the Ethiopia, which is the area in the curve or the endogenous thrombin potential and the peak thrombin being generated.

What about the effect of corn trypsin inhibitor

and C1-inhibitor on this assay. We know that the corn trypsin inhibitor specifically inhibits kallikrein and peak kallikrein and XIIa, and inhibits XIa, XIIa and kallikrein. We find that in this assay, the corn trypsin inhibitor did not appear to have much influence over the procoagulant activity of the IVIG. The C1-inhibitor was able to lower thrombogenic potential of Product Z but not completely, and I guess this confirms the data from our European colleagues.

So we finalized a method that we'd actually used and this is essentially a CAT method using phospholipids only. However, I think that since then we're now -- we find the method again and we're using very low concentration of tissue factor in order to get better inter-lab variability or lower inter-lab variability. Here, showing some results looking at 13 batches of a product. Some of them have been implicated in TEE, some of them not.

Here's four different type of substrate plasma and I'm sure in the lag time, without going to detail you can see that with a different plasma, we actually have different lag time. It's also difficult to say whether

there's any trend with the different plasma. Here's peak thrombin with a different plasma, again the same counter pattern. With a different plasma, we have different peak thrombin being generated by these different batches.

ETP, same picture. So here we're looking at some correlation and I've got quite a lot of the correlation graphs. I won't really go into them in detail, but I will go through them and then there is summary table at the end. Here we're showing NAPTT ratio to factor XIa and the correlation is 0.73. So it's quite a good correlation. And now I'll just skip through the -- all these correlation graphs because they're all quite similar.

Just here is a normal plasma TGT, lag time to XIa and then the peak to XIa, ETP and XIa in normal plasma, XI-deficient plasma, lag time to XIa. So I've done a whole different variety of correlation. So we skip to the end. This is the correlation table. We can say that for both the thrombin generation test in normal plasma and factor XI-deficient plasma correlation with XIa is very good with peak thrombin because we get to about 0.8.

It's not so good for the lag time with XIa content -- XI-deficient plasma. I think that the ETP is

kind of in between the peak time and the lag time. So actually we did -- so we do get some reasonable correlation of XIa content with the peak thrombin. So in conclusion, our investigation shows that the different products has different levels procoagulant activity as detected by different assay methods.

Thrombin generation test is sensitive to procoagulant activity in IVIG and some of the readouts correlate well with NAPTT and factor XIa levels. However, the thrombin generation test results are dependent on the source of trigger, substrate plasma and a whole bunch of other variables. So therefore it is very difficult to standardize and obtain good intra-lab agreement if absolute value has to be used as pass/fail criteria.

So I think that again, I'm waiting to see these results in a collapsed study and see where we are in terms of the different assay methods currently performed by the different laboratories and to see whether there's any way that we can improve the agreement between laboratory. So, I'll stop there. Thank you.

SPEAKER: We have time for some questions. Yeah.

SPEAKER: Elaine, hello. Two remarks to that, to

the technothrombin assay. You know in our hand, it has worked very well and we have standardized it and implemented and validated for all assay release. So we are somewhat below the 50 percent deviation, but it was inter-lab. On the -- two other remarks. What we have found during all the month of investigation right now is that it did not really depend on a full plasma, or a factor XI-deficient plasma.

It was the fact that the one was (inaudible 31:20 -6) and the other was fresh frozen. So the factor XI-deficient plasma, by whatever it means, showed to have smaller variations. And we think that it is due to the fact that it is not (inaudible). And it was also more active in peak thrombin we could achieve with that plasma.

So what -- another remark is in all these studies and all inter-lab studies, was it really very much controlled? How much time can we spend on dilution of the sample in deficient plasma or other plasma? We've seen that this is very, very crucial. If you don't standardize it very much, obviously in higher dilutions, the XIa is inhibited by a C1-inhibitor or other inhibitors. So you get a big variability in that. Has that been controlled?

MS. GRAY: Okay. I don't really disagree with you at all. I think that, you know, like all assays, if you do it enough, you've experience and you control the assay well and you control the plasma source, you will get very good within-laboratory agreement on a day-to-day basis and certainly that your validation packet shows that. The result I show there are actually inter-lab variability. So it's actually between laboratory variability.

And of course in those large collapsed studies, different laboratory -- but I've to say that those actually using the same plasma because they were not -- we were not adding anything else in there. And there are different proficiency, if you like, with interparticipants. And -- but I still think that is from the collapsed study, where we'll see how well can we agree between laboratory. I think that within one lab, all these assay will work well providing that we put it in the effort to make sure that everything is controlled.

In terms of using XI-deficient plasma, of course, we tried -- both we try out the freeze-dried as well as the fresh frozen and the fresh frozen plasma is something that we prefer. My thought about using XI-deficient plasma is

that it may not -- if we think that the procoagulant component is XIa, then there's no reason why we shouldn't use XI-deficient plasma. However, they haven't been proven for the other products. So if we want a global test for all products, then it's difficult at the moment for us to say we should just be using XI-deficient plasma.

Now, the other point I have on that is that if we think that the procoagulant component is XIa, it will be much easier for us to design a XIa assay which is much easier to standardize in the thrombin generation test. But without -- I'm not saying that, you know, we shouldn't be doing the thrombin generation test. As you know, you know, I really think the thrombin generation test is a wonderful test. It gives you a lot of information and in the right hands, it's extremely good.

But we've got to find ways of standardizing it and I think that, you know, maybe that if we have a good reference material, looking at the sample ratios rather than just absolute readout, we might be able to get a good assay after that for lot-release purposes. But I think that we need to do a lot of work on it.

SPEAKER: Is there time for a -- two quick



comments?

SPEAKER: Sure, absolutely.

SPEAKER: So, one thought I had about the source of variability is that when we were doing some assays with immunoglobulin preparations, we found that the ones that did contain XIa would tend to progressively activate the zymogen XI in the samples. So how they were stored and how long they were left unfrozen before the assay could make a big difference. And it's worth keeping in mind.

And then my other comment was that way back when we looked at some of this, not all of the thrombin generating or -- not all of the activity that resulted in increased thrombin generation in a cell-based model was due to XI or XIa. And so I think there's a potential for other things to be in there.

And I can understand why XI-deficient plasma would reduce the variability, but until we're sure that -- unless we decide we only want an assay for XIa or we're sure that's the only relevant factor, I think it's worth considering at least some other possibilities. Thanks.

MS. GRAY: I agree with that. And again, I think that your point about the stability issue of the XI, XIa is

very important. I think that when I first look at this and not worked on IVIG before, I -- you know, the first thing that came to mind was that this product is a liquid formulation and it's in glass bottle. So immediately, my thought was that if you have XI antigen in there, you could create activation continuously. So you can actually generate XIa continuously.

But of course at the same time, the XIa can be absorbed into glass and get degraded at the same time. So when I really look at the reference material side of things and certainly (inaudible) the freeze-dried material that we pushed out for the collapsed study was actually freeze-dried in a synchronized sample in order to avoid that kind of problem. But I think the manufacturers probably will have more data on the -- how the factor XI in the actual product itself behave over time.

SPEAKER: I think I need to add something here because we did a lot of studies, studying stability of different immunoglobulin products and factor XIa procoagulant activity in those products. And we found that the products that are currently on market now, they do not have the ability to generate more factor XIa with storage.

Quite the opposite is true. With storage, at room temperature, we see very rapid decrease in factor -- well, it's not very rapid but we see decrease in factor XIa-like activity in all the products that we tested so far.

In other words, if you have an old lot, it's more likely that the activity in this old lot will be decreased with time rather than the other way around.

SPEAKER: Okay. If I can make one comment on the thrombin generation assay and whether or not to have factor XI in it. In our experience if you remove factor XI from the plasma, you will actually lose the sensitivity to XIa because if you have particularly very low concentrations of XIa, you'll get the amounts of thrombin generation that may be difficult for the thrombin generation assay to detect. But you will get feedback activation on the factor XI in the plasma and you will get a delay in peak. So you can actually lose sensitivity.

MS. GRAY: Okay.

SPEAKER: There was a --

SPEAKER: I have just a brief -- I think (off mike) question was right. (Off mike) by the way, do you use automated systems, procedure in Europe (off mike) or

still (off mike)?

SPEAKER: It is taken care of from marketing to  
(off mike.)

MS. GRAY: I think in doing these assays especially the (inaudible), it's very important to think about the temperature of all your reagent, temperature of your plate. Because I think that Professor Hemker had actually just published a paper on the effect of temperature on the thrombin generation test. So if you want really good reportability, you have to control, all those physical parameters that involve the actual test itself. So, it's a little bit different to doing a, you know, a factor XI clotting assay on the ultimate machine.

SPEAKER: Thank you very much. Our next speaker is Dr. Dave Gailani and he will speak about thrombogenicity of factor 11a, background, animal studies thrombogenicity of factor XIa, concentrates the data that is available to academia so far.

#### FACTOR XIa AND THROMBOSIS

DR. GAILANI: Okay. Thank you very much for the invitation to speak. What I am going to talk about today is essentially how thrombogenic is factor XI or factor XIa, and I think that that can actually be looked at in a couple of different ways. From the standpoint, does factor XI participate in thrombosis in humans; I think the epidemiological data that I'm going to show you supports that. But the consideration for the discussion today is, is infusion of small to moderate amounts of factor XIa thrombogenic in itself, and that's of course a much more difficult thing to get a handle on.

This slide just reminds me to tell you that, from the standpoint of a hematologist, factor XI is a protein that's missing in people with factor XI deficiency. So that may seem like very circular reasoning, but factor XI was indeed first identified as a factor missing in a family of patients who had a prolonged activated partial thromboplastin time.

So the model you're seeing here, which is a sequential activation of plasma serine proteases -- and I can't really point to them here -- is the basis of a PTT assay that we use in clinical practice. It involves

exposing plasma to a contact-free activation reagent, which is usually -- oops -- a purified earth. It's not a physiologic substance, and this is widely used in clinical practice.

It's -- in this model, factor XI is activated by factor XIIa -- if I can get this going here. And then -- this is a really steep angle, I'm not sure I'm going to be able to do this really. But I'll point over here. No, doesn't seem working. There we go, (inaudible).

Factor XI is activated by factor XIIa and then in turn activates factor IX. And this model has kind of fallen out of favor as a model of hemostasis in vivo for the simple reason that while it depicts a chain, missing different links in the chain creates different clinical syndromes. So people missing factor IX, for instance, have hemophilia, a serious bleeding disorder.

Factor XI deficiency, as we'll see, is a relatively milder disorder, and people missing factor XII don't bleed at all. So I'm going to show you a model in a moment which we think probably does a better job of reproducing what goes on in vivo. But I wanted to bring up one point that people who have managed coagulation

laboratories have known for a long time, and that is that the PTT clotting time of plasma lacking factor XII is very long. The PTT clotting time of plasma lacking factor XI is not quite as long, although it's prolonged, and the PTT clotting time of plasma missing factor IX is shorter than that of factor XI or factor XII.

So, some of these steps seem to leak around the step below them. And I'm going to show you some data from animal studies and also from thrombin generation assays that that may in fact be occurring in some other systems. And so, I think that when we, for instance, mathematically model what it is factor IX -- factor XI does, we can't simply say, well, it might be activated by one protease and then only activates another. It may be doing multiple things.

Okay. So I think our current understanding of how factor XI works is as follows, and this is really been discussed a bit already. But what we have is, we have a blood vessel, it has tissue factor outside the endothelium. When the endothelium is cut, we're going to have factor VIIa and plasma, gaining access to tissue factor forming a complex, and that's going to be the trigger for hemostasis.

We're going to get thrombin generation through factor Xa activation, and we're going to get sustained thrombin generation through activation of factor IX. So these are the vitamin K-dependent proteases contributing to thrombin generation.

Factor XI, in the current models, is usually involved in supplemental activation of factor IX and this turns out to be more -- most important in certain tissues. So not in all tissues, and I'll go over that in just a moment.

While factor XIIa was the activator for factor XI in the traditional cascade model, the absence of bleeding diathesis in people missing factor XII suggests other mechanisms can do this. And this has been alluded to already. We think that thrombin and perhaps multiple forms of thrombin can actually feedback after the initiation of coagulation to activate factor XI in a manner that doesn't require factor XIIa and sustain thrombin generation over time.

So if we consider patients with factor XI deficiency, this is a relatively rare disorder in the general population. But in certain ethnic groups like the Ashkenazi Jews, French Basques, you find it in a fairly



identifiable frequency. It's quite frequent here, and for the most parts it's an autosomal recessive disorder. And bleeding here is usually trauma-induced or surgery-induced and it has a predilection for certain tissues, the oropharynx and nasopharynx and also the urinary tract. And what those have in common is very high intrinsic rates of fibrinolysis. So the process that tend to degrade clots are very active there, and XI seems to be important in maintaining the integrity of the clot once it's formed.

So these people have problems with surgeries on those tissues, but rarely have problems with spontaneous bleeding. Probably one of the best examples of this I can think of is a patient we had at Vanderbilt, who played professional football for a decade and didn't know he had factor XI deficiency and had no problems with it. And then had surgery on his mouth, and had extreme severe hemorrhage and had to be transfused to control the bleeding. So, very tissue-specific.

I think because of this relatively modest role in hemostasis, for a long time it was based on the premise that thrombosis is simply hemostasis in the wrong place, that factor XI wasn't going to really contribute

substantively to thrombotic disease. And I think we now know that that's probably not the case, and I would like to discuss some of that.

First of all, there are epidemiologic data from human studies implicating factor XI in both arterial and venous thrombosis. And the first studies were actually from Joost Meijers and Frits Rosendaal. They were colleagues in the Leiden Thrombophilia Study showing that people who had factor XI at the upper range of normal. And the normal range of factor XI actually extends over about three-fold protein range from about 15 nanomolar to about 45 nanomolar.

People up in the top 10 percent have about a two-fold increase risk from the general population for venous thromboembolism. And subsequent studies, including population-based studies in Holland on myocardial infarctions and a number on stroke, have also shown this sort of gradient -- as you go up in plasma factor XI level, your risk of thrombosis rises.

Now, there have been some studies mostly done in Israel by Uri Seligsohn and his colleagues on factor XI-deficient patients. And here they're -- while this is

difficult study to do because the -- and it's obviously very, very small -- it appears that there is some protection from venous thromboembolism and also stroke, but perhaps not myocardial infarction in people who are severely deficient in factor XI. So I think there's a growing consensus that factor XI can at least contribute to thrombosis if it's not a trigger itself.

Now, maybe more pertinent to what we're talking about today is some experience with factor XI concentrates. And since the bleeding in XI deficiency isn't that severe, these aren't used that widely. But there are -- there have been three that have been prepared over time. Two of them currently available, although only one of them can be obtained in the United States and it takes some effort to do that. And in 1991, there was a report out of -- from Israel of three catastrophic thrombotic events, of fatalities related to infusion of XI concentrate. And in two cases there was a marked elevation of D-dimer consistent with the consumptive coagulopathy.

And the most experience we have is with this product from Great Britain. And there, they also have noticed some thrombotic events. Usually these occur in

elderly patients, around the time of the infusions typically. There are -- some have a more tendency for arterial clots than venous clots. And because it was assumed this might be due to traces of XIa in the preparation, the samples were initially supplemented with anti-thrombin and then heparin.

The interesting thing is that those manipulations didn't necessarily seem to change the profile of how the product was prepared. Infusions of this material into individuals who didn't have thrombotic events would often show elevations of D-dimer, elevations of thrombin-antithrombin complex as if something was being triggered by the preparation. But I'm not aware of any systematic investigation as to how much XIa might -- residual XIa was in the product of other proteases or the product.

Recently, data from about 230 patients was -- 230 episodes or infusions in 161 patients was reported with this product and they had 21 adverse events, 12 of which were likely thrombotic. The preparation that we can obtain with a little effort in the United States is actually prepared in France. It's called Hemoleven, and this product actually has antithrombin C1 inhibitor and heparin

in it. And there was a report a few years ago on 31 patients, which involved 33 procedures, identified three thrombotic episodes, all in individuals who got relatively large amounts of the material, 30 units per kilogram. So the recommendation is usually to keep the amount that's given down. Okay.

So I'd like to over some animal data now, showing factor XI's contribution to thrombosis. And while we've worked a lot with mice, I think it's important to point out that the original demonstration in an animal model of the role of factor XI in thrombosis was made by Andras Gruber and Stephen Hanson, when they were at Emory, using a model within the olive baboon. And what they do is they actually create a temporary fistula between the femoral artery and vein in these animals and they introduce thrombogenic grafts. And these are usually pieces of Gore-Tex that have been coated with collagen or tissue factor or other things of that sort.

And they infuse the animals with radiolabeled fibrinogen and radiolabeled platelets, so they can use a gamma camera to actually detect the formation of thrombi within these clots. And then there are sampling ports,

where they can remove the material and measure various parameters. And this is data from a relatively more recent study that was done when these investigators moved to Oregon Health and Science University.

And what they did is they gave these animals a single dose of an antibody that blocks factor IX activation by factor XIa. So the antibody is the factor XI A and it binds the exosite on XIa at the center -- important for interacting with IX. And these animals essentially have an anticoagulant effect that lasts about 10 days. During that period of time, factor XIa antigen actually rises in the plasma of the animals, but it's completely neutralized. And the various surgical manipulations are done on these patients; they don't show any evidence of a bleeding disorder during any of this.

So, we're going to concentrate on the panels here. They are marked 01A6. That's the designation for this particular antibody. And what they see in animals treated with 01A6 is a marked decrease in platelet accumulation within the shunt. What is actually happening is the surface of the shunt is being coated with platelets, but the three dimensional growth of the thrombosis is being

interfered with. And so what you're going to see through the subsequent slides that I'm going to show you is a reoccurring theme. XI isn't necessary for starting the clot; it's necessary for making it really big and including grafts and blood vessels.

The antibody also depresses fibrin formation, and you can see the effects on thrombin generation here. These are thrombin-antithrombin complexes. This is what typically happens in an animal that's not treated with the antibody. The thrombin-antithrombin complex is blunted. And this beta-thromboglobulin, a marker of platelet release. It goes up when the graft is inserted, and if you have factor XI antibody on board, it gets blocked.

So we have studied this phenomenon in mice. And somebody pointed out that some of these models are artificial. I'm not going to make the argument that that is not the case. Mice don't like to thrombose, so you have to do pretty nasty things to them to make them do it. And what we are -- the model we're most familiar with is one where we actually expose the exterior vessels to concentrated ferric chloride, which essentially causes outside an injury and causes desquamation, loss of the

endothelium, exposure of collagen. It's a pretty significant injury.

And we actually use a modification of this technique where we try to titer the ferric chloride response to find out what the least -- the lowest concentration is that will produce an effect. So what you're looking at here are data that are generated on thrombosis in the carotid arteries, okay, of mice that are either normal -- the black bars; missing factor IX, the red bars; the factor XI, the white bars; or factor XII which are the cross -- which are the blue bars.

The point here is that wild-type mice will reproducibly thrombose their carotid arteries when 3.5 percent ferric chloride is used to injure the blood vessels. But factor IX, XI and XII deficient mice are universally resistant at 5 percent and partially resistant at 7.5 percent. What does that mean? To produce an equivalent effect of that type with heparin, you would have to give a dose that's super therapeutic and leaves the animal prone to bleeding to death in a tail bleeding model. So this is really actually a potent anti-thrombotic effect.

The point I wanted to make out -- make here is



factor XII-deficient mice actually are somewhat more protected than factor XI or factor IX-deficient mice. So we think of the intrinsic pathway being responsible in some way, shape or form for thrombosis here, XII must be doing something in addition to activating factor XI.

Curiously, we see the same thing when we consider factor XI and factor IX. The thought is, well, XIa is an activator of IX, that's what it's doing in the thrombosis model. But when we go and combine IX and XI deficiency to make double knockout mice, those animals are reproducibly more anti-thrombotic, if you will, in this model, suggesting that XIa is doing something other than simply activating factor IX. And I will give some data to suggest what that might be in all of the slides.

So we've seen a lot about thrombin generation assays, and I'm not going to go over this in a lot of detail. This simply shows these white bars are normal plasma. The black bars are factor XI deficient plasma. This is just to reiterate the point that, as you drop the tissue factor concentration, the importance of factor XI, the thrombin generation in the thrombin generation assay goes up.

What we see over here is that if we use a low amount of tissue factor to trigger thrombin production, and we block factor XI with an antibody that prevents it from activating factor IX, we largely wipe out thrombin generation. If we use an antibody as factor XI that's specific for interfering with the activation of the protein by factor XIIa, it has no effect. So what we think we're doing in these thrombin generation assays is that we're making some thrombin through tissue factor and it's feeding back on factor XI and activating the XIa so that it can produce more thrombin.

Now, you can actually clean this assay up and get no background activity from the VIIa tissue factor by triggering the assay with thrombin itself. Okay. So this is the effect of plasma that's induced to clot with a small amount of thrombin, not enough to measure -- to register in the thrombin generation assay. You get a nice peak, and if you block factor XI or the XI isn't there, this goes away.

Now interestingly, you can get an even better response if you use forms of thrombin that we don't typically think of. Not the alpha thrombin that you typically buy from various companies, but some of the

breakdown products of thrombin. So, pro-thrombin conversion to alpha thrombin actually proceeds through a series of intermediates -- meizothrombin. Meizothrombin does have one -- which have been shown to have some activity towards factor XI. Actually, meizothrombin, on the other hand, is a better activator than alpha thrombin.

Alpha thrombin in turn can degrade into beta-thrombin and gamma-thrombin, and these have been shown by Ken Mann's group to be present in clotting blood. And the key things here is that these two forms lack the interaction site with fibrinogen. So if you think of anything that thrombin's going to do in plasma, fibrinogen is going to be the major competitor.

Using beta and gamma thrombin in the thrombin generation assay, actually takes the competition from fibrinogen out and you end up with an enhanced thrombin generation. So whether or not that's physiologic is not clear, but if you're looking for a way of actually looking at factor XI, not XIa, but XI, gamma-thrombin and beta-thrombin can actually enhance some things, enhance that.

Now, this simply shows the effect -- the sensitivity of the thrombin generation assay to XIa.

That's been discussed extensively here. I just wanted to make one point. If you use enough factor XIa in plasma lacking factor IX, you can still get thrombin generation. And we've looked at this with human plasma lacking factor IX -- and you have to be careful because not all human plasmas that are commercially available truly lack factor IX. We've looked at it with murine plasma lacking factor IX, and XIa can somehow jump over the gap.

We've also used species of -- recombinant species of XIa that lack the factor IX interaction (phonetic) sites. They can do the same thing in IX deficient plasma, even though they're weak in normal plasma. Ken Mann's group has demonstrated that factor XIa can activate factor V and factor VIII, and that might be one way that this is jumping over the need for factor IX.

We've also been able to demonstrate that XIa is a weak activator of factor X. So if you think about the structure of factor IX, it's essentially identical to factor X or very, very similar. In fact, factor IX is probably the result of a gene duplication event of the factor X gene. So factor XIa may actually be more than just a IX activator, it may actually be able to activate or

prime to some extent -- one step down. And what that implication is for thrombogenesis is a little hard to say, but just something to keep in mind.

One other comment on the thrombin generation assay, most of the assays that I think have been reported here use corn trypsin inhibitor as a blocker of factor XII because contact activation is such an irritating thing to deal with thrombin generation assays. If you collect different plasmas, they're going to have different degrees of contact activation that have neither go on during collection or afterwards.

We think we may have identified one of the culprits in this that may help -- and I'm not going to say standardize this assay, but at least smooth some of the bumps. In an effort -- since we look --- we like to look at factor XI activation. We wanted to be able to compare factor XII-mediated activation to thrombin-mediated activation. So that means no corn trypsin inhibitor. And we hypothesize there may have been particulate matter or something of that sort in the plasmas that were contributing to this, so we simply put it through a relatively low-speed centrifugation for a prolonged period

of time, and there really wasn't very much in the pellet that came down.

But what that did that we hadn't anticipated is it took the low density chylomicrons, the lipoproteins, and they went up. Okay. So now we have chylomicron depleted plasma. And this plasma no longer had spontaneous thrombin generation in it, which was the main culprit. So what we have here is an example of a normal plasma. We simply recalcify it and there is some thrombin generation, and this varies tremendously from plasma to plasma. After we spin it, that goes away.

Now as it turns out, we probably have -- we've noticed before that while we add phospholipid vesicles to thrombin generation assays. Most plasmas don't need that; there's enough phospholipid already in there. There isn't after you spin the chylomicrons out. But if you put phosphatidylcholine/serine vesicles into the spun plasma, if you will, what you end up with is a plasma that can support thrombin generation after you stimulate with tissue factor or thrombin, but doesn't have a baseline activity from so-called contact activation.

As it turns out, chylomicrons are a very potent

activator of factor XII. And there's some old literature on this that triglyceride-rich lipoproteins such as chylomicrons and very low-density lipoproteins can actually trigger contact activation. So some of the variation that we're seeing may be due to the fact that they're different lipid levels, or it depends whether or not the plasma is collected shortly after someone ate (phonetic), as to the chylomicron levels, and where the process is trying to determine if there's activity, XIIa activity and XIa activity, that are associated with these chylomicrons.

Okay. So now, I want to finish up here by just going over some data we've generated in a flow-base model. And I think that obviously thrombus generation assays hold, I think, promise here for being sensitive for detecting factor XIa in products. But it's a static system; it's certainly not a physiologic system. When you go and stimulate plasma in a thrombin assay with tissue factor, the tissue factor is dispersed throughout the plasma. And that's certainly now, also what's going to happen in an injured blood vessel, where the tissue factor is going to be on the surface.

So what we have here is a flow model in which a

capillary tube can be coated with tissue factor and epithelial cells, collagen, you name it, and blood can perfused through it at different rates to produce different shear forces. And the blood can be reconstituted -- which is in collected into sodium citrate, can be reconstituted with calcium or it doesn't have to be. And what we're going to be looking at here using various microscopic techniques is deposition of platelets and deposition of fibrin strands.

And what we see, this is the collagen -- here is data for collagen surface. If you just perfused blood that hasn't been recalcified across it, you get platelet aggregation, but you don't any fibrin formation. Recalcification is going to allow fibrin strands to form, and this formation of the fibrin strands is absolutely dependent on the presence of the platelets in this flow-base system. This is flow at about venous shear rates, maybe 300 per second. If you block factor XI in the system with an antibody, you lose the fibrin formation. Okay.

Now, the thought was perhaps factor XII was activating the thrombin -- the factor XI in this system, but in fact that's only partially the case. While adding



an inhibitor of factor XII in the system seems to reduce fibrin formation somewhat, it doesn't block it completely. So factor XI is doing something in the system that it's not tissue-factor dependent, because there is no tissue factor on the surface of this particular system. But doesn't seem to be factor-XII dependent, and it requires the platelets. And it's conceivable the platelets are in some way, shape or form contributing to the activation. We won't worry about that.

Now here's, I guess, the final point I would like to make, and this again with this flow model. And what you're looking at here are platelet -- this is now confocal images. What you're looking at here is low shear in panels e and f, and high shear in these here. This is 300 per second, 1,500 per second. And what we have in the thinner frames is an image that's made with multiple confocal images, so that we're looking sort of across the cross-section of the tube.

And the point here is that when whole blood is perfused in the presence of calcium at low shear, you get essentially larger clumps of platelets -- here there's more smaller ones -- that completely occlude the tube. In fact,

in about 15 minutes, these tubes will completely occlude through these red-cell rich thrombi.

When you have an inhibitor to factor XI in place, you simply don't -- you have platelets depositing on the edges of the tubes, you just don't get the growth of the clots. So this is very similar to what we saw in the baboon and also in the mouse models. There's just a defect in stability of large clots, not clots, but large clots.

Now at high shear, fibrin is less important, and this is primarily about platelets. And what we see here is a very similar phenomenon. You get near occlusion of the tube in the presence of XI and it's reduced considerably when you use an antibody to factor XI. While I don't have an image to show this, we have some recent data that suggests that this process here may not -- that's XI dependent -- may not be all dependent on thrombin generation.

So if we don't recalcify this system and simply run platelets through this at high shear in the absence of calcium, which should prevent thrombin generation, there's still an effect of blocking the factor XI with an antibody, as if factor XI maybe contributing something unrelated to

thrombin generation of this assay, perhaps directly to the platelets.

So to finish up, I think that we can safely say that elevated plasma levels of factor XI are associated with an increased risk for arterial and venous thrombosis in humans. I think the epidemiologic data is becoming very strong on that.

If concentrates of factor XI can indeed be thrombogenic, particularly in patients with preexisting vascular disease, but whether or not that's XIa that's contaminating it -- and that's the assumption -- or something else, we're not -- I'm not aware of it. In animal thrombosis models, the lack of factor XI or inhibition of XI or XIa will interfere with thrombus growth.

And it really seems to be stability of these large -- they call them pathologically large thrombi -- that's the key here, which may indicate that thrombosis, at least from the standpoint of factor XI, is quite a different process from hemostasis.

Thrombin generation assays, as we've seen, can certainly be designed to be very sensitive to factor XIa.

It provides some evidence that factor XIa activities may be distinct from factor IX activation. But whether or not those are important for hemostasis or disproportionately important for thrombosis is really unclear at this point.

In the flow models using human blood, factor XI contributes both to fibrin formation and platelet activation and seems to be particularly important for the three-dimensional growth of thrombi.

So with that, I'm just going to give credit where credit is due. Most of the studies you've seen with the flow-base system, work done by Anton Matafonov in my laboratory and -- where is he here? Dimtri Kravtsov did a lot of the thrombin generation study.

So with that, I will take any questions. Hopefully, we're not too far out over time. All right, thank you.

(Applause)

SPEAKER: Sir, I am interested in trying to relate those cases where patients were actually given known amounts of factor XI and developed thrombotic complications, because that has obviously relevance to what is happening at the IGIV.

DR. GAILANI: Right.

SPEAKER: So two questions related to that. One is, you mentioned already that there were more arterial types of thrombi -- (cross talk) -- which fits --

DR. GAILANI: Yes. They're on both, but that's -  
- yeah.

SPEAKER: But the question is related to the timing. You know, we said with the IGIV product, it seems to be longer lag period before you develop it. But the same thing happened. The other question is -- that I have related to this is the dosing. I mean -- you know, I haven't been able to do it quickly, but you or Mikhail probably have done it -- is you know what are the dose of factor XI or factor XIa that were associated with thrombosis, when you know what you're giving and you know how much is in the vial?

DR. GAILANI: Right. So with regards to the second question, most of the thrombotic events occurred in elderly patients and there were at the higher doses. The issue is how much factor XI is necessary to get you through a procedure, and there is disagreement on that.

Those who point to patients with mild deficiency

having had problems with surgery, say you need to push it close to a hundred percent in order to get them to the surgical procedure, and it may have been those patients where they were trying to get the level up close to the physiologic level that ran into the most problems.

So at least with the French product, the higher concentrations seem to be the culprits. And I'm sorry, quickly, the first question again?

SPEAKER: Well, where there was a lag time of the venous thrombosis?

DR. GAILANI: Yes, that was -- most of the events occurred within a couple of days of administration. Some of the -- there were some venous events, and at least one of them, a pulmonary embolism, occurred almost a week out. But I don't know of anybody -- I don't know of the data actually presented in that manner. I just don't know. I'm sorry.

MR. SCHULTE: Okay, Stefan Schulte from CSL Behring. I have actually two questions. The first one is with regards to the characterization of the factor XI when you applied to your assays, how you make sure that you, for example, don't have contamination or already activated

factor XI?

DR. GAILANI: Right. So that's a major problem. What we typically do with the XI -- so in order to try to keep the systems -- all components of the system similar, typically what we'll use is we use a XI-deficient plasma and we'll add XI to it. Okay. The plasma-derived XI is treated extensively with DFP and stored at a PH5 -- or then dialyzed into a PH5.2 buffer. When we do that and add that back to the plasma, there's no spontaneous thrombin generation.

Can I say that there's not a little bit of XIa there? I don't know. But typically what we do is we add the XI, we allow to sit in the plasma for 30 minutes and then we test it and we don't spontaneous activation. When we use recombinant variants of factor XI, it becomes much more problematic because those are far more prone to spontaneously activating, if you will.

Any concentrate of XI is going to start to develop activated XI in it with time, because there's a slow auto activation rate. So we usually use DFP inhibitors -- DFP inhibition -- and then we test the molds to make sure they don't have spontaneous activity in them.

MR. SCHULTE: Okay. That's also my experience. The other question is, when we analyze our product, we also found significant amounts of kallikrein. Can you please comment on kallikrein as a potential thrombotic risk as well?

DR. GAILANI: Okay, that's -- that's a complex question. Certainly, kallikrein -- if you leave corn trypsin inhibitor out of a thrombin generation assay, kallikrein can do its thing. It make XIa which can make XIa which can produce -- whether or not -- there's really almost no human epidemiologic data to suggest that PK levels high or low are pro-thrombotic or anti-thrombotic.

There's a couple of studies in mice where people used a chloromethyl ketone and came up with different results. One group which claimed that the chloromethyl ketone was specific for factor XIa concluded that it had an antithrombotic effect because it blocked XII, in the mouse models I showed you.

Another group, taking that same chloromethyl ketone inhibitor, declared that it was specific for alpha kallikrein or at least more potent for alpha kallikrein, and showed that it had an anti-thrombotic effect, but using



a different thrombosis model.

So the one that showed the anticoagulant effect was the ferric chloride model I showed you. The one which had the pro-thrombotic effect was the -- was a laser injury model. So we have these data going in different directions. We don't have prekallikrein deficient mice that have been tested in these models yet, but that work is in progress.

MR. SCHULTE: Thank you very much.

DR. GAILANI: Okay.

MR. SCHULTE: Thank you very much. One more -- one more question.

SPEAKER: One more question? I'm curious in your -- the factor XI and platelet aggregation flow studies that you did, what the platelet concentration was and if you varied that all in those experiments, and also what the diameter of your tube was?

DR. GAILANI: Yes. So the diameter of the tube, it's -- those experiments I showed you, diameter isn't the right word because they're not circular, okay.

SPEAKER: Okay.

DR. GAILANI: These are actually more

rectangular. And I think it's a 2 millimeter by -- I want to say 0.2 millimeter, something like that -- 0.2 by 0.4 or something, something like that. The platelet count is adjusted. We use two different systems. One is one where we'll take a plasma, and we'll reconstitute it with red cells and platelets, and there the platelet count we use is 250,000.

If it's whole blood, the platelet count is going to be whatever the platelet count is of the person involved, which is usually somewhere between 200,000 and 350,000-400,000. So that does vary.

SPEAKER: And did you see differences in the formation of the platelet aggregates with the different people's blood?

DR. GAILANI: Actually, in this particular -- unlike some of the other thrombin generation assays that we've used, the thrombus formation here actually seems to be fairly reproducible. I don't have an explanation for that, but the result -- the timeframe of formation of the clot is relatively similar between different individuals.

SPEAKER: Really cool. Great talk, thank you.

DR. GAILANI: All right. Thanks.

SPEAKER: Anymore questions? Thank you very much. This now concludes session I, and please come back at 1 p.m., in 40 minutes. We'll have box lunch -- lunches to facilitate the discussions. And in the interest of time, please come back in 40 minutes. Thank you very much.

(Whereupon, at approximately 12:20 p.m., a luncheon recess was taken.)

## A F T E R N O O N   S E S S I O N

### PANEL DISCUSSION

SPEAKER: -- and participate in the panel discussion.

SPEAKER: Just two housekeeping announcements. One is, if you're leaving today please remember to fill out the evaluation form in the back on the desk. The second announcement is, for the sake of time and to get people out here on time to catch their planes, tomorrow we plan to start at 8, 8:00. Maybe there will be doughnuts.

SPEAKER: So we have two topics for the panel discussion: what are the most likely biochemical causes of thrombotic events in IGIV recipients, and what other potential causes of IGIV-associated thrombotic events should be considered. We have two presentation prepared to facilitate the discussion. The first presentation will speak about the clinical causes of thrombotic -- the clinical picture of thrombotic events and the second presentation will cover biochemical root cause investigations.

But I want to remind that the purpose of this panel discussion is to discuss the actual questions. So, short -- these presentations will be short and we will have time to discuss the data that was presented in the morning. Thank you.

#### IGIV AND THROMBOEMBOLISM

DR. STIEHM: Speaking after lunch is always a challenge, not quite as bad as speaking at 8:00 'o clock in the morning. And the house staff asked me to give a little talk at 8:00 'o clock in the morning on what I was going to talk about here. So I did. But as I gazed around the room, I felt like I was the superintendent of a graveyard. There are a lot of people under me, but no one was listening.

(Laughter)

Okay. So, just a brief summary of the side effects that we see with IVIG. We have a number of minor side effects: headaches, malaise, blood pressure changes, tachycardia, site swelling with use of subcutaneous. And then we have four major rather common serious reactions.

One are anaphylaxis or anaphylactoid reactions, renal insufficiency, headaches or aseptic meningitis, and what we're going to talk about today, is thromboembolic events.

And we have a number of very rare complications, hematologic changes, dermatology, hyponatremia, arthritis and pulmonary, and some potential but never yet identified, are HIV or Crutzfield Jacob disease, and in the past, hepatitis C. As we all know, there's been lot of reports of thromboembolism, but they are really diminishing if you consider the increase of the use of IVIG, which is going up dramatically. And this is from Canada from 1997 to 2007; you can see that something between 7 percent and 9 percent of the youth. And this is present in all over the country, but United States used more gamma globulin than all the other developed countries, and it's still very poorly used in developing countries.

The historical case that Dr. Scott referred to was by Woodruff, Grigg, fatal thrombotic events following treatment of autoimmune thrombocytopenia in 1986. And they were using 400 milligram per kilogram for three to five days. And they postulated that the patient had underlying arteriosclerosis, but the rising platelet count might have

caused this or they thought that possibly having antibodies to platelets caused increased platelet aggregation.

In my field -- I'm a pediatrician -- thromboembolism in children is extremely rare. There was one case in 2006, when a child was found with Kawasaki disease, another child with ITP, and third one with agammaglobulinemia that developed cerebral thrombosis 10 days after the infusion. And another patient with recurrent ITP given large dosages of IVIG developed cerebral venous thrombosis 10 days later. And note that these time intervals are late type of things rather than the kind of event you'd expect immediately.

I made emphasis to look at a series of patients that started out, without rather than with the FDA and many of others have done and looked at cases and then caused -- I tried to do some studies of patients who were given IVIG in terms of the incidence of thromboembolism. And one study was 150 patients who had received stem cell transplants from HLA-identical donors, and they gave them 50 each -- 50 milligram per kilogram, 250 or 500, and gave a controlled group of 50 no IGIV.

And you can see that venoocclusive disease

occurred in very many of them, up to 17 percent, and grade 3 venoocclusive disease occurred very commonly at 500 milligrams per kilogram at 11 percent, which is highly significant. And their conclusion that you should not give IGIV; it has no benefit and has an increased risk of grade 3 venoocclusive disease.

In looking at patients with multiple sclerosis given 1 gram per kilogram per months for 27 months, and compared to a 159 patients given albumin, six of them, 4 percent, developed deep vein thrombosis or pulmonary embolism. And it was ineffective and may have long-term events, particularly occurring in one patient after a long-distance flight. And so they recommended these patients are immobile and were given extremely large dose.

Caress, in 2009, looked at risk factors for thrombotic events. And here, again, there is a high incidence occurring. Thirty-eight patients -- 19 patients had a TE event, and this was quite common. Eight had neurologic problems and the mortality was about 17 percent. And we've -- as been referred to before, looking at case control studies in which risk factors were occurring, the big risk factors were coronary artery disease, cigarette



use, hypertension, cardiovascular disease, diabetes and hyperlipidemia.

And he did this analysis showing that the more risks that you have, the much more likely you are to have a thrombotic event. So if you had four risk factors, your chances of getting it were extremely high.

Foster et al. reviewed 145 ICU patients that were receiving IVIG, and the overall incidence was 3 percent. And it was interesting he compared the various adverse effects, and acute renal insufficiency was very common, 81 percent, and thromboembolism was only 4 percent. So of course, it's much serious but very commonly is acute renal insufficiency, which may have coagulation role.

One thing I noted going over this is that hemolytic anemia may be a cause of or contributing cause to thromboembolic event. Comenzo et al., in 1992, described a case of immune hemolysis with intravascular coagulation and serum sickness, with Kawasaki disease, who developed thrombosis. Another patient also had the same thing. He had acute hemoglobinemia or hemoglobinuria following Rh illness or ITP, and described that in 2005.

And in pediatric patients, a recent series of

giving IVIG for hemolytic disease in newborn due to either ABO or Rh incompatibility shows that 6 percent of them developing enterocolitis -- of necrotizing enterocolitis, one of the most severe things that CAN happen to premature, and is associated with small clots in the wall of the intestine.

Huang et al. studied 200 -- studied 67 patients with antibody-mediated rejections given IVIG, which is one of the standard therapy for antibody-mediated rejections. And he found again about 4 percent incidence of antibody-mediated rejection. And so they did a very interesting study, which is shown in next the slide. They decided that they had to do something about that, so they felt that the first thing that they would do is give all of these patients aspirin. A second got Lovenox enoxaparin in a standard dose of 1 milligram per kilogram, and all of them get pre-IVIG infusion hydration.

They gave the gamma globulin slowly, 50 milligrams per kilogram per hour, increasing to 100 milligram per kilogram per hour so that the entire infusion was done over about a five-hour period. And then they gave them post-infusion bonus of saline. And they then found

that the incidence was zero. They completely prevented thromboembolic events.

So to summarize, the risk factors that we see and many of you have -- we've already about is older age, immobility and air travel, high blood pressure, cardiovascular disease, diabetes, dyslipidemia, proteinemia, gammopathy, smoking, prior thrombosis, anemia or polycythemia, the use of -- estrogen use, hypercoagulable state and the presence of hemolysis. And the infusion risks are large does, brand selection, no prehydration, no premedication, fast infusion -- or the first infusion is much more common.

So what should you do about this? Well, the first thing that is done is use a slow infusion rate, 50 milligram per kilogram for the first hour, 100 milligram per kilogram per hour thereafter, a total dose over 5 hours. Avoid as tolerated dose escalations. We see this all the time that if they tolerate the first hour, increase the dose, get them out of the hospital more rapidly. Consider pre- and post-infusion hydration. Use aspirin or heparin there. And I think that this probably is a very good idea for any patient over 55 or 60, particularly that

are in the hospital, is to heparinize these patients and given them aspirin.

And, of course, what are going to just study today is should we do coagulation studies on these patients, should we do blood viscosity levels and should we do protein levels looking for multiple myeloma or gammopathy. And another good recommendation: if the patient is bed-rested, do a Doppler for clots in a bedridden patient. And the references are there. Okay.

(Applause)

SPEAKER: I think in the interest of time, we can go on to the second presentation and then we will discuss the biochemical --- possible biochemical root causes of thrombotic events.

SPEAKER: Should I?

SPEAKER: Yes. Thank you. We really need to keep these presentations short so we will have time for discussions.

#### POTENTIAL CAUSES OF THROMBOTIC EVENTS IN IGIV TREATMENT

MR. ROMISCH: Good afternoon, ladies and

gentlemen. I just want to go over the first slides because they have been repeatedly shown. And I would like to share with you some information of the time we had with Octagam through the last six to eight months, where we were confronted with the thromboembolic events and how to look for the root cause -- or the root causes, first of all the biochemical. About technical issues I'll come back later on in the second talk.

I don't go into that. You have heard about it. I focus on activated coagulation factor, which finally turned out particularly than the Factor XIa as the biochemical root cause.

So the situation has been addressed already. And now we are in a situation in Europe that the CHMP has recommended to lift suspension of the marketing authorization last month, and here in U.S., it's under review by the FDA. So first of all, of course, if you know nothing about what has caused your thromboembolic events, you have to have a strategy how to tackle that. And the one is the biochemical reason. Secondly, how do you ensure that this somehow applies with an in-vivo situation and look for a thrombosis model. Some out there; we focused on

a Wessler model, but that will be addressed in more detail by colleague tomorrow.

So certainly, then we had to address the root cause. We had to exclude it. That was even the more difficult part, to exclude other factors which would have contributed or may have been hidden by Factor XIa activity. As you heard this morning, it's very difficult to differentiate. You have to puzzle together all the pieces. And then finally, we had to implement the corrective and preventive measures.

I'm going to that. I just show that we used certainly the TGA. It's a technothrombin-based assay. It works very well. I haven't heard the word thromboelastography the whole morning. I was surprised to do, because that is information which gave us, at least in a qualitative way, much information in addition, because it appreciates both the thrombin generation but also the dynamics of clot formation.

And so, we started from the broader way, where we used, again as also Elaine said -- I mean just not to miss something -- the whole plasma. And when we learned what the main -- the major root cause was, then we went down to

the specific plasma, namely, the factor XI deficient plasma, and specific assays. And finally, here, we used then -- established a factor XI assay, which is based on activation of recombinant factor IX and then based, using the Hyphen assay, which is a (inaudible) factor Xa activity, amidolytic activity.

So that ensures at least that we introduce not other factors here. Of course, it's not specific. If you have IXa in there, obviously you would measure it as well.

So briefly, I just -- don't go through this list. We have very extensive investigations from all kinds of perspectives which we could imagine that could support or initiate thrombosis. So we started with global assays like PTT and so on that didn't regard to be sensitive enough. So we had got to TGA and NATEM, which have a certain time, certain sensitivity, and they worked out very well and helped us a lot to understand to go ahead.

The Wessler test as well, although more than 50-years old is -- and you see that tomorrow -- and is very still instead of the other model, to our opinion, if it is handled that way very well and standardized. And then, of course, we worked also with deficient plasma and to exclude

other factors. The -- from Dr. Wolberg, obviously, the XIa was in the room and we, of course, also had a first look on that and did inhibition studies by -- inhibitors by -- that had been addressed this morning already, and also monoclonal antibodies.

And last, not least, we observed even the coagulant, procoagulant principle and characterized it. And did even -- or did also spiking studies with Factor XIa, which totally mimicked the effect of what we have seen in those affected batches. We also found, as in line with all presentations this morning, kallikrein activity in a couple of clots, more or less. I'll come to that in a minute.

Then the other part of the story was to exclude other factors. How did we do that? Of course, again, with absorption studies, depletion studies, we even had a growth factor in cytokine arrays and so and so forth. So I'll just go ahead. It would take too long to go into detail, but we can discuss that if you like to.

Just some -- only some key slides. This is an inhibition curve of two of the concerned batches. You see that we also spiked -- buffered with 50 milliunits per ml



of Factor XIa and we used a CI-inhibitor to inhibit. Nice inhibition characteristics, which brings it down to baseline which is now the assay is around 30 to 50 nanomolar of thrombin, if you use a Factor XI deficient plasma.

And the same old results we achieved if we used a monoclonal antibody against factor XI, not XIa, XI than you obviously need more, but it worked.

This is the depletion study. We immobilized this antibodies to a resin, then we passed certain lots around it, circled it over the column and we got a total depletion of factor XI, nothing else. Then the procoagulant activity was gone. The supernatant was free from the procoagulant activity. I think you can get more specific than with antibodies. They can cross react but you have to exclude it, and we did.

That is, we used a certain adsorption. Then we removed the procoagulant activity from the solution. We had a Western blot, and you see the outcome very clear. We had factor XI in there. It's run under reducing conditions. It's the Elliot of the curve. The upper bend shows you the pro enzyme. Factor XI non-activated and

nicely deactivated (phonetic) form; heavy and light chain of both -- of factor XIa.

So that clearly was in there, and the major part was not activated. And I can tell you, you cannot activate it anymore, also not in the final container. We have never seen that, and it even decreases over time. We do not find factor XI activity in there. But we also -- we have also confirmed that by MALDI-TOF and so on and so forth.

So this is a key slide and I would like to spend a few minutes on it, because that shows you the whole picture. I know it is a very busy slide, but please follow me through that. On the X-axis, you see the factor XIa activity in milliunits per milliliter. On the Y-axis, there is a peak thrombin concentration. So the first thing you see is that even at below one milliunit per ml of factor XIa, you have a very steep increase of the peak thrombin generation signal.

Here in the factor XI deficient plasma, here it starts around 30. The circle there at 30, at 0.11a, and it steeply increases up to one milliunit per ml, just around to 350 nanomolar. And then you can even add more and then sometimes you reach a plateau, of course.

So I think this is a very impressive -- it's very impressive and shows the potential of the thrombin generation assay and the differentiation power in here. It shows you very tiny amount.

So now, I'll start with boxes. I'll take the pink one there, and that shows -- in that box are all the cases which had been -- and all the batches that had been associated with thromboembolic events. So what we saw is that at that time -- and we have to be careful with storage conditions of the final containers because room temperature -- we know that room temperature decreases factor XIa. But at this point in time, we saw that the first batch that had the lowest factor XIa concentration was 17, 17 milliunits per ml factor XIa that had caused. All others were higher than that.

So that is the pink box. Then go to the blue box then. It is -- that shows the results from the Wessler test. It shows that -- from the Wessler test we saw that a concentration of larger than 7 milliunits per ml of XIa had some signal in the Wessler test that will be elaborated tomorrow. But we did not see any result. It was all scored zero, if we are equal or below 2 milliunits per ml.

So the threshold in the Wessler tests must lie somewhere between 2 and 7 milliunit per ml.

So what did we do? If we look at the -- in the beginning, we had -- our first limit was 500 nanomolar of -- due to experience we had gained and we learned over time obviously -- was 500 nanomolar peak thrombin concentration. That is this yellow box. And it still holds 2. However, as we see, that it gets relatively close to the other boxes. So it was then agreed, and certainly we down to get -- to increase the safety margin for that. So we went stepwise down from 450 to 350 nanomolar, and that's the dark green box where we ended up now.

These are our release criteria. You see that this is smaller than 1 milliunit factor XIa and it's smaller or equal 350 nanomolar peak thrombin concentration. And we also added here -- and that they all have -- they have to fulfill all this criteria. Here is the time to peak, and the lag time and the peak thrombin concentration. So this is a validated assay that we have implemented it in our quality control department, and that is now regularly affirmed for all release batches.

That gives you a picture where we are and what

the rationale was to come to this kind of green box, and how that fits to the clinical experience with also the Wessler test. I think it is very important to understand how we came to that figures: the 350 nanomolar and the time to peak and so on. Certainly, if you use the CAT (phonetic) or even other modifications, the total numbers will vary. The important thing is that the discrimination power between normal batches and those with increased coagulant potential can be peaked, and I think that that is the case here.

So just how -- you know, this system is very complex. And how to exclude these other factors and cross contamination? Yes. Okay. Kallikrein was addressed. What we saw is -- we used recombinant kallikrein because we wanted to avoid any cross contamination. We see a recombinant kallikrein has an impact on TGA. Yes. Don't ask me why, there must be bypasses. Also in the factor XI deficient plasma, that's a matter of research. But in the range we're moving here -- and we're moving in a range of now below 0.05 microgram per ml of kallikrein activity, there is no impact on the TGA.

Yes, you have, but then you have to have massive

kallikrein in there. On the other hand, it's good the TGA can detect massive contamination with kallikrein.

Last, not least, this is where we are now with the kallikrein activities. The pink line is where we are. And what we experienced is the blue bars were from Pharma Octagam batches, you see that were detectable kallikrein activities.

Here we have 11 other immunoglobulin preparations, different lots. You see that there's one -- the reds are still significantly detectable in there and they're still available. And the validation batch is three out of seven because I couldn't bring all here, is now that we are below that pink line with all the validation batches so there is no impact on the procoagulant activity.

So briefly, TGA turned out for us the most important test-system sensitive, adequate for that what we have identified. Kallikrein has an -- may have an impact, but a very minor extent contribution to procoagulant activities. We have not found any other factors in there that could contribute to the activity like factor VII. They are all gone. And finally, we have implemented now a step that reduces and removes XIa early enough in the

process. I'll come back to that in the second talk. Okay. Just want to state here to the discussion this morning.

XIa as an inducer of coagulation, yes, no, or just for propagation of the clot, I mean, to literature. Yeah, we know that XIa does not only activate IX, it can do other things. It can even, on the other hand, delay clot lysis, which also has an impact on prorogation. And it has been infused even in monkeys, in '96, and it had a significant increase of coagulation markers.

From that what we know -- I mean, with all care, we think that XIa can induce thrombosis if it can hit the target in unfortunate patients.

Okay. Thank you very much for your attention and just appreciate the whole team that worked on it.

(Applause)

SPEAKER: So I think the purpose of the panel discussion was to address, I think, a couple of questions which were listed here on the handout. What are the most likely biochemical and maybe biophysical causes of thrombotic events in IGG recipients? And I think we've -- the talk so far have targeted in on XIa, perhaps kallikrein and the question of viscosity.

But I'd like to ask the panel members what their thoughts are on other potential causes that we may not have addressed in the talks, and specifically, the possibility given the incidence of arterial events that perhaps platelet activation is a direct consequence to something in the preparation may be going on. Would anybody care to speculate?

SPEAKER: If I may briefly answer. I mean, if you have an activated coagulation factor in your preparation, then platelet activation is not far. So I think -- I think the question is, if there's no activated coagulation factor, whether then there is platelet activation. Whether there's something else, I don't know. We didn't find anything so far.

SPEAKER: So given the incidence of our connection between elevated factor XI levels and arterial thrombosis are not -- those are inconsistent. So the XIa, I think, had -- does this easily explain the arterial as the venous thrombosis. Also, given the timing of the two, although some of the earlier talks discounted --- well, so whether viscosity is the only issue in the venous thrombosis or whether XIa is contributing there as well may



stem from difficulty in separating whether XIa is present at the time that the venous thrombosis is detected or the time at which it was initiated. And so, the XIa could easily be implicated in both of those situations.

But that obviously doesn't preclude any one or all of these other central risk factors as mitigating the event.

SPEAKER: Has anybody actually looked at the effects of the IVIG products on platelet aggregation, perhaps in a system where hirudin was used to block thrombin to see if there might be something else within the preparation that could directly affect platelet function?

MS. GRAY: I think we have to look at the effect of the IVIG which contains XIa on collagen-induced aggregation and also arachidonic acid aggregation. And we didn't actually see any effect. And the IVIG on their own doesn't seem to be having effect in a system that we use. But we haven't really gone into look at what happens at least, for example, if we initiate maybe the -- to hit platelet (phonetic) aggregation using ATP or other agonists.

SPEAKER: Elaine, in that assay, was thrombin

generation present when you were doing the collagen and arachidonic acid stimulation?

MS. GRAY: Yes. But you know, it's very -- we didn't do a lot work on that because the preliminary work was -- shows that we didn't have positive result. But I think that now if we have a little break in the times working on the other bits of IVIG, we'll go back and look and say -- because I think it's certainly worth in exploring, seeing the effect on arterial thrombosis.

SPEAKER: Well, I think it's pretty clear that IVIG administration will lead to cellular activations through IL-6 increase, TNF-alpha increase, on the one hand. On the other hand, you have all the issue of regulation of apoptosis by Fas/Fas-ligand interaction. And there is still a debate whether IGIV is inhibiting or increasing apoptosis. So you could imagine that IGIV could lead to endothelial cell apoptosis with a whole sequel of pathogen effects.

And I think it's out in the literatures that IGIV induces PAF synthesis and secretion from neutrophils through Fc gamma receptor interaction. So there's a whole range of potential interactions, which could contribute, I

think, more so to arterial thrombosis than only identifying one single culprit, namely factor XIa.

SPEAKER: Please?

MS. GRAY: I'll make a couple of points. One is that I think most of the audience has realized by now that it's possible to have lots that do not appear to have a, I would say, elevated peak thrombin or elevated amylolytic-activity, but have been implicated in thrombotic adverse events. So whether that is actually caused by the immunoglobulin infusion or not, there's a moderate number of those, potentially. And it would be very interesting to study more lots that were associated, perhaps especially with arterial events, only because those are more clear cut and recognizable at the time. So that's really a research comment.

I just wanted to read something from the Lancet 1986 paper by Woodruff. It's just interesting that they thought of platelets first. They say, "The effects of the IVIG preparation that was -- seemed to cause thrombosis in these ITP patients -- and on ATP release from normal human platelets were studied at a concentration of -- and it gives some details: 9 grams per liter the approximate

plasma level predicted at the completion of a 6-hour infusion most of the -- if most of the IVIG remains in circulatory compartment. IVIG alone did not cause platelets to aggregate in platelet rich plasma at 37 degrees C, but did cause some enhancement of ATP release at sub-threshold levels of ADP or adrenaline.

Evaluation of the response of platelets in whole blood in a Chrono-log Lumi-aggregometer, the IVIG revealed release of 0.21 picomoles ATP pertaining to the XI, platelets relative to normal controls in which phosphate-buffered saline was used. And 15 -- I forget what that -- omega, impedance or aggregation relative to normal controls over 20 minutes."

So there was this concept and this still might relate to factor XI though that platelets are somehow activated or stimulate by immunoglobulin in that setting.

SPEAKER: This -- maybe now I can just ask this question. Are there lots of IVIG that have been associated either with arterial or venous and not both? Has that been looked at?

SPEAKER: It's a difficult question to answer mainly because clusters of these events don't frequently

occur. So what we more -- most typically see is one arterial event or one venous event. And we have seen, though, in the occasional patient -- a combination in one patient of an arterial event and a venous event.

SPEAKER: During the discussion, it was brought up that platelets bind to factor XIa and prolong its half life, and I'd like someone to comment on that?

SPEAKER: Those studies, I think, depend on which inhibitor you're looking at, particularly when you're studying factor XIa or in the vicinity of platelets. One of the most potent inhibitors isn't one of the plasma serpins. It's protease nexin-2, which is released from the platelet granules. And so, under those circumstances, when the factor XI does interact with platelets, they seem to be protected to some extent from protease nexin-2.

I'm not aware that a lot of formal studies have been looked at to see how well XIa is inhibited by antithrombin heparin, for instance, or C1-inhibitor heparin in the presence of platelets. I don't know if somebody else knows of any studies of that sort?

SPEAKER: And to the inhibition, I mean, that has been in the -- I don't know whether I pronounce it

correctly. Burke, in 1996, or so described, just putting XIa into plasma and looking what happens to that. And it takes about 10 minutes until there's a plateau reached, but that has been measured by going into inhibitory complexes, not by activity. And 30 percent went into antitrypsin complex, 30 percent in antiplasma and 30 in C-1 inhibitor and 5 to 10 in antithrombin complexes.

Although I don't know whether there's XIa left in there, but that is, in a static system, just putting XIa in there. So we don't know what happens if there are cells around and whether there are atherosclerotic plaques around where it could stick to -- it could be protected and slow circulation certainly.

SPEAKER: If you're studying the biological effect of immune globulin after an infusion, you measure the antibody level because that's what the purpose is. And I'm wondering if such studies would appropriate in patients given gamma globulin in terms of their coagulation profile, in addition to see how long the elevated factor XIa persists.

SPEAKER: It is difficult -- is it difficult to inject a lot of product with known procoagulant activity?

SPEAKER: I think he was referring to a product that was given for therapeutic indications.

SPEAKER: Yes. My understanding is that current products or future products that will be available -- that will be on markets after this workshop wouldn't have procoagulant activity. So this study doesn't seem possible.

SPEAKER: Yes, in addition --

SPEAKER: Another issue that came up is if you give subcutaneous gamma globulin, does that change the coagulation profile of the product?

SPEAKER: What --

SPEAKER: What we have to do is -- we have this publication from Tim Carter (phonetic) from '96. He -- that was, I think, after the evaluation of discussions about factor XI concentrates and whether XIa as traces have caused thromboembolytic event. So obviously, he went and injected monkeys with XIa and just followed it, and just looked what happen. So obviously, they were healthy monkeys and not diseased anyway. But there were clear increases of coagulation markers. I mean, I don't have in mind whether it was TAT for F1 + 2 or something like that.

But they really went up. So I think that says all what happens if that hits then a plaque or whatever.

And just to add, I mean, there are many patients -- it's not that a lot just is thrombogenic as such. I mean, if that -- obviously, we have patients here and they have a different history and underlying diseases and vascular diseases. So there are batches that have even a high or moderate XIa and may never develop or show up with the TE. And others, who really hit the wrong patient group, do. So, there's a not a lot that provokes in all patients having receiving the lot NTEs. So we really have to appreciate the patient situation here as well.

SPEAKER: Very briefly, because I think we're running out of our time here.

SPEAKER: I was just saying (off mike) and certainly that's (off mike).

SPEAKER: Okay. All right. With that, I think that's the end of our session. I'd like to thank all of the speakers and all of the people who put in the questions. Thank you.

(Applause)



SESSION II -  
PARTITIONING AND ACTIVATION OF CLOTTING FACTORS BY  
MANUFACTURING PROCESSES

MS. GRAY: And a good colleague of ours, Dr. Gross, is going to begin the discussion, from the Paul-Ehrlich-Institut. And thank you very much.

RISK REDUCTION AND MANUFACTURING

DR. GROSS: Thank you. I wasn't aware of this slot in the meeting today and I had the chance to talk about until yesterday evening. So I have just seen the revised agenda yesterday evening, so I tried to prepare at least something.

Just to start with, our workshop goals. The goals were to identify the most likely causes of the thrombotic adverse events which were observed, to identify promising test methods that can be validated and which are reproducible and robust and -- and this is depicted in red here -- the potential of the manufacturing process to reduce such potential procoagulant impurities and the

validation of the removal of such factors is one of the major issues which has to be addressed.

The proposed outcomes -- and here I would like to change the order a little bit of the ranking, because I guess we should have here 1a and 1b. 1a would be the ranking order of the most likely biochemical causes of IVIG-related TEs, and 1b, I would call, the process understanding how it is possible, how can be -- such impurities be introduced into the IVIGs during the manufacturing process.

And just the ranking number is number three, is the identification of tests which are likely to have predictive value and assessment of the validation potential. I guess without such tests, it would be difficult to identify such impurities, but at least we should think about whether specifications should be introduced into the, let's say, release specifications. And therefore, also in the bottom I've depicted a rather as a questions and requirement, whether they should be a revision of the monograph on human normal immunoglobulin for intravenous administration be, let's say, requested by introducing test methods or specifications.

What are the reasons? This was mentioned several times this morning so -- it's our current experience with immunoglobulin preparations which causes thromboembolic complications. And the current discussion was focused on the potential of certain test systems -- TGA, NaPTT, antigen-specific assays -- which might be able to detect certain impurities, but we should always keep in mind that we cannot test quality into certain products. Therefore an understanding of the manufacturing process is much more essential than we thought until now.

We should look -- or companies should look at the potential influence of the manufacturing steps on the removal or even activation of impurities, and these should be considered on a risk basis and approach and this should be analyzed on a scientific basis. And the risk assessment should be the basis for the design of the manufacturing process in all the different optional steps and step combinations.

What we faced during the recent years is that we have obtained less and less information which is provided on the in-process controls and the manufacturing process details and process validation, or even on the analytic

methods, in the submissions for marketing authorization applications, even for variations.

Another important point is that the globalization of the manufacturing process has dramatically increased all different options what manufacturers have in their manufacturing processes. So a really important, let's say, issue is process harmonization and the adaption of the manufacturing processes to different facilities. And what we observed during the recent years is that the usage and the quantity of alternative approved steps are changed over the last years, so it has increased frequency and different ratio of plasma mass capture steps to plasma pool size and all the different things.

So as the manufacturing process became much more complicated, options became much more multiple. So it's really difficult for us as regulators to have here the right, let's say, insights and views on the manufacturing process.

This is a just a scheme of a usual manufacturing process. You start off with the plasma pool. You have many optional adsorption steps, coagulation factors. You can purify factor IX, factor VII. You can remove

antithrombin III, PPSB. Then you have the cold ethanol fractionation. You have several possibilities for virus inactivation, and you have further purification steps, which is for polishing or other limitation steps. They remove aggregates or dimethyl (phonetic) or something like that.

You should also think about a completely different, let's say, approach for manufacturing immunoglobulins. There are, let's say, methods around which even do not use fractionation or precipitation. There are filtration methods around -- and we have no idea what filtration contributes to the removal or which does not contribute to the removal of certain, let's say, procoagulant coagulating impurities. So we should also have a look at the new, let's say, approaches to produce immunoglobulins.

I try to avoid the term "quality by design," but actually the manufacturing process for immunoglobulins is a really huge quality by design approach. So all the different, let's say, critical parameters which are necessary would include the pharmaceutical development, the validation, which includes the risk analysis and also which

includes the proper quality control and the quality assurance should be included here in the application, let's say, dossier and should be scientifically based.

Our goal is, and that's also the goal of the manufacturers, to have a safe product. That means that this really required that a validation of the process with all the different optional steps and all the different combinations will be required in the future. The focus should not only be on one impurity. We have heard this morning about factor XIa, we have heard about kallikrein, but we have also discussed quite recently, activation of platelets. So the focus should not only be one impurity, all potential impurities should be addressed and considered.

Of course, the influence of each step, each introduced material and the removal of potential contaminants should be discussed, and the identification of critical process parameters and the setting of these is really important for the future. Overall, these, let's say, measurements, will lower the risk of thrombosis generating agents in the final product.

And the first step to address this was done in

Europe at least by the Group 6B, which has proposed to revise monograph on immunoglobulins for intravenous administration and this change will be introduced in the production section.

And there a new writing (phonetic) was introduced. The method of preparation also includes a step or steps which have been shown to remove thrombosis generating agents. Emphasis is given to identification of activated coagulation factors and their zymogens, and process steps that may cause their activation. Consideration should also be given to other procoagulant agents, which could be introduced by the manufacturing process.

And by doing -- by having introduced this paragraph into the monograph on the immunoglobulins for intravenous administration, we have the basis to ask for these-- let's say, for the documents, for the results. And actually what we have done over the last year was during upcoming variations or new applications, we asked for these issues.

As I said before, it is necessary to further revise the monograph on human normal immunoglobulin for

intravenous administration or also for the subcutaneous administration, and to include specific test methods or specifications. I have to say it is still too premature. We have heard this morning that the number of tests, assays is around -- the TGA was mentioned several times, what we have heard this morning several times that performance of the TGA is not that simple that simple as it seems to be.

We have heard from our colleagues from AFSSAPS that there are some -- they have some trouble with setting up the TGA. We have seen the correlation what Elaine provided, which is not always linear. We have heard from Johannes Dodt that TGA is at a -- at least the TGA performance that applies to in-house assay at certain threshold level of factor XIa, you cannot distinguish between peak thrombin concentrations. So there are certain limits with the TGA, and therefore, I guess a really big step forward would be the outcome of the collaborative study, which is ongoing.

And I guess the first results were delivered last week to Elaine, and we delivered our results yesterday to Elaine. So I guess she's really busy during the night to present at least some data tomorrow to you as well. And of



course, also the discussions which are ongoing during this workshop will help to address whether there should be an assay at all established or whether it is necessary to include an assay into the monographs.

This is just one picture what we have seen or what might give, let's say, my represented basis for discussion. What we have here compared is NaPTT results against the results of then the TGA. And this is time to peak and time to clotting. And what you can see here is we have collected many, many more data now. Well, I guess we routinely test all the different IVIGs what we have now separate for batch release. And what we see is that the left down corner, we the Octagam batches, which were involved in TEEs.

Then in the middle we have another, let's say, cluster of Octagam batches which were -- this was the quality what we seen before, say, have now implemented the correction actions. And on the right side in the blue, let's say, circle, you have now the 24 new Octagam batches. Now, we have 36 new Octagam batches, and they're all in this upper cluster. And the quality of this Octagam batches is now close to our IVIGs which we're going to

market.

But we have also included here, and we have discussed this already this morning, is Vivaglobin , where the market authorization holder himself detected some procoagulant impurities. And if we look at the data here, we can also demonstrate that, let's say, that impurities are high as it was in the Octagam TEE batches. But we have to mention that this product is given subcutaneously, so the effect in patients cannot be really correlated, at least now. Therefore -- yes, I guess the clinical -- as a clinical meaning for what does it mean, if you have such impurities in such subcutaneous IVIGs, is still to be investigated a little bit more.

And the question is whether -- what the picture also demonstrates to you is that TGA through the sensitive method it has a wide range of sensitivity, and whereas NaPTT is not as sensitive, but both assays are able and capable to distinguish between good and bad batches. And the question what we have is, which sensitivity do we need to identify, let's say, bad batches and to, let's say, distinguish between bad batches and good batches.

So it might be -- it might already be the case

that NaPTT would be sufficient in order to identify bad batches and then we would have an already validated assay which is already in the monograph, which would be critical to, let's say, discriminate between good and bad batches.

And another thing what we should mention here is that also the NaPTT is able to unspecifically detect impurities, whereas antigen-specific assays would only detect specific impurities. So we should look for an assay which is able to detect unspecific impurities and which is sensitive enough to distinguish between good and bad batches. Thank you very much.

(Applause)

MS. GRAY: Maybe we have time for just one question for Steffen. Anybody? Or we can wait till the panel discussion?

DR. GROSS: Yes, we should.

MS. GRAY: Okay.

DR. GROSS: Just one.

SPEAKER: So were you able to identify any steps in the manufacturing process or the plasma characteristics that you associated with a high XIa? Or you can't talk about it?

DR. GROSS: We didn't identify it, the company did. And we know which steps are -- were introduced and which steps were responsible for removing such factors. But, yes, I cannot talk about it.

SPEAKER: Okay. I'm an academician. I have to ask.

MS. GRAY: Okay. Our next speaker is Albert Farrugia, representing the PPTA. And he promised me that he's going to give a very interesting presentation.

MANUFACTURE OF IMMUNOGLOBULIN THERAPIES - RELATIONSHIP TO  
THROMBOGENICITY

DR. FARRUGIA: No, I didn't. Good afternoon. It's been some years since I spoke at an FDA Workshop. I think it was 2004. I was 47 years old, just a kid with a crazy dream. Incidentally, that's a Leonard Cohen joke.

Okay. I am charged today with introducing the discussion involving manufacturer, a question of which has just come up. And this is a summary of what I'll speak about. I will talk about the first phases of classical plasma fractionation. The companies will then take this up

individually to discuss how they individually address the finishing processes.

I will share some reflections like any speaker, late in the day. Some of what I have to say will have already been touched upon, but I'll share some reflections about immunoglobulin in products as being distinct products. I'll share some very personal thoughts about the issue of causality and thrombogenicity, and I'll make some concluding remarks.

This is one of the great Americans of all time, Professor Edwin Cohn, who in fact developed this industry. He was charged to do this by the U.S. military during the Second World War after a very distinguished 20-year career in which in his department, in Harvard, he developed a lot of protein chemistry with other distinguished protein chemists such as Scatchard and Edsall.

Frequently, I hear people nowadays, many of them younger than me, disparaging Cohn fractionation as being bucket chemistry and non-scientific and stuff like that. This is absolute nonsense. Cohn approached the fractionation of plasma proteins based upon very scientific principles, ultra protein chemistry, much of which has

withstood strongly the test of time. And this is actually a very scientific process.

I just want to say again in relation to tribute to Edwin Cohn, that Cohn never took a patent for his work. He considered it as part of public service, and he insisted, like all good scientists, on the right of republication. So that many of Cohn's papers while developed for war-time purposes during the Second World War were published during the war, and we know that in the opposing countries, including Germany, Cohn fractionation also occurred because of this. Truly a great man; twice nominated for the Nobel price, never got it. So I will leave you to reflect on the justice of that.

This is the most familiar version of Cohn's initial work, the so-called Method 6. Cohn fractionation consists of the fractional precipitation of proteins from plasma utilizing alcohol in order to decrease the dielectric constant and make the proteins less soluble. Other parameters, including the salt concentration, the ionic strength and the pH, are also adjusted to bring proteins close to their isoelectric point and induce the precipitation.

You can see from the slide that we first get the classical fraction I being precipitated. This is composed mostly of fibrinogen. The alcohol concentration is then further increased to result in this first version of Cohn fractionation in the precipitation of so-called fraction II + III, which includes the immunoglobulins and a lot of other globulin proteins. This is then further fractionated in order to result in the ultimate albumin fraction.

I want to remind you that Cohn fractionation was initially developed in order to purify albumin as a stable blood substitute for use in the conditions of war. Along the way, Cohn, of course, generated other fractions which could be used therapeutically, and this Cohn Method 6 did in fact yield gammaglobulin, which was used therapeutically in the sense it was from Cohn Method 6.

I also want to remind you that all this occurred in the 1940s and '50s before the coagulation cascade was indeed developed and before most of the clotting factors we know about today were actually discovered. At the time that Cohn actually developed this process, only two or three factors, including fibrinogen and so-called antihemophilic globulin, had been characterized.

So when you, as you need to do, go to the original Cohn papers in order to assess this question of where the proteins and the procoagulant fractions actually partitioned -- what's going on? -- you read a lot the word, prothrombin. Of course, at this time prothrombin was not even purified. By prothrombin, what the investigators meant was basically all the procoagulant fraction of plasma, which under suitable conditions could coagulate fibrinogen. It's important to realize that.

As the therapeutic potential of the IG fraction was realized, Cohn's group, led by his colleague Oncley, developed further the fractionation in order to result in a fraction II precipitate, which was more stable and more amenable to working into a therapeutic product. And this is shown on this particular slide, and this you can see here the fraction II + III is subfractionated so that the fraction II goes down the route to developing a more purified IG product, and the so-called globulins, including the procoagulant parts, are partitioned into a fraction III, which was subfractionated and investigated also for potential therapeutic use.

Over the 1950s, the Swiss fractionators Kistler



and Nitschmann developed their own version of Cohn ethanol precipitation, which was basically the so-called Kistler and Nitschmann fractionation scheme, which is essentially an abbreviation of Cohn fractionation. By studying carefully the precipitation conditions, they managed to abbreviate the whole sequence of sequential precipitations into just three. It is shown in detail in this slide and in the following slide, and this resulted in a gammaglobulin precipitate which could also be utilized.

This happened in Switzerland, where very early on, active work in gammaglobulin use was initiated and led to many of the first clinical observations on the use of IgG. I thought it apt to cite from Kistler and Nitschmann's original paper, two interesting points. First of all, that the method was aimed at obtaining yields as high as possible, while achieving purity such as seemed necessary and reasonable -- observe the wording.

And very importantly, I think for the purposes of our discussion here today, that slight deviations in ionic strength and other parameters wouldn't influence yield and purity of the final gammaglobulin remarkably, and a rigorous standardization of the processing is therefore

indispensable. These words are more than 50 years old.

Some clues on what causes thrombogenicity in relation to Cohn fractions. There are some indications that the removal of fraction I itself is necessary or adds to the removal of thrombogenicity. This is some work which I was involved in many, many years ago when I was but a youth and had a life, in which in one other aspect of fractionation which has included a strong focus on thrombogenicity, the production of prothrombin complex concentrates. We observed at that time that a removal of fraction I and the utilization of fraction I ethanolic supernatant rather than cryosupernatant led to lower thrombogenicity in the product.

I think that this might have some role and might cause us to think about a bit in relation to the fact that some fractionators in order to, as I say, abbreviate the fractionation have taken to the custom of occasionally co-precipitating fraction I, II and III together by including a large concentration of ethanol immediately in the fractionation. Some more clues -- and this is important, I think, and has already been touched upon in relation to what is being boxed into corner of being the culprit in

this issue, which is factor XI.

It is important to know that under the conditions of Cohn fractionation and in the initial precipitation steps, besides the fact that factor XI has a molecular weight which is quite close to IgG and the way in which the pH and the ionic strength are manipulated, the isoelectric point also approaches that of immunoglobulin. And therefore, it's not surprising, it has been published, that it co-purifies with IgG in preliminary steps.

It's also important to know that this has been noted as well today, that factor XI is also absorbed with heparin-agarose. And in one particular concentrate, which I think has been mentioned, it is found as is antithrombin III, in equal amounts with antithrombin III. So it is important to note that that is one way of addressing the issue of factor XI.

So to summarize on this particular issue, coagulation factors with the exception of fibrinogen and factor VIII, possibly factor V -- there are some indications that factor V also goes through fraction I -- partitioned to essentially the first Cohn fraction II + III. Fractionation of II + III results in most of the

globulin, except for the gammaglobulin, being precipitated into fraction III, away from fraction II.

Nevertheless, this is inevitable. Some factor content in fraction II must be expected, and further removal of these factors must rely on finishing operations. And these include ion-exchange chromatography, diafiltration, different (phonetic) activation processes themselves, which introduce other purifications into the fractionation sequence. And these will be the subject of company presentation after I finish this talk.

I just want to make some points now in relation to the question of IgG and the extent to which IgG can be considered the single class of products. I was struck by this publication recently, which along with many other publications, given the current interest, described an episode of thrombosis following IVIG treatment in ITP. And what struck me most about this publication was that the product was not actually specified by name, it was just called immunoglobulin.

And I think that this one aspect which requires some comment in the sense that there is a kind of ethos in some areas that all immunoglobulins are equivalent and are

the same. I think that this is something which is not justifiable when we consider biologics in which the individual process, which can vary according to the extent to which fractionators vary their processes, can affect the product itself.

I'm obliged to Dr. Scott. I can remember her giving the following slides in a visit she made to Australia, to my then organization 11 year ago. And I used this slides, which actually includes work from CBER a long time ago, with her permission. You can see here one particular very minor modification in the precipitation of fraction III from fraction II + III, which I've already described.

When this done a pH 5.4, a stable immunoglobulin fraction results. When the pH is just 0.3 over pH units less, we get an unstable fragmented immunoglobulin fraction. This is now known to be due to the fact that plasminogen's activation into plasma is best at the lower pH and then results in a fragmentation of the IgG, when plasmin is accidentally generated.

Another interesting bit of data, again, from Dr. Scott, shows this issue involving PKA this time, not factor

XI, in which the stirring of the filter aids -- and we've heard about contact activation and you'll hear more about fractionation conditions later. In which the stirring of the filter rate time was varied, more contact activation and more PKA was generated, and this led to a product which led to reactions.

So we can see how relatively modest changes, not necessarily immediately appreciated, in the fractionation sequence leads to different products. And I think that this is an important point.

If you look at the patients who receive immunoglobulin, these are the potential in relation to adverse events. And you can see the compositional variation, which is possible to sodium, sugar, osmolarity, et cetera content. And you can see how this kind of affects patients. This is a somewhat, in fact, impossibly complex slide, but it seeks to make one very simple point.

And this is in relation to product tolerability. This is from a publication which compared patients on two individual products. I refer you to the original publication, which actually indentifies the two products. And the point to be made here is that as with many similar

publications, patients who switched from one product to another exhibited strong tolerability and adverse event differences. This supports our contention that immunoglobulins in terms of safety and adverse event profiles, need to be considered as very distinct individual products. The same applies, I think, in many aspects of efficacy.

I apologize that I didn't put the reference from which this slide is extracted. This is a publication from Taiwan from some 10 years ago. I believe it is from the Journal of Pediatrics. It again compares a number of products which are not identified in the slide, but which are identified in the original publication in relation to efficacy of IVIG in avoiding the vascular malformations in Kawasaki disease. The point to be made is that products in this study show the different efficacy, this time, profiles. And this was deductively assessed as being due to the different manufacturing processes which were used to make the products.

And I think already we've heard the word "class effect." Today I think it's a bit challenging ascribe to products which are so variable and different the concept of

class effect, and we've already heard about the basic etiology of thrombosis and the issue of Virchow's triad. And I'm struck by the fact that in what I might call the pre-Octagam literature, with the exception of in-vitro type studies which doesn't look rigorously and try to assess causality in relation to patients, the clinical reports in this phase of the literature always seem to emphasize issues related to a and b in Virchow's triad. In the sense that they looked at -- and we've heard today about patient comorbidities and characteristics and the issues like viscosity changes and so on.

And so I wonder whether we're actually looking at two convergent issues here. The pre-Octagam era, representing what is accepted as a recognized but very rare adverse event, and the post-Octagam era, mostly characterized by problems with one or two products. I'm kind of intrigued, and perhaps the FDA will comment about this later on whether the FDA is interested in trying to solve the problem going from the Octagam level to the zero level. And we can discuss that later on.

I just want to share -- and this is I suspect something a bit controversial. But some personal



reflections on a thrombogenicity plasma products issue, which, like some of the people here, I was reasonably involved in early years, and this is the question of the causality of the thrombogenicity of prothrombin complex concentrates. This was a big problem in the treatment of hemophilia A, 25 to 30 years ago when we didn't have the single factor IX products.

And several tests -- in fact the tests which you've been hearing about today have their inception in the development of ways of addressing this problem. This is why the NaPTT was initially developed and embedded in the European Pharmacopoeia and the first coagulation based versions of these thrombin generation tests.

The strong hypothesis at this time was that this was due to entities detectable by these general screening tests which were representative of activated forms of the complex of coagulation factors which made up this product. Eventually, I think that much of this was disproved -- if I can use a word which actually I don't like using in terms of science -- in the sense that it was the actual zymogen overload, which was shown to be the issue more than the actual content of activated coagulation factors.

And I'm showing here a complex set of data from two publications extracted from the Paul-Ehrlich-Institut. You've already seen this type of thrombin generation curve. And I don't have the time to enter into a detailed description of this very elegant scientific data. You'll have to take my word for it and go to the original publications.

But what was actually shown by the Paul-Ehrlich investigators here in these two sequential publications was that it was not the actual factor IXa within the prothrombin complex concentrates which causes thrombogenicity, because they could show that the clinically thrombogenic concentrate had less factor IXa than "cold product." But that by -- with elegant studies involving the supplementation with purified factors, shown on the left hand part of the slide, that was actually the zymogen prothrombin.

The point I seek to make is that causality is difficult to ascribe on the basis of immediate laboratory data and we need to be aware of the sophistications needed.

So I just want to come to a conclusion. I hope haven't overstepped time, Elaine. As I've said, the

procoagulant globulins invariably co-fractionate into the combined fraction II + III in Cohn fractionation, and then the vast bulk partition into fraction III upon further purification of the immunoglobulin fraction.

Factor XI in particular, but not alone, but in particular, has physical chemical properties which lead us to expect it to co-fractionate with IgG in these preliminary steps. As indeed it does.

Now, we have seen much data at this meeting and in publications over the years, but there are some levels of factor XI in the vast majority of products. These are very small, and this shows that subsequent steps effectively clear much of the factor XI from IgG. Fraction I, I suggest, has possible implications and contributing to thrombogenicity, and perhaps it is not desirable to immediately co-fractionate it into the immunoglobulin fraction as a starting material.

The different IgG products have different safety and efficacy profiles; causal linkage therefore to particular effects is difficult and it is not desirable to talk about class effects in the first instance. I have spoken of my advocacy, as have many other people, of

remembering that thrombosis doesn't just come from activated coagulation factors. And that in other types of fractionation experiences, such as prothrombin complex concentrates, we may find some lessons to address the current problems.

So I thank you for your attention, and I hope I haven't overstepped my time. Thanks very much.

(Applause)

MS. GRAY: Okay, we have two minutes for questions for Albert.

DR. FARRUGIA: What do you want, Bernie?

SPEAKER: I like you to return to your youth when you had a life. And one of the subjects you (off mike) most of the other speakers have not talked about (off mike) cellular content (off mike) plasma, or the cellular fragments that may present (off mike) and whether they have an effect at all?

DR. FARRUGIA: I will engage in a pure speculation because I'm not aware of a lot of data. It has been a source of intrigue to me as to whether this is a contributor. One should expect that plasma harvested from, for example, whole-blood type collection might have a

higher cellular content.

I can remember when working on thrombogenicity in PCCs -- this is 25 years ago -- we were also looking at different plasma freezing systems, systems based on centrifugal versus systems based upon filtration principles. And we could observe some in-vitro, NaPTT type, measurable differences in these products.

I think that deriving a more robust answer to your questions these days would be extremely difficult, unless one were to look at a lot of batches derived from particular types of plasma. And given the fact that this is an extraordinarily rare event anyway, I think it will be very difficult to answer. But it is a good question.

If you, for example, look at plasma itself, irrespective of whether it is still in contact with blood, and you store plasma in the cold, for example, you get activation of things like complement and the contact activation system itself, in-vitro in the plasma. So it could result in some level of contributing to this type of problem if you get it following on a fractionation. Can't say anymore.

SPEAKER: Would you mind reviewing again the

evidence that that factor I precipitation reduces thrombogenicity?

DR. FARRUGIA: This is purely an extension from, as I said, work which I myself did in relation to another clinical thrombogenicity problem, which was the generation of prothrombin complex concentrates. And this is extremely old data, and in reaction to current practices, it is of limited relevance. But we had found out that by absorbing Cohn fraction I supernatant from which the fraction I obviously had been removed, the subsequent prothrombin complex concentrate had lower in-vitro thrombogenicity as assessed in tests than had been the case with product manufactured directly from cryosupernatant which had not been modified.

I noted in the literature that there is a practice occasionally -- I don't know how widespread it is, and individual companies will comment about their individual processes -- of co-precipitating fraction I + II + III in some processes. It could be that the co-precipitation of fraction I immediately into the ultimate immunoglobulin leading fraction might contribute to some procoagulant activity. But again, this is semi-

speculative.

Dough (phonetic)?

SPEAKER: (Off mike.)

DR. FARRUGIA: I can't remember. I'm jetlagged,  
Dough.

SPEAKER: (Off mike.)

DR. FARRUGIA: Of course you have, Dough, you  
work for the FDA.

(Laughter.)

SPEAKER: (Off mike.)

DR. FARRUGIA: It's very interesting. I wasn't  
aware of this. I leave it to the young people to continue  
the work.

SPEAKER: Okay. Thank you.

(Applause.)

PRELIMINARY PRODUCT TESTING AND METHODS DEVELOPMENT -  
MANUFACTURER PRESENTATIONS

MS. GRAY: Okay, I think that we're now on to the  
manufacturers' experience. I like to welcome Peter Turecek  
from Baxter to present Baxter's experience in this area.

MINIMIZING PROCOAGULANT IMPURITIES IN IGIV PRODUCTS -  
BAXTER'S APPROACH

MR. TURECEK: Thank you, Elaine. Good afternoon, ladies and gentlemen. I would like the next fifteen minutes or so to show you what we at Baxter do to minimize the procoagulant impurities in IGIV products. I will show you which tests we have established. I will talk a little about in-vivo thrombogenicity. I will show you one practical example of removal of factor XI or a factor XIa during the commercial Cohn fractionation. I would like to end up then with some of our pharmacovigilance safety assessments for one our IGIV products before I come to my conclusion.

I think I can skip the contents of this slide, because everybody in this room is aware where we were coming from. I only want to highlight that although we had not a similar signal with our products, we proactively started an investigation to address this further and we



were building here really on experience that we had been establishing for the last 40 years in this business, where we always had a focus on procoagulant impurities testing in any of our new plasma products that we were developing.

The test methods that we had established is not very different from what you had already heard this morning. We have of course the non-activated partial thromboplastin time. We formally performed it mainly in normal plasma since this issue came up also in factor XI-deficient plasma. Then prekallikrein activator assay.

What we did not hear so much about this morning was we also do protein and protease profiling, which means that we have established and validated ELISA test methods for -- with specific antibodies for all the proteins that could be contaminating our plasma fractions. We also look into proteolytical activity using different chromogenic and fluorogenic substrates, so that we do get an idea of what kind of protease we do have in our products.

Then of course, we focus on thrombin generation assay. This was not implemented because of this issue. Those of who you have been hearing from us on our hemophilia products know that we had been working on this

for the last 20 years. So we do have at least two variants of validated thrombin generation assays in our laboratories. But for this purpose we're building on the calibrated automated thrombograph based on the so-called Hemker Method.

And then of course, we have a factor XIa activity assay, and I have the details in this slide here. This is an assay that builds on a published method from 1994 from Von dem Borne and co-workers. The specificity or the specialty of this assay is that it uses factor IX as the natural substrate of factor XIa. So it is very factor XI specific. It employs purified -- other proteins, factor VII, factor X, phospholipid, calcium, and the read out is activation of factor X.

And this factor Xa that is generated in this assay is measured with a •NAPAP-chromogenic substrate for factor Xa. So it's a simple chromogenic assay, but this is again very specific for factor XIa.

The most relevant in vitro methods that we use for assessment of the thromboembolic potential of IGIV products are the global assays, the NAPTT, as I mentioned before, where we have also routine in the quality control

for some of our even older immunoglobulin products that are no longer on the market, but our quality control is able to handle this method quite well.

In the meantime, we have also even for IGIV, validated the thrombin generation assay. As I said before, we have experience with this methodology for the last 20 years. This specific assay I just presented to you, it raises the notion that factor XIa is one of the primary candidates for causing the thromboembolic impurities.

And now, let me show you some results here. What we did here, we compared products from several manufacturers. None of them we know that would be causing thromboembolic events, because we simply purchased them on the free market and compared them with four batches of our Gammagard Liquid or Kiovig product.

And what you do see here; first of all, the TGA results. This is the third column here. We display the result here as percent of a normal plasma control at 5 percent protein that we adjust in our sample, and there are three products where we found elevated levels; product H, I and J. J had the most prominent thrombin-generation activity.

This product was also containing most a factor XIa product. Product I as well had 1.95 milliunits factor XIa per ml. Product H, by the way, did not show any factor XIa content. And one of the three products was also depicted by the NAPTT assay, which we display inversely to some other tests. We'll see probably later on.

We have released -- it's not a release test for us. But historically as we had as a release test, we displayed a milligram of protein at 180 seconds. So this product J had 1.44 milligrams, and all the other products were negative in all the assays.

This slide here shows the correlation of these assays between factor XIa, TGA and NAPTT for various immunoglobulin products. This contained a data set that you had seen in the previous slide, but also additional samples that we put in here, and this is not for the purpose to show you the products or product qualities, but simply to show you the correlation.

And what you do see here on the left panel, correlation of factor XIa and thrombin generation, peak thrombin as percent of normal plasma, as I explained to you before. There is quite a good correlation between factor

XIa and TGA.

On the middle graph, NAPTT versus factor XIa, there is also correlation which is somewhat weak, and this because in the high clotting times, there is some variability in the NAPTT assay. And we also do see one outlier here which has a relatively high factor XIa content, but still along NAPTT. And if we correlate NAPTT with TGA, the picture's not unexpected because it's a combination of the first two graphs.

Let me talk about in vivo thrombogenicity. And what I'm showing you here is really very, very new results. What we did here, we spiked factor -- pure factor XIa into an IGIV product. We spiked a 5 percent IgG solution, and the goal was to reach the similar concentrations as were found in the samples A and G of the collaborative EMA-FDA blood cluster trial that was just finished two weeks ago. This is why we only could perform this study within the last six days.

So we took our results here, and I would not like to show too many details because that is -- would be Elaine's purpose to summarize the results of our collaborative trial. But in these two samples, we found

about 30 milliunits factor XIa per milliliter that equals to 600 milliunits -- milliunits factor XIa per gram IgG. And when we spiked this into our IGIV product and applied it to the classical Wessler model with the scoring system, as you can see here, we found the following results.

The IgG without any spike turned out with relatively high Wessler scores, which -- I think I have an explanation since I had the opportunity to discuss this with Marietta Putz, who is probably the most advanced expert on Wessler test in this room here, since she is now working for a year or so on this test only. And she told me that one should very much carefully differentiate between real clots that occur in the Wessler test and erythrocyte agglomerates that can disintegrate upon gently shaking the samples.

So this relatively high background figures are probably not relevant clots here, but what is relevant is that when you spike 600 milliunits factor XIa per gram IgG into the IgG products, you see solid clots. So we all found fours (phonetic) for the two lots that we spiked here with. So there is a clear, at least in this model, a clear correlation between factor XIa and clot formation.

Let me turn to the removal of factor XIa capacity during our commercial Cohn process. Here is a small manufacturing chart from the Cohn pool to the precipitate G, which we investigated. We looked into the intermediates here and tried to figure out where factor XI or factor XIa goes. And here are the results. If we set the Cohn pool at 100% of factor XIa, then we find almost 90 percent in the supernatant, but then the factor XIa and factor XI precipitates at the fractionation -- a fraction II + III, and further down it's further removed through the filtration step until precipitate G. So in the precipitate G, you will find about a little bit less than 2 percent of the factor XI, XIa zymogen that is contained in your product.

I always say XI, XIa, because the ELISA that we use here is not able to differentiate. The further reduction of factor XIa occurs during the downstream purification of precipitate G to the final product via conventional chromatography.

Let me close up with showing you briefly some of the pharmacovigilance experience with our Kiovig/Gammagard Liquid product from 2005 to 2010. We collected all

spontaneous reports of thromboembolic events. Solicited, clinical and literature reports were excluded. The data are presented during the next two slides separately for the U.S. and Europe. Limitations here is, of course, underreporting in spontaneous reporting schemes occur to an unknown extent. Reporting frequencies may not represent the incidence rates. Due to a missing denominator data, no incidence rates can be calculated from spontaneous pharmacovigilance data. And of course, we do see regionally different reporting cultures, which is reflected here, the U.S. experience.

Gammagard Liquid was marketed here from 2005 on. We currently are reaching about 12 tons of IgG that we are distributing here, and the number of adverse events here is -- this is not percentage, this is single numbers -- 0, 2, 1, 2, 1. And in 2010 there were four. We do think that is because of the alertness to the issues that occurred with this product. So the numbers are fairly small.

The numbers are little bit higher in Europe. This is maybe because of the different reporting culture. In Europe, the total number of IgG distributed of this product in Europe is smaller, but still the numbers are



fairly overseeable.

So to summarize, this one here, as I said, the absolute numbers of thromboembolic reports are low for Gammagard Liquid. The reporting frequencies have remained low and consistent during the time that we analyzed here, so we do think that non-product risk factors -- and this was discussed earlier this morning extensively -- as listed in our product insert are also likely to play a major role here. So we think that this is addressed and labeled adequately.

So to summarize what I was able to present here, Baxter has proactively evaluated the IGIV products by in vitro methods indicative for thromboembolic potential. We measured factor XIa in different IgG products and showed the correlation between TGA and NAPTT and factor XIa. The three assays showed a very good correlation level sensitive for factor XIa. Historically, NAPTT had been used for IGIV product characterization, therefore this is our preferred method out of the array of different method because it's easy to perform. It can be validated and, as I said before, we do have experience with this assay to the -- to more extent than with all the other assays.

Spiking of an IGIV product with pure factor XIa makes it thrombogenic in the Wessler stasis model. I was also hopefully able to show you that the commercial Cohn fractionation process is able to remove 98 percent of factor XI or factor XI zymogen or factor XIa present in the Cohn pool.

And finally, the number of spontaneous thromboembolic reports with our Gammagard Liquid product is fairly low. And as I said before, we do think that the risk associated with this product for thromboembolic complication is adequately labeled. And with this, I would like to thank you for your attention.

(Applause.)

SPEAKER: Just one question. We have time for just one question.

SPEAKER: Yes. You have mentioned very few cases of thromboembolic events. Did you investigate the batches implicated with this thromboembolic events and with the TGA or any other biochemical tests?

MR. TURECEK: Only partly, because the earlier thromboembolic events that were occurring in 2005, '06 -- so this single cases, of course, could no longer be

investigated since the product is no longer there. And for the last manufacturing year, of course, we looked into all the batches that we had manufactured last year with all the assays that we were using and there was no correlation at all.

This was very carefully assessed. And as I showed you, since we do not have any factor XIa contamination in our Gammagard Liquid product, we of course didn't find any correlation.

MS. GRAY: Thank you, Peter. So I think the next speaker is Stefan Schulte from Behring. Thank you.

CHARACTERIZATION OF PROTEOLYTICAL ACTIVITY IN IGG -  
VIVAGLOBIN

DR. SCHULTE: CSL Behring. That's correct.  
Thank you.

So, yes -- Hello, everybody. I would like to give you a presentation about the efforts we did at CSL Behring to characterize the proteolytical activities in IgG products and with a focus on Vivaglobin. As you heard before, it contains proteolytical activity. I think it

serves the purpose of this workshop very well.

If I give you some insights on our investigation with regards to the assays applied, root cause analysis and also how we fixed it eventually. Before I start, I have to disclose my conflict of interest. You can either read this slide or I'll give you a short version.

So I'm obviously an employee of CSL Behring, and therefore my conflict of interest are the product manufactured by CSL Behring. Okay.

So Vivaglobin is a liquid IgG product. It's exclusively used for subcutaneous application, and that's based on our understanding the reason why the proteolytical activities which are present in the product did not cause any clinical harm, just because of this route of application.

The regulatory status of Vivaglobin is: it's approved in the U.S. since 2006. In Europe, it was approved 2004. But it's actually a very old and established process because it was approved in Germany under the name of Beriglobin, I think, in the '60s. I could not find out the exact date, I admit that. And as I said, it's an established manufacturing process, and therefore, during

development, nobody paid attention to proteolytical activity at all, as you can imagine. And also, we did not detect them before, and historical data also indicated no safety concern.

So let's start with the root cause for the proteolytical activity in Vivaglobin. We have actually a very good understanding what caused the problem, and just to explain it, I have to give you some insights with regard to the manufacturing process of Vivaglobin.

On the right side, you see -- the big boxes show the Cohn process which we are running at CSL Behring in Marburg. So we start with plasma. We isolate cryo, which results in a cryo poor plasma. Then we do the fraction I precipitation, and finally the fraction II + III precipitation, which then gives the starting material for the Vivaglobin process, the IgG paste.

What is very special for our process is that we have an improved Cohn process. So we have incorporated so-called optional adsorption steps. So once we have collected the cryo, we can do adsorption for PCC, prothrombin complex concentrates, and C1-inhibitor. And we can also do after the fraction I precipitation, an

adsorption for antithrombin III with a heparin resin.

So what we basically do, we add anion exchange resins to either collect PCC and the C1-inhibitor and a heparin-fractogel to isolate the AT III, and that can happen before the fraction II + III precipitation step. And all these optional part of the license, so they're are approved process steps for Vivaglobin.

And what happened over time: that we increased the anion exchange adsorptions of PCC and C1-inhibitor, and in parallel, also reduced the heparin step for AT III adsorption. And what we observed actually was that if you perform in the Vivaglobin process an anion exchange adsorption for PCC and C1-inhibitor, the proteolytical activity increased. And when you did an additional adsorption with a heparin resin for AT III, they basically reduced the proteases significantly.

So when it comes to the scientific explanation, it's actually fairly easy to my mind. So when you add the resin, for example, the PCC resin to a cryo pool plasma, it triggers a contact activation. So factor XII gets activated, and this factor XIIa then generates kallikrein and factor XI from the respective proenzymes. And this

protease is -- actually this activated proteases are then detectable in the IgG paste or in Vivaglobin eventually.

So what we found out here is that we have approximately a maximum 20 micrograms factor XIa and approximately 60 micrograms per ml kallikrein in Vivaglobin at maximum. And that is, for us -- was a very, yes, interesting finding. And also when you look at thrombogenicity, I think from a Vivaglobin perspective, I would not underestimate kallikrein. Kallikrein also has for sure a potential to cause thrombogenicity when you injected it, for example.

And what happens -- and the heparin step then completely removes it. So when we have a fully adsorbed Vivaglobin, you basically cannot detect the proteases.

And the next slide shows this to some detail. Here on the left side. And on the X axis, you see the degree of AT III adsorption and the chromogenic protease activity, which is one assay we use to detect the two proteases involved.

And here you see a nice almost linear correlation. And basically, if you come closer to 100% AT III adsorption of all fraction II + III paste, you

basically can't detect any proteolytical activity anymore.

So we also used a whole battery of assays to characterize the protease when we started our root cause analysis. So we also use the NAPTT, because NAPTT can pick up both proteases; kallikrein and factor XIa. We also applied thrombin generation assays, which where we use different mode of activation, intrinsic and extrinsic activation, and also applied it in factor XI deficient plasma.

We measured the factor XI activity based on an NAPTT assay in factor XI-deficient plasma. We applied chromogenic protease activity, but we use only one protease substrate, which is the one which is used for the PKA assay. And we also applied ELISA assays for the antigens factor XI and prekallikrein. And here we use commercial tests which we have not validated yet.

So what did we do? So as I said, there was a shift over time. So we randomly selected lots from different period of time. So we select lots from 2007, 2008 and 2009 and then we test them by the different assays. So when I start from the left, here you see the chromogenic activity. You'll see an increase in



chromogenic activity between 2007 to 2009.

And in the middle you see the thrombin generation assay results. Here it's the thrombin peak, and you also see an increase of the thrombin peak over time. And for the NAPTT you see, yes, a shortening of the clotting time when you use this.

So more or less what you can see from this, each assay we applied basically picked up this proteolytical activity. So there's also a very good correlation between different assays, and I'll just show briefly some examples.

For example, here you see the thrombin generation peak and the NAPTT, and you have a very nice linear correlation of the clotting time and the TGA results. What I omitted here actually were some lots which had a 100 percent AT III adsorption, because basically they give no signal either in the thrombin generation assay or in the NAPTT.

There's also a correlation with the time to thrombin peak and the chromogenic protease activity. Again, it looks, to my mind, very linear and shows a good correlation. And what we also found, when we used the ELISA and tested different Vivaglobin lots, we could see

that the ELISA -- and, for example, the Kallikrein-like activity also shows a linear correlation here, which looks, to my mind, very convincing.

With the factor XI antigen ELISA, we also see this correlation, although the curve doesn't go through the zero point. So we, for example, detect it in some lots still around 5 microgram antigen, although there was no proteolytical activity detected. But also when we used the factor XI activity assay, we couldn't get a reaction here. We currently don't why this background is so high. I assume it's actually background. I don't know exactly.

So when you think about all the data I present to you, so which is now the best assay? I can say -- you can pick and choose basically. But let me give you a few points to consider.

As you have heard from me, the Vivaglobin proteolytical problem is a mix of kallikrein and factor XIa, actually more kallikrein than factor XIa. So when it comes to assay, I would think you have to address the specificity. So you cannot use, for example, an assay which only detects factor XI or factor XIa. And that, to my mind, questions the thrombin generation assay and also

the factor XI activity assay by itself. You can also argue against a chromogenic protease assay. It's not a functional correlation assay. The aspect of method validation is also important.

So, for example, thrombin generation assay we use just for comparison purposes, not for measuring some, let's say, exact amounts of -- or give it a specification. So, for me, the rationale for a specific assay is very difficult, and also you have to establish a kind of link to the thrombotic risk.

And here I can show you some data which we did in the Wessler model. So we have a similar model as Peter presented. So we have a -- our thrombosis score goes only to 3. That's maybe the only difference. And we compared Vivaglobin. So we compared an AT III adsorbed and a non-AT III adsorbed. We also tested Vivaglobin in IV application, which is not the licensed application. And we also designed a Wessler model for the subcutaneous application to see if there is a safety risk.

And the table on this slide shows you -- maybe I'll start from the right. On the right side you see thrombus weight and thrombosis score. Zero means no

thrombosis and three means occlusive thrombus. And when you look at the results, there's only one result which shows a clear thrombosis result and that is Vivaglobin lot, which had 100 percent PCC adsorption and basically no AT III adsorption.

And we selected a dose of 150 mg/kg, because higher doses it even more prominent. So 150 mg/kg was sufficient. And when you look at the bottom, there's another Vivaglobin lot which has also a 100 percent PCC adsorption, but also experienced 100 percent AT III adsorption and basically you see no thrombosis score at all, even if you apply it in the non license route of administration IV. So that was for us very convincing that the AT III adsorption really removed the problem.

In the middle part of this table here -- we also tested the Vivaglobin lot which was highly thrombogenic when it was applied IV in a subcutaneous setting. What we did, we applied a dose of 150 mgs/kg, which was derived from the maximum clinical dose. And we measured -- we did perform the Wessler test after one day, two day and three days post application to see if there's a thrombotic signal. And again, also this lot when it is administered

subcutaneous did not give any signal here.

So when I look at all the investigation we did, to my mind -- we could have, yes, picked it up fairly easy by just applying the NAPTT assay. So that one reason why we recommend the NAPTT assay as the most appropriate. It clearly detects the proteolytical activity in IgG. It's a very sensitive method. It's already established for thrombogenicity in PCC complex concentrates and factor IX products. And what I think makes it a bit more convincing than others -- other assays is it detects actually both protease; factor XIa and kallikrein.

And it also established cut-off levels and specification, which are derived from the PCC factor IX products. And when we do 100 percent AT III adsorption, we meet this -- the same specification, the same dilutions as PCCs.

So let me summarize my little talk. So from in terms of the proteolytical activities in Vivaglobin, we I think have identified the proteases and we have also found the root cause. And we changed the process that -- we made the previous optional antithrombin adsorption now mandatory to avoid this proteolytical activity. We also have done

some significant work on assays for the proteolytical activity. We test and we use assays and method. And what was very interesting for us, all the methods detected actually the proteolytical activity.

There was a high degree of correlation, and part of this correlation is -- also that what I noticed was different Vivaglobin lots was that the ratio of kallikrein and factor XIa was almost the same. So it was always, you know, a ratio of three or more kallikrein compared to factor XIa, very close to this. And yes -- and the NAPTT is a preferred method for us, and I think that should be. Thank very much.

(Applause)

SPEAKER: Question by asking you which TGA method do you perform in your --

DR. SCHULTE: We use a TGA method which we closely adapted to the FDA protocol, just depending on our machines. But we -- I think we had basically the same method.

SPEAKER: Sorry, I didn't -- you mean the thrombin generation test method you use --

DR. SCHULTE: Yes, thrombin generation assay we

also did in factor XIa-deficient plasma.

SPEAKER: Yes. And which method? Is it in-house method or is it --

DR. SCHULTE. In-house method, yes.

SPEAKER: It is --

DR. SCHULTE: We have also our own method, but we just had to change our method to meet the FDA protocol.

SPEAKER: And it's a fluorogenic based method?

DR. SCHULTE: It is, yes.

SPEAKER: A question regarding Wessler test. So to my knowledge, there is not that published -- there is not much published in Wessler test when the (inaudible) is administered subcutaneously.

DR. SCHULTE: True.

SPEAKER: Do you know that, you know, your test is optimized to detect subcutaneous thrombosis?

DR. SCHULTE: Of course I cannot say, yes, because we didn't detect anything when we applied it subcutaneous. But we want look first into this and elaborate in more detail now. For example, also apply the protease themselves and can measured by an ELISA in the rabbit. But so far we only got a negative result with the

worst case lot we ever had; was in terms of Proteolytical activity.

SPEAKER: So you didn't have any positive control or anything like that?

DR. SCHULTE: No.

SPEAKER: Did any of the heparin bleed off of the heparin column into your product?

DR. SCHULTE: Yes. What you want?

SPEAKER: Did you test for heparin in the product after you did the heparin -- the extensive heparin adsorption?

DR. SCHULTE: So the heparin adsorption we always used in our manufacturing process and it's done before the --

SPEAKER: Is there any heparin in the product?

DR. SCHULTE: No, no. Not all. Sorry.

SPEAKER: You're sure?

DR. SCHULTE: I'm pretty sure.

MS. GRAY: Last question.

SPEAKER: I have also a question regarding the Wessler test. I just also wanted to ask for the positive control group and I want to ask you for the negative



control group. What was it?

DR. SCHULTE: It was just saline solution.

SPEAKER: And so, I also would agree to test at T-max, if you know the T-max of your product, because I have no experience with subcutaneous administration and the Wessler too. But I think it's a good approach to test at approximately at T-max of a product.

MS. GRAY: Okay. Thank you.

(Applause)

MS. GRAY: Now I'd like to welcome my co-chair -- want to talk about the experience from Grifols.

#### IVIGs & TAEs PROCOAGULANT ACTIVITY ELIMINATION

MR. JORQUERA: Hello. Good afternoon. I wanted to thank the organizers for giving the opportunity to Grifols to present some of the studies and some of the thoughts that we have for this -- around this subject.

This is a conflict of interest statement as Grifols manufacturers and distributes plasma derivatives and the (inaudible) on behalf of the research group that has been working on this subject.

I want to follow a little bit from the presentation of Dr. Farrugia. In this first slide, I wanted to emphasize the point that, well, you could use Cohn-Oncley or Kistler-Nitschmann for obtaining the classical intramuscular immunoglobulins. You can choose to separate Fraction I, as Dr. Farrugia mentioned, or you can chose to have Fraction I together with Fraction II + III or with Precipitate A in Kistler-Nitschmann.

I wanted to just focus here in two points. That there is a -- sorry, minor difference or rather there is some probably relevant difference between the Cohn-Oncley method and the Kistler-Nitschmann, which is the concentration of ethanol that is used for precipitation of the impurities, precipitation of the impurities that are contained in Fraction III or the equivalent to Fraction III. While Kistler-Nitschmann uses 12 percent ethanol probably in an attempt to increase yields and improve yields, with that process Cohn- Oncley uses 17 percent ethanol, which is about one-third more precipitating agent.

We all know that intramuscular immunoglobulin gave rise to severe adverse events when infused intravenously, so Fraction II or Precipitate GG coming from

any of these two ways of doing plasma fractionation need further processes to obtain IVIG.

And what the relevance of a starting material, also Dr. Farrugia mentioned before -- well, we know that Fraction I contains complement components and also some other clotting factors like clotting Factor VIII and fibrinogen. We know that Fraction II + III contains clotting factors, essentially the proenzymes. So if you chose to have them together, well, you are putting enzymes that are proenzymes that when activated participate in inflammation and blood clotting processes.

We also have seen several slides, probably more complicated than this one, saying that the coagulation proenzymes have the capacity to have feedback activation, especially probably when you have partially purified materials like the fractions that we use to produce immunoglobulins.

Very recent publications relation coagulation and complement and demonstrate that coagulation and complement serine proteases have the capacity to interact and activate each other. And that mixture is what could be present in Fraction I + II + III if you have -- when you (inaudible)

in order to start fractionation.

Well, the initial strategy is to prepare IVIG counted on acid pH treatment. Already in 1962 Barandun and his group from Switzerland demonstrated that if you treat with acid pH the immune serum globulin, then that increased the tolerability for the intravenous infusion, and acid pH based formulations like a 5 percent IgG in 10 percent maltose were already described in 1983 here in U.S. patent.

Another strategy to obtain intravenous immunoglobulins is to use an anion exchange purification. And a typical procedure uses DA (phonetic), for instance, and then in that case you have resins with positive charge in a column, and these resins are bound to eliminate impurities with negative charge depending on the isoelectric point. But other impurities, including Factor XI, thrombin itself and maybe others, can go through with IgG that have positive charge and then you can find these residual impurities in the airflow together with the IgG.

In the case of our IVIG production process, we have a Flebogamma DIF 5 percent and then we made a process change, which consisted in increased the concentration of the product to 10 percent. And for that purpose, we went

through a full clinical development. And we started our production from Fraction II + III, so we previously separate Fraction I. We employ a polyethylene glycol and ion-exchange chromatography followed acid pH, pasteurization and solvent-detergent treatment.

The use of pasteurization requires removal of the aggregates that are generated during the heat treatment and that leads to a first here, polyethylene glycol fractionation, second polyethylene glycol precipitation, leading to a very high purity products. So we end up with a 20 nanometer Planova Nanofiltration before we end up with the final product.

So for our research we use the techniques that you have already heard along this morning, so I'm not going to go through them. These are regular techniques that we have in our laboratory. These three are essentially as described in the European Pharmacopoeia monographs. But we also incorporate a thrombin generation test using a commercial kit and some ELISA for Factor XI antigen, and we use the one-stage APTT or prothrombin test assays for the main coagulation proenzymes.

And I wanted to emphasis here that these tests

were not designed to test partially purified materials like the ones that we've tested in this study, but rather to test plasma from patients. So there's also -- these tests have to be interpreted with caution.

Well, so -- and the first thing we did is to analyze the industrial extraction or Fraction II + III that, as I say, is the material that we use for our production at Grifols. And we analyze the total recovery of proenzymes in industrially resuspended Fraction II + III versus the plasma pool, assuming that plasma contains 1 U/ml of all these factors. What we found is that we recovered, as it had been previously described, important amounts of all these proenzymes in the -- and we don't know what degree of activation they have there. But we suspect that Factor VII, at least, and perhaps Factor XI are partially activated just coming from these results.

And to explore this a little bit more, we analyzed the industrially extracted Fraction II + III with NAPTT. Here we express the result as a ratio of the sample versus the normal plasma. So a ratio of 1 will be normal and a shorter ratio indicates a possible activation. So we have here batches that indicate that the industrially

extracted material is probably already activated.

We also detected thrombin presence with this European Pharmacopoeia test in one of the batches, with very high values of prekallikrein activator. The specifications for the final product are in the range of lower than 30 something. We also detected important amounts of kallikrein activity as defined as the increment of observance of the chromogenic substrate. And we also detected thrombin generation capacity when we compare to the vehicles both with plasma, platelet-poor plasma and Factor XI deficient plasma.

So we have important amounts of coagulation proenzymes, and probably they are activated to some extent in the starting material. Then what we did was to follow the production -- the purification process. These are the different processes, steps, and these are the starting values. They have been compared here in international units per gram of total protein. What we found is that essentially at the following step, we could not detect any of these proenzymes. The only one that we could detect was Factor XI, when we concentrated the product in one of the steps. We were able, again, to detect Factor XI.

This Factor XI was still detectable after the acid pH treatment that we have in the process, but it was not detected anymore after pasteurization. You can see here that is not quantitated, and when we concentrate the bulk up to 5 percent or 10 percent, still it is not detectable.

We know that from the development process already, when we're developing the product more than 10 years ago, we knew that we had some serine protease activity. We controlled that by the chromogenic substrates. You have already heard about them this morning. We saw an important effect of the pasteurization -- I'm sorry, because I'm trying to see now with a pointer and then the slide jumps. So we saw that along the purification process, the proteolytic activity gets reduced, especially during the pasteurization step, but not during the acid pH step.

But we need to know how robust was the pasteurization to deal with the procoagulant activities and the activation marker that we had detected. So what we was to generate an especially activated sample from the Fraction II + III in the laboratory. And we spiked with



different volumes, 1/20, 1/10 and 1/5, into the intermediates before the acid pH treatment or the pasteurization.

What we saw is that even with the lowest spike volume, that's the 1/20 spike in the left of the slide, we see that the correction of the NaPTT is small when you compare to the control that is around 300 seconds. And the kallikrein also gets not corrected or the thrombin generation test or the Factor XI antigen practically stay as they are.

However, the prekallikrein activator is inactivated. So probably that's the mechanism by which the acid pH treatment favors the tolerability of the intravenous immunoglobulins for the intravenous administration in some products. On the other hand, in case of the pasteurization, we saw a marked effect of activation factors. Even at the highest spike concentration, all the results, the NaPTT results, got normalized to the controlled level. The PKA also was negative. Kallikrein was negative again -- I'm sorry. The thrombin generation tests got negative and even the Factor XI antigen was disappeared to the test probably because of

inactivation of the antigen.

We did some kinetic experiments. We saw that most of the material that we had spiked in the laboratory - - this is specially activated material -- was inactivated by half of the pasteurization treatment. And at the end the pasteurization, there was negative results in this test, including the thrombin generation assay with Factor XI deficient plasma, prekallikrein activator and kallikrein-like activators.

So that was about the process, the production process. And coming to the final products, we did a comparison of the products from several competitors that we could reach. We found that product A, we had one batch from year 2007 that gave some signal in the thrombin generation assay. But the signal was much higher in products that were released to the market in year 2010. We found another product from a different manufacturer that also gave some signal in thrombin generation assay. But essentially all the modern products are negative, the ones that we found, including the Flebogamma products from Grifols.

We also did the NaPTT assay with Factor XI

deficient plasma and here the profile is different. As I said, the value that we want to see is 1 and that's the kind of value that we see for the products that are negative in a thrombin generation assay. But we see a shortened NaPTT times for the products that give high values in the thrombin generation assay. I want to emphasize that the lots tested here were not among those published as involved in thromboembolic adverse events.

We also did the NaPTT tests and the thrombin generation test with platelet-poor plasma, not Factor XI deficient plasma, and again, with this test, we were capable to differentiate products or batches from product A released to the market during the year 2010 because they were -- they had a slightly shortened NaPTT times and they showed capacity to generate thrombin generation test, while all of our pharma products did not show this capacity.

To finalize, we analyzed by one-stage assay the content of factor -- clotting Factor XI. And here this test could not differentiate between activated and non-activated Factor XI because it activates everything. And what we found essentially is that the lots from manufacturer A from the year 2010 were capable to -- we

were capable to detect about 1 PEU unit per ml in the product, and this correlated very well with the plasma equivalent units that we found with the ELISA assay. I insist these lots were not among those published as involving thromboembolic adverse events.

With this final products additional one-stage tests for Factor II, VII, IX, X and XII, and we did not detect any of these factor in neither in Flebogamma nor in the products from manufacturer A. Manufacturer B1 also was not detectable for Factor VII and Factor XII. We did not test other factors and we did not test other manufacturers by one-stage assays.

So, in summary, among all tested products only products A and B1 show indications of coagulation activity markers, with high thrombin generation test and NaPTT values and with Factor XI deficient plasma. Product A has high thrombin generation test with platelet-poor plasma and has lightly shortened NaPTT platelet per plasma and a slightly shortened NaPTT platelet-poor plasma times. And product A also shows Factor XI clotting activity by the one-stage clotting assay.

We did some estimations and the amount of Factor

XI are least partially activated, and we know that for the other test that we will be infused in an adult receiving 2g/kg will be approximately 250 units. And previously, it has been mention in studies I think at the -- where only 50 units of Factor XI were capable to trigger clotting activation in chimpanzees.

To finalize because tests, laboratory tests, are nice, but what is really important is the clinical results. We are doing here a comparison of the pharmacovigilance data for Flebogamma, Flebogamma DIF to the result that has been published by the European Commission concerning Octagam. And the basal rate of Octagam during the years 2005-2007, that's previous to a manufacturing change that they applied, was 1.49 to 1.60 million grams sold or marketed per case of thromboembolic events. This rate increased to 0.52 approximately during the years 2008 and 2009 and reached 0.18 million grams per case during the first half of 2010.

By comparison, the data we have with Flebogamma from 1993 to 2010 is 6 million grams per case, and in the case of Flebogamma DIF that has been marketed for a shorter period of time, is quite coincidental, from May 2007 to

September.

That was all, and I just want to recognize the help the group that has working at Grifols with me, especially Marta Jose who is present here today, Marta Carretero who helped with this pharmacovigilance data, but also Maite Lopez and Nuria Marzo, Perra Risto (phonetic). Thank you.

(Applause)

MS. GRAY: One question? Maybe we will leave it to the panel discussion. Okay.

And we now welcome Claudia Nardini from Kedrion.

PROCOAGULANT ACTIVITY TREND ANALYSIS DURING  
THE MANUFACTURING OF IVIG

MS. NARDINI: Thank to the organizer to give me the opportunity to present Kedrion strategy to prevent and to mitigate the risk of procoagulant activity in immunoglobulin products.

Here is the declaration of conflict of interest as Kedrion manufacturers and distributes plasma products and the Kedrion products portfolio includes immunoglobulins

for intravenous administration.

And as Kedrion is not present in U.S. market, let me spend some words about the regulatory status of IgV preparation produced by Kedrion. The trade name is Ig VENA and was first registered in Italy in 1984 and currently is registered in 33 countries. And Ig VENA is a liquid formulation, 5 percent dosage, and it is formulated in amounts at pH 4. Basically, it's the most used human normal IG for intravenous administration in domestic market, which is Italian market, and it is available and distribute in many EU and extra EU countries.

For give you a more precise data, we -- or I reported here the quantity which were globally distributed worldwide -- and in the Europe and in Italian market during the period ranging from 1st of January 1995, and we distribute over 27,000 kg of Ig VENA. And the starting data is associated with the requirements of established Italian law, which implemented the Community European Directive in which procedure on obligation and guidelines regarding pharmacovigilance to be carried out in new EU territories are indicated.

The ranging period was between 1995 and August of

2010. You can have also a more precise data referring to the market contribution, Kedrion contribution in domestic EU market and worldwide market.

I don't wanted to go deeply in this slide, because I've -- this is -- the content of and the value of, therapeutic value, of IGV is widely discussed today and the second day of this workshop. I only would like to pick up that intravenous immunoglobulin preparation were developed as plasma derivatives between 70s and 90s as a first, second and third generation and were also considered as a life saving therapeutic for different congenitally immunodeficiencies.

Indeed, for purification of immunoglobulin, we can use different intermediates deriving from plasma, and mainly either Fraction I + II + III or Fraction II + III intermediates derived about from ethanol fractionation are mainly used for purification of immunoglobulin. And in this slide, I reported the recovery percentage of immunoglobulin in each different intermediates and in respect to immunoglobulin purity.

Kedrion use for the purification of each sample preparation the Fraction II + III intermediates attained by



Cohn fractionation. You can see briefly in these slides the flow diagram for the methods reported in the upstream process and the downstream process. I indicated also the sample where -- had been taken from the process in order to monitor the process to see in which part of the process the procoagulant activity is reported.

Here the description of the samples, samples from A to D describing the upstream process. And in the last column of slide, you can find the process operating parameters, which are basically the basic Cohn fractionation. And from sample E to G, we have the downstream process, which is basically the resuspension of Fraction II, S/D treatment, and finally, the formulation at low pH and quarantined at temperature of approximately at room temperature.

The samples were taken to be measured by means of different -- a battery of different tests, which were deeply discussed and described this morning. And I only would like to remark that we performed the following tests by using a Factor XI deficient plasma and PPP plasma. We performed thrombin generation assay; thromboelastography, with Natem system; NaPTT, not activated partial

thromboplastin time; and aPTT, in order to check the Factor XI content.

Here are the results, the raw data that we obtained for different samples taken from along the process. And the clue is that definitely we have repartitioning regarding Factor XI as shown in sample B by the test of aPTT. Indeed, we have a concentration of Factor XI after the removal of Fraction I, which is below 5 percent and during the downstream is below 1 percent. For the other tests, we observed that the critical point in which we remove the procoagulant activity is the sample D in which we have -- after the precipitation of Fraction III. This is shown by the lapse longer thrombin clotting time and the low thrombin generation, that you can see in the other column.

And I would like to remark the clotting time that we attained for TEG test in sample C, which suggest us that we have a coprecipitation of activators, coagulation activators, in Fraction II + III, which is being removed during resuspension and precipitation Fraction III. So the results that I showed before was used to build a graph. And as you can see, this graph simply we reported that in Y

axis the measurement, the value measured for different assay, and the X axis, the samples we -- drawn by the process.

And we can see that, okay, the trend referring to aPTT test with the removal Factor XI content and we can see that for the -- due to the prolonged time, clotting time of NaPTT test and TEG test, we remove all fully complete -- at least, completely, the procoagulant activity in sample D. And this is confirmed by TGA.

I use also these graphs because I would like comment with you some results. And, okay, the trends showed the same results. And I see colored in red the sample D, the removal procoagulant activity, and for aPTT, the removal of Factor XI.

And the points that I would like to discuss with you is, if we have a look at the time, coagulation time of the TEG test and the NaPTT test during the removal of Fraction I, famous sample B, from the prolonged coagulation time of TEG, it seems that procoagulant activity is removed during Fraction I precipitation.

We know that this is not true and it is confirmed by the time, coagulation time, issued in a NaPTT test.

Maybe in these samples the ethanol concentration may affect the clot mechanical properties and elastic properties of the clot during the TEG test. Another point that I would like to discuss is that, as you can see, the TEG content in sample D and in the final product are similar, and this suggest to us, in spite of the fact that the concentration, the final product, is 10 time more than in sample D after the removal of Fraction III.

And this suggest to us that probably during downstream purification, it is possible to removal more procoagulant activity. In order to investigate better the thrombin generation during Fraction II + III, we simply make these samples -- this session, in which we -- we resuspended Fraction II + III as it is, and we measure the thrombin concentration versus time. And in the second session of experiment, we measure the same resuspended sample in presence of Factor XIa and Factor XIa antibody and with the monoclonal antibody specific.

And we -- the blue line represent the TGA for Fraction II + III resuspension without antibody. The purple line represent the variation on TGA after incubation of Factor XIa antibody. So we see that there is a

reduction, a partial reduction, in thrombin generation, and due to the presence of Factor XI and -- but it is only partial. Thrombin remain in the resuspended sample. And these drive us to conclude that the pro coagulant activity is partially due in this stage to Factor XI content but is measured due to the other coagulation activators.

And this confirm also what we found during repartition. Indeed, Factor XI, I told you that, is removed in our process during Fraction I removal step. TGA is capable to give a more information, more screening of minor concentration content in Factor XIa. And so as the first attempt to validate this test, we will look at the linearity of the test. And we perform a test in which we see the concentration of thrombosis versus a serial of diluted sample containing different concentration of Factor XI activated.

And -- okay, we plot the thrombin concentration expressed in log versus Factor XI concentration expressed in log, and we found the linearity of the curve in four concentration with the limit of quantification approximately in Factor XI activated of 15.62 picomolar.

In order to increase the sensitivity of the test,

we followed a suggestion according -- a suggestion reported in the publication issued in November 2010 by Grundmann and others, in which they suggested to perform the tests without the tissue factor activators. So we performed again the test with phospholipids only without tissue factor and the results was that we increased the linearity and the sensitivity of the calibration curve. And, okay, we reduced the test -- the limit of quantification of Factor XIa around 3.9 picomolar.

Another interesting test that we performed was the thromboelastometry on IVIG preparation, 5 percent, and we use 5 percent preparation as it was just as control and we test the thrombo -- we test the thromboelastometry coagulation time and we use it as a control. Then we had a certain amount of Factor XI activated to the same preparation and we test again the clotting time, and we observed a reduced clotting time and an increase in firmness of the clot and an increase of the surface area of the clot. After that, we spiked the solution with the monoclonal specific antibodies and we reestablished the situation as in the control.

So this suggests that these tests could be useful

in -- when you have some thrombolic -- there's some thrombolic -- sorry, coagulation system, coagulation activity in the preparation. And by using the specific activity is possible to check which activator factors is responsible for that.

Okay, if you know the problem in your process, you are able to control the process and to understand more of the process. So after verifying that we have a repartitioning of procoagulant activity during the precipitation of Fraction II + III and -- resuspension II + III and precipitation of Fraction III, we investigated the robustness of our process. And we challenge as the first trial the operating parameter, process operating parameter, referring to the pH. It is well known that by lowering the pH, we increase the yield of gammaglobulin, but we increase also the impurity profile.

And indeed, when we resuspended the Fraction II + III with a pH drastically out of our targeting operating parameter, which was a pH 4.8 instead of pH 5.5, we found that an increased protein content, but we found also an increased generation peak of thrombin and also an increased content of Factor XI.

Why an unexpected value was evident for NaPTT test? We don't know. We have to investigate. We have performed just one trial, and so these tests will undergo in further investigation during the next plan for -- to study the robustness of the process, which will include further parameters such as ethanol and temperature.

So coming to the conclusion of this presentation, I would like to summarize the outcomes. And the outcomes is that the production needed for -- to obtain Ig VENA is capable to remove the procoagulant activity, including Factor XIa and thrombin. We have a battery of specific tests for the detection of coagulation activators, mainly Factor XIa and Factor XIa-equivalent activities that can be applied to characterize the final product to monitor the manufacturing process and to check its robustness.

And this, as a part of these tests, will be included in Kedrion quality global system for the quality assurance system to control -- to apply during the change control. Therefore, the next step will be the standardization of modified TGA and NaPTT tests, and we addressed these tests to improve the sensitivity in order to detect minor level of activators, again, Factor XIa or



equivalent activities. And other tests, such as Natem, may be used to investigate which activators are responsible for the triggering of the coagulation cascade.

Thank you for your attention.

(Applause)

SPEAKER: Okay. One question before we go to break. Maybe I can ask a question. Do you use whole blood for your thromboelastography?

MS. NARDINI: Yes. Blood, yes.

SPEAKER: You use whole blood?

MS. NARDINI: Yes.

SPEAKER: Okay. Because I think that that's perhaps one of the limitation. We need to use whole blood for the test.

MS. NARDINI: Yes.

SPEAKER: Okay. In which case, I think that we're going to have 10 minutes for the break, and we will restart right on time at 4:00.

(Recess)

MANUFACTURER EXPERIENCE: PRODUCT AND INTERMEDIATE TESTING  
ON PROCOAGULANT ACTIVITY

MR. ROMISCH: So, ladies and gentlemen, now I'm back now with a talk about assay systems and our experience, what happened during manufacturing, and what corrective measure we have introduced. The first part, a bit about, again, cold ethanol fractionation, Cohn, Kistler-Nitschmann. But and directly go now to the XIa and partitioning and which corrective and preventive measures we have introduced.

And then, more in general, I mean, the experiences we've made during development and during assessment of the processes; which kind of assay would be useful, which are reliable, suitable, makes sense, in that environment we're living in, and the matrix in ethanol and so on.

A bit about activity in antigen assays and commercial, and we did a lot of tailored assays approaches just to investigate what we can do to appreciate these complex matrices.

I don't have to tell a lot about that. You've seen that now a couple of times. We have also optional mass capture; factor II, VII, IX, X, protein C, protein S,

antithrombin. I come to the point that where we know that our factor XIa is generated, maybe on the filter aid surface. We know that it is -- and that is in line with what we heard before, that XI and XIa is present in the fraction I, II, III, that it is precipitated in the fraction I, III. And that is known. This is nothing spectacular. So that factor XI binds and is generated and activated on filter aids and diatomaceous earth.

That is known here. For example, one of the old fractionators knew that already, 1962 citation that factor XI goes into Cohn fraction III and that it is celite bond. So this is nothing spectacular. It may have been forgotten through all the years where fractionation became just very well-established method, and that has been -- has to be rethought now. So I always like to dig in these old publications because there was already a lot known.

Our surprising finding, and I come to that, and that is more or less is in line with some differences, which Stefan Schulte talked before, that it is dependent on the extent of the upstream mass capture. So that is what we had in the discussion before, factor XI is adsorbed to surfaces like the filter aids; diatomaceous earth, filter

aids.

It's activated on that surfaces that has been published. And if do that, and you measure in the plasma supernatant, this group -- that group in 1995 published that and showed that that it goes into serpin-inhibitor complexes. And those are suicidal inhibitors, means that they inactivate factor XIa in a bath. But we have to say here, that we do not actually 100 percent know is what happens at the surface, if it is bound to the surface, is it partially protected. A couple of proteases we know are protected from plasma inhibitors if they're bound to surfaces like platelets and so on.

Anyway --- if they threw the XIa into plasma and follow the inactivation, right, in terms of going into an inhibitor complexes, you'll see that they --it takes about 10 minutes to reach a plateau and to inactivate factor XIa, in a static system, certainly, and it's not bound to something.

It's known that for quite a time that factor XI undergoes autoactivation at negatively charged surfaces, and it is prone to cold activation, which is called the kallikrein-like activity where it can be activated. And

again, it's found in Cohn fraction III or equivalents also from Kistler-Nitschmann.

So coming back to that picture -- so what actually happens on a vessel wall is if the subendothelium is exposed due to injury, then it's the job of the contact phase to act here and just to initiate reactions like you see them on -- in the right upper quadrant that is XIa is activated, one of them, factor is XII is activated at the surface and so on. And you see the feedback loop not only at the surface but also through thrombin, and so that is physiologically relevant and meaningful. So this is nothing spectacular.

What we do know is, when we introduce our filter aids into a manufacturing process -- and we well in knowledge that we can activate factor XII, that we can have prekallikrein activator which is XIIa activated and inactivated and also kallikrein activity in a very efficient manner. At the same time -- and that was not really on the, I would say, radar that XIa as well here is activated.

So this is nothing. That always happens. It happens day-to-day. This is nothing new. Factor XI is

activated at diatomaceous earth filter aids.

This is -- by the way, it's not a shower device. This is really kind of this diatomaceous from -- it's sand. It's more or less 95-98 percent silicon dioxide, so sand. So what we experienced here is, and what happened is that, if we extend the mass capture very early in the process beyond a certain limit, then our precipitation efficacy in I + III precipitation is not that effective.

That is one very important part. So there is a correlation between mass capture and efficacy of reducing during the whole package of fraction I, III precipitation. So and -- obviously if that is not 100 percent perfect, then we carry over some of the XI, XIa in the fraction II and then there is not a very significant minimization during the final process.

So what did we do? I mean -- we introduced an additional adsorption step early in the process after optional mass capture so that we remove factor XI, and thereby obviously, by removing XI, we cannot have significant amounts of XIa later on. So I think it's quite easy to understand what we did. It took us some time to understand all of that and to optimize that, but now we

have implemented this in our manufacturing process.

In addition, what we introduced was in-process testing. We test factor XI antigen before and after this adsorption step to ensure that each and every of these batches now reaches a certain limit of factor XIa antigen in it on that stage, and finally also, for the TGA in the final container, as we have discussed before.

And there are some other aspects which I have not time to go into detail, but certainly there are other things that can influence the precipitation behavior -- that is not new to you -- like matrix effect, ethanol, buffer conditions and so on. So that has to be very carefully limited and investigated.

So now, I come to the assays and what make sense to our -- what we think what we can measure. So what we have seen certainly, and we obviously have to appreciate here, if we take the sample as such on that level, we have the cryoprecipitate removed. So if we use that sample originally, we have totally different behavior than we have a plasma. We have a factor VIII (inaudible) factor depleted plasma. So that has another basis if you use the original sample.

However but -- certainly, here we can test the single factors, as they are depicted here. On that stage, we can only test activated factor activities. We cannot 100 percent say this is XIa or so because this is a mixture which is very complicated and it can -- your signal in the different systems, NATEM or thrombin generation assay, if even you use factor XI deficient plasma, if they is traces of IXa in there or Xa, you cannot differentiate. You just can measure the sum, the procoagulant potential of that fraction. But we cannot 100 percent define what it is because these factors cross-react and vice versa in the test systems.

So on that stage when also sample B and so on has been presented before is -- yes, before ethanol addition, we can test factors, also activities II, V or VIII. But even at that stage, they are below the limit of detection in these activity assays.

On that stage, I really have to say careful after any ethanol addition because that seriously impacts all these kind of activity assays, and that includes TGA and includes NATEM and everything. So we tried our best to do -- to find out a way to do that. But if you -- if you



concentrate or try to filtrate out the ethanol, then you don't know what you introduce, other artifacts and so on and so forth. So here we tried to go a different approach. Unfortunately, we did not succeed but. And finally, on the NCH (phonetic) after fraction II reconstitution, you can more or less measure all factors; antigen or activity. But as we have seen in all these factors, we are now below a limit of detection. Factor XIa below 1 milliunits per ml and TGA and NATEM as discussed before.

So I don't want to stress too much time on that. But certainly, addition -- the other, the global tests we have addressed, they are not useful, you cannot use them, in that they are sensitive enough, you cannot use them.

The TGA, we validated, implemented it for the Octogam release. But to our understanding and knowledge, it does not make a lot of sense in-process. The data variation is incredible. And additional information comes from the ROTEX system, the NATEM system, as well I also appreciate that in terms of getting more principal information about, but it's not quantitative in a way.

Specific factor assays -- the factor XIa assay, as discussed before, is also prone to cross reactions due

to factor IXa and so on. You cannot be sure that this is XIa, only if you do additional inhibitor studies and so on. And the kallikrein, yes, of course, the chromogenic, flurogenic assay in terms of combination of all kinds of inhibitors and substance.

But what we did -- and that holds true for both, the factor XIa and the kallikrein. I come back to that. We tried to address that in more specific way. The TGA, I just jump over it. But either in standard human plasma, but also in factor XI deficient plasma, it's a very sensitive assay and we appreciate the assay very much.

I'll jump over it. Just to see -- and here is our system. If we spike factor IXa into it, you see that there is obviously also an impact on the peak thrombin concentration which is not a surprise. However, if you look at the designated milliunits, you see that the factor XIa has a more dramatic impact than the IXa spike, which came to us as a surprise. But that can be discussed separately.

But certainly, it has to be acknowledged if we're talking about XIa. In certain samples, we have to -- we really have to say XIa-like activity.

So finally, I have to say that that we tried to tackle this issue in in-process samples with a different matrix and a more complicated matrix. And what we did is also based on, as has been addressed before from Dr. Dodt, I think that there was a kind of an immunosorbent activity assay. Just taking an analyzer plate to capture XI, XIa out of that and use that -- wash like you do in an analyzer and then add the fluorogenic or substrate on that. And in principle, it works nicely. Also, you can differentiate.

There are two things. One is that these antibodies capture XI and XIa. So if you have 3 percent XIa and 97 percent XI or even more, then obviously your sensitivity is significantly decreased, and on that stage, also the samples are diluted, so. Unfortunately, yes, in principle it works, but it did not work out in these in-process samples.

And that held true for XIa and also for the kallikrein. So in principle, to investigate the root cause and so on, this can be used really well and gave us a lot of information, but for in-process testing, it simply was not too sensitive, it was too insensitive. That will change, maybe, if you have a monoclonal antibody specific

to XIa that you can increase the signal. So the same held true for the kallikrein test we did, and also here it has to be taken care that there are not cross reactivity for the assays.

This gives you a brief appreciation of more than 20 lots of Octagam from the new process, starting with a cryo-poor plasma. You see here the -- and this is antigen, factor IX and factor XI antigen per milligram of IgG. And so the -- you see that that after the factor XI adsorption, obviously the XI is down to a very small residue and after that, it moves almost at baseline. So it's gone, cannot be activated.

That's where we are. And again, that is the work of a huge team of coworkers and helping hands, which I have to acknowledge here. And thank you very much for your attention.

(Applause)

SPEAKER: Is there any question for the speaker?

MS. GRAY: Can I just make a comment before --

SPEAKER: Yes. Sure.

MS. GRAY: We just saw the slide that mentioned factor XI antigen IU/mg. I just want to say that at the

moment, we don't have international unit for factor XI antigen.

MR. ROMISCH: Okay. Now, we've made it international. But -- no, I understand, Elaine. And the same holds true for the ELISA.

MS. GRAY: Yeah, of course.

MR. ROMISCH: We should call them plasma equivalents units or something like that.

MS. GRAY: But of course, if there's a demand for international unit, we will consider this.

MR. ROMISCH: So more work for you.

SPEAKER: Jurgen, I appreciate your sharing this with us and helping us to learn from this experience and further ensure patient safety. I miss the piece in the story, though. Something changed in Octagam between 2008 and 2010. Could you explain that to me?

MR. ROMISCH: Yes. It's very simple and it's very close to that what Stefan Schulte told. The mass capture has increased over the years. There's more demand for octaplex, octanyne and so on. And these kind of -- this kind of increase is, at least, one part of the story. And we see that increased mass capture, so then we more and

more ran into this kind of elevated XIa. So --

SPEAKER: And --

MR. ROMISCH: Yes. It is part of the story, I have to say.

SPEAKER: And then that overwhelmed the capacity of fraction III step to remove it; is that what you said?

MR. ROMISCH: It is the efficacy -- you have to see that as a package. The I, III, III reconstitution and the I, III precipitation is different in efficacy compared to a non-mass capture package. But now we have -- now we have -- the XI is now gone, and so I think that will have no further impact, as I mentioned.

SPEAKER: Thank you.

SPEAKER: Any other question? No? In that case, we'll move to the next speaker. It is Dr. Roni Mintz from Omrix Biopharmaceuticals.

#### TOWARD A LOW THROMBOGENIC IVIG

DR. MINTZ: Good afternoon. First I would like thank the organizer for inviting us to take part in such an important workshop. The presentation that I will give

today was based on the work conducted at Omrix R&D, which is now a part of Johnson & Johnson.

Omr-IgG-am is a 5 percent IVIG solution, which was voluntarily recalled from the market in November 2010 due to recent reports indicating an increased in thromboembolic complication following treatment, including Omr-IgG-am. Recent evidence in industry has pointed to a connection between such adverse events and high levels of factor XIa in IVIG product. Changes were introduced by Omrix to the manufacturing process of the IVIG enabled a specific removal of factor XIa.

As part of Omrix's root cause investigation, Omr-IgG-am sample was sent to evaluation to FDA and NIBSC laboratories, and to further evaluate Omr-IgG-am batches and to be able monitor production changes, three methods were developed or obtained from these laboratories and one in-vivo assay, among them. The in-vitro TGA assay which we used was based on the thrombinoscope, which was first from Stago.

The specific assay for factor XIa is based on the protocol kindly provided by Dr. Ovanesov, and then vivo assay is based on the thrombogenicity in animal

Wessler stasis, thrombosis rabbit model.

First when we started to work with the TGA assay, we compared the use of deficient versus normal plasma. And we found out that using the deficient plasma, we obtained more sensitive results and we couldn't detect factor XIa levels in batches which -- in the deficient plasma which could not be detected when we used a normal plasma. Therefore, we decided to use the deficient plasma.

It's sounds trivial, but in order to validate the TGA, since the thrombinoscope manufacturer said that assay is for research use only, we needed to validate the hardware and the software. So we used special contracts via Thermo Scientific, and the software was validated using Omrix's validation team. In addition, we evaluate plate uniformity using thrombin calibrator and the uniformity was with RSD of about 5 percent. And in each test, we added systems suitability, which was a sample with known value of TGA with -- actually it was intermediate value and the RSD was about 15 percent between tests.

This is a description of the assay. I won't go



over it. Just that the main thing that we use is a 1 picomolar of tissue factor and 5 -- 4 micromolar of phospholipids, and the calibrators was added to samples and plasma. Okay. The value that we used for the evaluation was a thrombin peak.

When we compare the results we obtained from FDA to the results obtained in Omrix, we can see that there is a very nice correlation; almost identical results were obtained when we compared the batches. Similar results obtained when we compared the results from NIBSC to our results. Again, very good correlation.

So when tried -- when we wanted to qualify the TGA assay, we decided to qualify it as a limit test since factor XIa is a contaminant and our manufacturing process post-changes resulted in signals below detection level. So we checked for specificity, and which was done by addition -- increasing the concentration of one of the ingredient in the factor in the IVIG, and recovery obtained was about 90 percent. And increasing the protein concentration resulted in recovery of 93 percent for the in-process and 87 percent for the final.

The limit of detection was done by spiking

negative IVIG in-process sample or final with low amount of factor XIa, and the limit of detection obtained was about 0.3 nanogram, which is equivalent to about 2 picomolar. The linear range was between 20 to 100 nanomolar of thrombin.

I just wanted to add before that in our assay, we subtract the baseline from the data. So the baseline is actually plasma, deficient plasma, with buffer -- with relevant buffer.

The next assay was the factor XI kinetic assay, which was kindly provided by Dr. Ovanesov. Again, samples are diluted in buffer and also the calibration curve was done with factor XIa, first from HTI, and the samples were then transferred to another plate which contains a substrate and then transferred to an ELISA reader. We qualify this assay, again, as a limited assay from the reason that was described above.

The linear range for this assay was between 25 to 140nanogram per ml and the limit of detection was about 10 nanogram per ml. We checked for specificity for factor IX and factor XI and factor XIIa, which generated nearly no signals in the concentrations that were tested.

Robustness was tested by using various pH and evaluating the effect of the result. About more than 95 percent recovery was obtained with these samples.

Now, when we look at the correlation between Omrix result to the FDA and NIBSC, we can see that there is correlation between the laboratories also. The assay that produced by NIBSC is different from the assay we used and the FDA. These two samples that seems very high is actually, when you dilute the samples, you can get the actual concentration. They were very high, contained very high concentration of factor XIa.

The correlation between Omrix TGA and factor XIa result, we can see again that there is a very nice correlation, low concentration and generated low signals and vice versa.

Now, the in vivo model we used is based on the Wessler rabbit model. Like in the literature I briefly said, the rabbit is anesthetized, the jugular vein is exposed. Samples are pre-warmed to 37 degrees. Sample is infused through the marginal ear vein at 20ml per minute about 10 to 15 seconds. After the end of the infusion, the exposed jugular vein is isolated and the isolated

jugular vein is incubated for 10 minutes.

Then the segment is dissected and its content is emptied into a Petri dish containing sodium citrate, and the content of the dish is examined for the presence of clots and clots are scored at a scale between 0 to 4; so a sample of the clots and the score.

And now when we look at the correlation between the TGA assay and the factor XIa assay, we can see that there is a correlation. Batches which contained a low concentration of factor XIa generated a low score in the Wessler test and vice versa. In both assay you can see this result.

The next thing we did, we used a batch which contained high concentration of factor XIa and we diluted it in batches that was subjected to modified process and showed to contain factor XIa values below detection level. And you can see in the table that dilution of 1 to 80 resulted in TG value of about 80 nanomolar for the TG, and below detection level for the factor XIa assay. And the score that was obtained was 1.6, which is equivalent to the value of the batch which did not contain factor XIa in our testing.

So based on the results above, we can see that batches which contained TG value of above 180 or above 40 nanogram of factor XIa generate a clot score -- with a score of 4. Lower than 180 resulted in a clot with a score of 2 to 3, and below 80 nanomolar of thrombin resulted in a score below 2. I just want to emphasize that a 10 percent solution of maltose produced a score of 1 in the in vivo assay.

So just to summarize, we find a good and direct correlation between TGA results which were obtained from FDA and NIBSC and Omrix for a series of batch tested. Direct correlation was found between factor XIa results that were obtained from FDA and Omrix Lab, and direct correlation was found between Omrix TGA and factor XIa results and direct correlation between the in vivo and in vitro assays. Thank you.

(Applause)

SPEAKER: So yes, please. Dr. Farrugia?

DR. FARRUGIA: Can you provide us with some information about the manufacturing method for your product (off mike)?

DR. MINTZ: Unfortunately, I was told not to -- I

can't. As a --

SPEAKER: Yes, please?

SPEAKER: What specification are you using to release on your product?

DR. MINTZ: Currently, we're not releasing it.

SPEAKER: Any kind -- I noticed that in the Octa presentation, their specification was 315 nanomolar. But in your Wessler assay, you clearly saw lots of clot formation at that point. Any comments on the difference between yours and the Octa results?

DR. MINTZ: Can you repeat on the beginning? I didn't --

SPEAKER: So the Octa -- so go back -- go forward one slide again, sorry. The Wessler -- the final --

DR. MINTZ: You want the Wessler or the one --?

SPEAKER: So I was interested in your stoplight, where it compared the Wessler results. Yes.

DR. MINTZ: Yes.

SPEAKER: All right. So Octa proposed a cut-off of a 350-nanometer thrombin by the TGA assay, which obviously would give a 4 in your -- in this particular --

now, obviously, theirs is different. But I'm just curious is does it give us any insight on the assays?

DR. MINTZ: Yeah, I just mentioned it before, but it was very short. What I meant to say that in our assay, we subtract the baseline which is generated by the plasma itself and we are adding buffer in the same volume of the tested sample. So we're usually getting around 140 nanomolar -- between 100 and 140 nanomolar of baseline, which is just the deficient plasma with the buffer. And if we subtract it, you can get your -- you're getting close to this volume.

SPEAKER: I'm not familiar enough with the FDA protocol. Is that the FDA protocol?

DR. MINTZ: No. We used the TGA based on the manufacturer protocol. But it's not specifying that to subtract or not subtract the baseline. So --

SPEAKER: Okay. Thank you.

MS. GRAY: Maybe I can help here. I think that the difference -- there's difference between the two different thrombin generation methods being used. The one used by the Omrix laboratory -- actually, the CAT, which is a different method to the Octapharma method. And

Jurgen maybe can throw some light on that.

MR. ROMISCH: Yes. Just to comment on the 180 nanomolar, we do not see something at all in a Wessler test and we have done a lot at 180 nanomolar. And as I said, we have an equivalent of 7 milliunits factor XIa per ml, it started to score in the Wessler. And that equals in our test system of more than 500 nanomolar thrombin. So one has to be very careful -- and when always -- if you state these kind of figures, one has to say what the assay looks, what the characteristics are and so on. Otherwise, we're really getting confusing about the total numbers.

DR. MINTZ: I agree. I just want to say that in our Wessler I'd say -- when we measured factor XIa, our value of generating -- of getting clots was about 12 -- above 12 milliunits you starting getting clots.

SPEAKER: (Off mike).

DR. MINTZ: And at 60, you get 4.

SPEAKER: I mean, I've to mention that the Wessler is not exactly the same in every lab. So it depends how much -- what's the speed you're infusing the IVIG and the amount. So that's one -- something we have to correlate before jumping into any kind of conclusion



what should be the limit.

SPEAKER: (Off mike).

SPEAKER: Can you please go to the microphone, please.

SPEAKER: At Octapharma, we used a dose of 1 gram IgG per kilogram body weight, and this is -- yes, we use -- this is the maximum feasible dose with a 20 ml per kg, and in approximately 10 ml per minute. Yes. It's a bolus injection. And you can do it faster via the marginal ear vein. So it's limited.

SPEAKER: Really fast.

SPEAKER: Dr. Calderon (phonetic)?

SPEAKER: I would assume that everybody is doing their pyrogen tests with a Limulus or some other in vitro test. Are any of the companies bothering to take advantage of the fact that if you do the pyrogen test in rabbits and you have a procoagulant, you'll probably have a dead rabbit on your hands? Interesting test, but it does work. And particularly, if you want to make it more sensitive, put in a catheter and monitor the platelet count of the rabbit.

If you give him something with XIa in it, that

platelet count's going to drop to the floor in the animal. If you put in your antithrombin III and heparin, the platelet count doesn't move at all. So I think sometimes we've gotten so enamored of simple laboratory tests that we can control and standardize that we forgotten that we're dealing with biologics, with whole organisms here, and occasionally there is a good reason for using whole animal tests.

We've used them for years and they served us well. Sometimes, we shouldn't run away from them as quickly as we have.

MR. ROMISCH: I commented it. You cannot at all compare a pyrogen test in a rabbit with a thrombus model. You give a rabbit, a healthy rabbit, that dose, that does not do at all. The stasis is the inducer. This is why we're doing the stasis. This exactly 10-minute stasis is enormous, enormous inducer of thrombogenesis. I mean, you hurt the whole vasculature and you clamp it for 10 minutes -- I mean that -- so why has Wessler done that 50 years ago then?

SPEAKER: Because it's a venous stasis test. The Wessler is a venous stasis test.

MR. ROMISCH: Yes.

SPEAKER: It's done in the jugular segment with venous stasis. It has nothing to do with arterial thrombosis at all.

MR. ROMISCH: Yes. But if it correlates with in vivo findings, I don't care. It does -- it correlates in our findings, as I showed you before. It shows even more sensitively than the human did, unfortunately. And I mean, we cannot do more right now than trying to correlate and finding different limits where the system start to react.

If there's another better model -- I mean -- this is why we're here, to discuss.

SPEAKER: Well, I would suggest that you might want to take some of these active lots and put them into a couple of rabbits in the old pyrogen test and I do have a feeling you'll have some dead rabbits on your hands from XIa thrombosis, and it will be virtually instantaneous. If you give them something that is reactive in humans, you'll have a dead rabbit.

MR. ROMISCH: Okay. Yeah.

SPEAKER: It's worth trying.

SPEAKER: Okay, yeah. But the -- okay.

SPEAKER: I would like to make one comment.  
From an animal welfare perspective, this is a no go.

SPEAKER: Okay. But if you -- if you're ready,  
I think we should move into the panel discussion. But  
maybe we can still have one more question to --

SPEAKER: I just had a --

SPEAKER: Is it a question to the speaker?

SPEAKER: No, just a quick comment. Maybe a  
little bit premature; I will show those data tomorrow, but  
talking about the concentrations of XIa. So 1 milliunit  
per ml is about 30 picamole. XIa in blood, fresh blood,  
at 2 to 5 picomolar, which is much lower than 1 milliunit  
per ml, gives about 3-4 minute clotting time in the  
absence of tissue factor, in the presence of CTI.

So 1 milliunit per ml, at least, on the other  
hand, what we're seeing is relatively very high  
concentration. And now we're talking about 7 milliunits,  
30 milliunits per ml. I agree that rabbits are humans --  
hard to believe that they would survive those levels of  
XIa.

SPEAKER: May I briefly comment on the gentlemen

before. I fully agree. Animal welfare -- no, really, I have to say that. I mean, for -- in order to substantiate and find the root cause, its one thing and we did it. But so far, we will not do that for routine assay release. Also because we've found in in-vitro correlate that really is predictive and we don't need the Wessler anymore in routine batches and later on, if it turns out that this really correlates well.

And also for the same reasons we did not further doing spiking experiments and so on just to show the correlation, because from those batches we had to test in the Wessler, we could really conclude that there is a concentration dependent, an XIa dependent, effect in the Wessler test. So that is what we skipped, and I agree that that has to be limited to a certain extent.

#### PANEL DISCUSSION

SPEAKER: So if you are ready, I think we should move into the panel discussion. So I will ask the session speakers, Dr. Gross also, to join here in the table, because we have to try to approach, if not answer, at

least four questions that are the panel discussion topics.

And I will propose a way -- devote maybe five minutes to each speakers. We already -- maybe five or ten minutes to each before we close the debate.

SPEAKER: I have the questions here.

SPEAKER: And Dr. Gailani -- I'm sorry. I forgot you are also joining. I'm sorry.

DR. GAILANI: Thank you.

SPEAKER: So shall we try to address the first question: which manufacturing parameters may confer greater risk of procoagulant activity in immunoglobulin products? Is there anybody who can make a first attempt?

MS. GRAY: Maybe I can start by saying that I think that Dr. Roemisch has carefully laid out the fact that one of the major parameter there is actually the increasing mass capture. And do we have any agreement on that?

SPEAKER: Well, I think he has also -- or there have been a couple of issues also that have mentioned, which are fraction I precipitation together with fraction II + III or not. And the other thing that has already been mentioned is that the use of filter aids during

fraction I + II +III precipitation. So I would say that the manufacturing parameters conferring risk are specific of each production process, but still those three ones that have been mentioned are there for discussion.

DR. GROSS: I guess it's difficult to pinpoint this to a certain step. And just to clarify here, one issue that was raised before, we never have, let's say, raised the question that we have a class effect. We even refused to have a, let's say, a class referral in the European Community because we think the manufacturing process still defines a product. And these are biological, so we never considered a class referral. Not only because we think this is always specific, it's also a workload for certain authorities to deal with these things.

So what I want to say is we will look for each product in the specific manufacturing process. And then it's really difficult to define a certain, let's say, manufacturing step which might have a higher risk to have, let's say, introduced procoagulant impurities, but as it was now raised several times, the mass capture steps -- and we have to say steps, because there are many options

to have mass capture -- is one of the important factors.

And you shouldn't forget the filter aids which are introduced which may contribute to the contact activation.

SPEAKER: Any further observation around this point?

DR. FARRUGIA: Well, I just like to express some reservation, because the adsorption in order to yield, you know, the factor IX family of products and the other products, what's being designated as mass capture, is not unique to Octapharma. Practically, every company does this in order to generate a whole family of products. Neither is the use of filter aids. Every company is using filter aids.

So I can't wholeheartedly endorse the suggestion that is what effectively made the change.

SPEAKER: But if you allow me, I think this is also processes-specific, because, yes, practically all the manufacturers will use the resin adsorption for prothrombin complex concentration or for antithrombin III. All the manufacturers use antithrombin III from fraction IV, which is precipitation. But we do not do it at the



same stage. In our company, we do the prothrombin complex concentration in the supernatant of fraction I instead of the supernatant of prothrombin complex. And we do the antithrombin adsorption after the fraction II precipitation. So we'll come back again, that this process is specific. These are things to consider, but still the main thing to consider is that each process is different.

SPEAKER: Yeah, just to comment on that. I mean, it's true that this is nothing specific to Octapharma's processes. But as I said, it's the extent of mass capture what you're doing. It's the presence of filter aids and the generation of XIa on diatomaceous earth filter aids. It's the impact on the I, III precipitation, and there are other effects from the I, II, III to II which have to be considered. These are matrix effect. These are buffer effects, and we know without going into detail that they have a significant impact on, for example, the XIa precipitation or reconstitution behavior.

So that has to be appreciated and it has to be limited and has to be validated. That is very clear.

DR. GROSS: I guess the approach quite -- changed quite a lot. In the beginning, I thought it was to maximize, let's say, yield and to get as much product as possible. But meanwhile, during the course of your investigation and our evaluation of today which were provided, we think that a scientific approach to assess certain steps and to see what goes out and what is purified and what was precipitated is increasing.

And we -- what we've done here during the workshop, we're quite happy about this; that almost, I guess, every manufacturer started to investigate their manufacturing process. Some companies even started 20 years ago, as I have heard from your side. And our companies, at least, started last year, so we're quite confident that the data available to provide these over to the regulatory bodies.

And as I tried to point out during my presentation is that, in Europe, let's say, the monograph on the immunoglobulins is getting to be changed, and the manufacturing process should be described in detail.

And we were asking ourselves and also this was discussed during the BWP meeting last time, how we should,

let's say, introduce this change in the monograph. It was a dossier. And usually in the variation regulations it's written that such a change to be compliant with the monographs should be a type II variation. And we're asking ourselves, how could we address this. How should we ask the manufacturers to provide this data?

But as I have seen here is that all the data are more or less available. It might be quite simple to submit these data to the regulatory bodies.

DR. JORQUERA: Well, here I think we might move to the second question: what are the challenges of test calibration (phonetic) for intermediates or final products? Few things have been mentioned like the absence of standards, the variability among laboratories, the matrix complexity of the intermediates and also maybe the final product.

SPEAKER: Yes. Jorquera, you said it. I mean, as Dr. Roemisch pointed out, obviously process intermediates that contain ethanol are not suitable to be used for the thrombin generation assay, while other tests like ELISA probably would be applicable to dose matrixes. And he also said one of the caveats, at least for the

thrombin generation assay, is that there is a reference of preparation or even the definition of what is a normal thrombin generation missing.

The thrombin generation assay working partly -- and Elaine, I think you can comment on this much more explicit than I can because you have been heading this for a long while -- have not yet come to a conclusion what a normal thrombin generation in a normal human being is. And as long as we don't know this, this always relates a test that can be used for one specific product to compare maybe batches, to compare manufacturing cycles. But there won't, at least, to the current situation, not be any possibility to compare final products from different manufacturers.

MS. GRAY: I agree. I think that it is very, very difficult to work out exactly what the possible (phonetic) criteria is using the TGA test as such. But I think that we-- I think there will a possibility if we think of the TGA test as a limit test rather than actually a quantitative qualification -- quantitation of the actual procoagulant activities in the product itself.

And maybe that I think that the collaborative

study result will show us some way forward. Tomorrow, hopefully, that we can get some data out and see where we are. But I think that it is very difficult in terms of matrixes, because if the intermediates are very different to the final product itself and if you have a reference material in there and if you use the reference material for connotation, then the ability of the assay is very important. So we need to really have more data to show what we're going to get.

The working party for the -- the SSC working party on the thrombin generation test addresses a slightly different issue, and that's actually addressing the issue of if you're looking at different type of plasma. But here we're looking at the effect of products. So there is a possibility that if we can standardize the plasma being used and also maybe the reagent we're going to use, that may also help towards the inter-lab agreement. But we have to see.

SPEAKER: Let me also add a comment. So what we also should not forget that we may also not have all the same problems. So we focus a bit on factor XIa and thrombin generation assay. But when I look at from our

perspective at this problem, kallikrein is more abundant in Vivaglobin than factor XIa. And we also, you know, need to take this into account that, for example, different manufacturers may also have different proteolytic activities, and that also means that we have done maybe different assays just to monitor and also control this.

DR. GROSS: Just to make it maybe a little bit more provocative, we're not sure yet whether we will require or request and release assay anyway. And I still think if during the, let's say, manufacturing process validation, the data will be, let's say, obtained as is the case for instance for our recurrent proteins, where impurities come from the manufacturing process such as host cell proteins, host cell DNA or protein A, for instance. Or even for the virus removal, you have to validate the virus removal for different steps.

So if you consider such procoagulant impurities in an equal way as an impurity and you can demonstrate that your manufacturing process is able or capable to reduce these impurities to a certain level, then it's independent from the impurity. You have to define your

own assay what you think it is reliable to, let's say, to show the impurity, and then you're on the safe side.

So you can define your own assay. You have to validate your own assay, and then you're free to use it.

SPEAKER: I think we would agree to this approach, and also it's a good introduction to the next question that we have. Because you know, if I would have to answer this question for, let's say, any new IgG product, you know, a significant change, I think we as a company would apply the whole battery of assays and really see, you know, does it have an impact, what is the difference and do a comparability study with these assays.

SPEAKER: And I only can agree to this. So we should use the whole panel for characterization. Or if we have some information upfront, if we do a process change, then we should focus on a certain assay which we do think is most appropriate to address a certain question. But overall, really -- and we're already using everything that we can. Yes.

SPEAKER: That's based on our knowledge about the process, of course.

DR. GROSS: But there's just one remark. I

guess you cannot simply apply the limits which are valid for factor IX or these PCCs for immunoglobulins. If you want to specify a certain limits or cut-off limits, these should be defined according to the product you're testing for and so forth. In immunoglobulins, you should have your own cut-off limits justified on a scientific basis on animal studies.

SPEAKER: Yes. Fully agree.

SPEAKER: So practically, we have already approached the third question and we have talked so far about laboratory tests and Wessler tests, but maybe needed also some clinical safety data. Anybody who would like to comment on that, of course, for your products? But what about changes -- depends, I suppose, from the profile that one obtains during the characterization and validation of the process and also from preclinical tests.

SPEAKER: I think what we're trying to get at here actually is not ask you what to do with respect to clinical studies, but what information might be available or potentially available either in humans or in animals other than the Wessler test. In other words, how does one approach that limit value? Is there some kind of data



from the clinical or preclinical work that might be relevant or useful? I mean any clinical work.

DR. GROSS: During the breaks, we already realized that, obviously, different databases are used. And this is, I guess, the first problem what we have. The companies and we, as regulators, use different databases, and sometimes we come even to different conclusions. So a common database and better exchange of the data would be quite helpful. And as I tried to figure out, to point out, is that we will come back to the manufacturers to ask them again for, let's say, evaluation of their databases to provide data, more data, better data on the amount of it that was distributed or what was even administered because there's a difference between administration and distribution.

So I could imagine that we here have even under or overestimated some of the TEEs for some products because they were simply put in hold and were not distributed anymore. So I guess better cooperation between or better, let's say -- yeah, cooperation between manufacturers and us, as regulators, would be quite helpful.

SPEAKER: I will just briefly -- I mean, what we have learned through the last six to nine months that -- and when we started off -- that we used a panel of assays which may not be fully independent but supplement each other. And so to cover all aspects we could really estimate what could happen. And those turned out to be successful to identify the root cause, to monitor it and to remove it.

And I think that that should be done also in consideration of what has been talked, but it still goes by quality by design aspects, risk assessment of certain steps, which we know now where that -- which have to be observed with more and then apply these assays or look at them. And what finally makes it to be most important that you cannot predict in the beginning. So I think, yes, we should do these where applicable, I mean, in in-process sampling.

And as I alluded on, I mean, not everything can be done at each step. That's not possible due to technical limitations.

DR. FARRUGIA: I think due to the rareness of thromboembolic events, we probably will never come to a

cut-off level by looking at all manufacturers. The case that we're currently discussing with Octagam gives us the unique opportunity to assess all individual cases because it must be known how much IgG the recipients received. It is I think known how many -- how much factor XIa was, if this is the causative as it turns out in the discussion here, was in this product and they should give the opportunity to calculate the cut-off.

And if there's a dose linearity there, that is what we currently don't know. But probably that can be derived from the results that are available.

SPEAKER: This is very difficult because if we discuss stability of factor XIa, you have to take in consideration that room temperature stored whatever sample shows decline of factor XIa over time. If you measure a batch -- I don't know -- two years after application and the TEE has come up, you just can try to extrapolate how the level was at the time point of administration.

So I think that is extremely difficult to do and to correlate. And again, as we said, we haven't seen any increase of XIa activity in these batches which we have stored, so there is no generation of factor XIa. The XI

antigen that is in the samples is not active. We haven't seen any increase over time, but only decrease. And that depends, I guess, then also for the different products on the formulations they have and so on -- storage conditions.

DR. JORQUERA: I have one comment here is that if there is the right strategy to try find a cut-off based on the Octagam data or we will rather try to find in case we have to have a test and we have to establish a cut-off. I think it will be more probably appropriate to be based on the results that are obtained from products that have a baseline very low rate of thromboembolic events, rather than relying on the specific Octagam data. I think we have many products that are showing very low rates of thromboembolic events, and I think I would rather try to derive cut-offs from those kind of products than the other way around.

And we have one question from there.

SPEAKER: Yes. I wanted ask -- just from wanted a broad perspective. We have set of production lots of certain products that have been associated with high TEE rates, and I assume that those certain lots have been

identified. And I also heard today that the NAPTT assay was endorsed by four companies but wasn't mentioned by two others. And we heard from the FDA earlier today that the TGA test is fairly specific for XIa.

So you --an integrated question might be, is it possible for FDA to assemble a set of production lots, some of which are blinded and some are not for TEE and some are not and learn whether different assay actually are sensitive and consistently sensitive or not? For example, is TGA enough or is a second not activated partial thromboplastin time assay useful. And I just wonder if there's some value in having sort of a blind test using known production lots, since these are rare events?

DR. JORQUERA: Do you want to take that one?

SPEAKER: Do you want to?

SPEAKER: I think that it wouldn't be fair to ask FDA to do that because we don't have all the information, we don't have access and I think legal power to request all the information. And this should be a collaborative decision, a result of collaborative studies and/or workshops like this one.

SPEAKER: To Elaine and Mikhail, wouldn't it be fair to say though that there is an effort afoot to obtain lots that we know have high titers and that we know are related with thrombotic events for the standards work and the collaborative activities?

MS. GRAY: Yeah. I mean, the study we just carry out, certainly that -- in that panel of non-material (phonetic) we have some high activity, some intermediate activity, some extremely low activity. Products from, you know, quite a few different manufactures. So I'm hoping that, you know, at least that -- although it's a very preliminary study, it will be the first study we have done. It will give us some idea exactly, you know, how the different assay work on these products and also that how well we agree or not agree.

And then, from there, we can actually move forward and see whether that reference material actually helped. But the thing is that we don't know that whether the reference will help or not yet, until we actually analyze the results from the study.

So yeah, I think that we do need to have collaborative work because it's not always a robust way

for standardization to rely on data just from one laboratory. So the more laboratories that take part in these kinds of collaborative work, the results -- you know, the decision we make will be more robust. Because it's very important that we don't -- well, from an IBSE point view, it's definitely that it's important with the test that we agree with should be good really for all the products.

We shouldn't come up with something that, especially, a reference material, that will only be good for one product and not for the others.

SPEAKER: I also want to mention a slightly different approach that is possible here. Any clinical data that is available in some sense is a result of passive -- this is a retrospective analysis of data that wasn't collected proactively, therefore, there are deficiencies to this kind of analysis. However, one can imagine a different approach. Now that we know that factor XIa is likely to cause thrombosis if it is present in immunoglobulin products, it is reasonable to implement changes to the manufacturing process of products that might have XIa in them.

And then, by doing that, we would reduce the amount of -- the likelihood of known procoagulant contaminant to be present in the product or market. So instead of implementing a cut-off value on products without making any changes and hoping that the cut-off value would limit the rate of adverse events, we would rather implement changes to the manufacturing process that would remove the known contaminant.

And then the point of further testing would be to ensure that the manufacturing consistency of the product rather than to ensure the safety of the product using an arbitrary cut-off value.

SPEAKER: Mr. Ovanesov?

MR. OVANESOV: You know there was a discussion earlier about whether this is a -- it should be regarded as a class effect or not, and I don't if that discussion was resolved. But it has some implications, regulatory implications, in terms of: do we need to ask all manufacturers to have testing in place, which is very important. So I think it might be worthwhile to just revisit that for a minute. What I'd like introduce to that discussion is that -- the idea is that when you make



these products, if you're making them from plasma, the starting material is very common to all these products. The starting material has clotting factors in there.

And it's true that the manufacturing processes are different and they could influence what you have at the end, the final product, differently, but your starting material is the same. That's the one point.

The second point that I think is important is that we've seen across the board over the years, that all these products are associated with some level of adverse events that maybe higher in some product or another product, but we're still seeing these adverse events with all these products.

So there is, at least to my mind, a reason to think that we should consider it to some extent a class effect and across the board we should consider this type of testing, whether it's part of the manufacturing process or the release.

DR. JORQUERA: Any further addition to the discussion?

SPEAKER: And I have one more comment regarding the availability of reference preparations and standards.

I mean, as a consequence of the last year, at least factor XIa became very prominent, and I think it would be a call for having an international reference preparation, independent of immunoglobulins, just that we can measure an international unitage of factor XIa.

That shouldn't be such a big deal, and I think there's a need for that. Many people will test factor XIa for whatever purpose.

MS. GRAY: I think that it's not a bad idea to have an international standard for XIa, but I think that we need to have some preliminary work on this because a purified preparation of XIa may not necessarily be a good comparator of XIa in a IVIG product. So we need to look at the data, you know, again to make sure that that is the right direction to go, because making international standard is quite resource hungry and we need to make sure that, you know, that is a good way to go forward.

And of course, in the end, it might end up that we need a XIa standard as a purified preparation, but may well also need another reference material that's traceable to that in order to give you a good comparison of XIa in IVIG. But I think those things will come out in, again,

in the collaborative study results.

DR. GROSS: Just two remarks. We are still collecting data from TEE reports, which we are increasing in 2010. So I'm quite confident that we will still obtain some additional information about batches material which might be involved for different kind of products. So I'm quite confident that we might be able to define certain cut-off limits either for TGA or for NAPTT or whatever for these immunoglobulins based on TEEs which were reported.

And the second remark is also for stability. We were quite focused on factor XI again -- we have done some CSL. So maybe kallikrein might contribute as well. And kallikrein is much more stable in these products, so you might be able to detect kallikrein in these samples as well as maybe thrombogenic potential connected to kallikrein impurities in this older aged samples.

So isn't always the case that you cannot test older samples. But you're right, with respect to factor XIa, older sample they will lose their activity.

SPEAKER: Okay. Sure.

DR. JORQUERA: So with this, we finish. Dr. Scott, please? Yes, go ahead.

DR. SCOTT: Okay. Anyway -- I think we'll  
finish now and thank you to the panel and the speakers and  
the audience for all your participation.

(Applause)

We'll see you tomorrow at 8:00 'o clock.

(Whereupon, the PROCEEDINGS were adjourned.)

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