

Plasma Exchange in the Treatment of TTP/HUS with Long-Term Follow Up and Prognosis

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About the Speaker

Dr. Gail Rock, whose training is in hematopathology (blood disorders), is Chief of Hematology and Transfusion Medicine (Department of Pathology & Laboratory Medicine) for Ottawa Hospital, and Professor of the Department of Pathology and Laboratory Medicine on the Faculty of Medicine for the University of Ottawa in Ottawa, Ontario. She is currently the Editor-in-Chief of the Journal Transfusion Science, President of the Canadian Hematology Society, and Chair of the Canadian Apheresis Group.

Her publications include: 168 publications in peer-reviewed journals and 49 review articles and/or chapters in books. Books edited include: Apheresis and Quality Assurance in Transfusion Medicine (Volumes 1 and 2).

Prior to joining Ottawa Hospital and the University of Ottawa, Dr. Rock worked as an Emergency Room Physician and she has also been in private Medical Practice. She was employed by the Canadian Red Cross as the Medical Director of the Ottawa Centre for close to fifteen years during which time she redeveloped local and national programs in Apheresis and carried out many studies on blood and blood components. She has served as a Consultant to the Department of Agriculture, to the Office of the Surgeon General of Canada. She has been a Special Advisor for Hospital Blood establishments of the Bureau of Biologics, Health Canada and has been appointed to the National Canadian Blood Safety Council reporting to Minister Rock.

Dr. Rock received her Ph.D. in Biochemistry in 1966 followed by two years of NRC Fellowship in the Biophysics Department of the National Research Council and two years of Postdoctoral Training on an MRC Fellowship. She obtained her M.D. from Ottawa University in 1972 and later her F.R.C.P. in Hematopathology from the University of Toronto. Dr. Rock is Chair of the Hematological Pathology Residency Training Committee.

**PLASMA EXCHANGE IN THE
TREATMENT OF TTP/HUS WITH
LONG TERM FOLLOW UP AND
PROGNOSIS**

GAIL ROCK, PH.D., M.D., FRCP

TTP MAHA

1924 Described by Moschowitz

**1998 Tsai, Furlan
 Inhibition of Protease**

TYPICAL LESION

**HYALINE MICROTHROMBI
OCCLUDING
SMALL VESSELS
PLATELET AGGREGATES +FIBRIN
± ENDOTHELIAL PROLIFERATION**

TTP PENTAD

- **THROMBOCYTOPENIA**
- **ANEMIA**
- **FEVER**
- **NEUROLOGICAL SIGNS**
- **RENAL ABNORMALITIES**

CAG

1998 BRITISH JOURNAL OF HEMATOLOGY
n = 135

- all Schistocytic hemolytic anemia**
- all Thrombocytopenia**
- 86 CNS**
- 30 Fever**
- 24 Renal**

PATHOPHYSIOLOGY

- 1. Platelet aggregating factors**
37 Kd ptn, calpain
- 2. vWF abnormalities**
UivWF
- 3. Antibodies plts/EACS**
- 4. Antibodies metalloprotease**

TTP

Multiple forms

- acute
- chronic relapsing
- congenital

CHRONIC RELAPSING

**ULVWF multimers
enhanced binding
to platelets/adhesion**

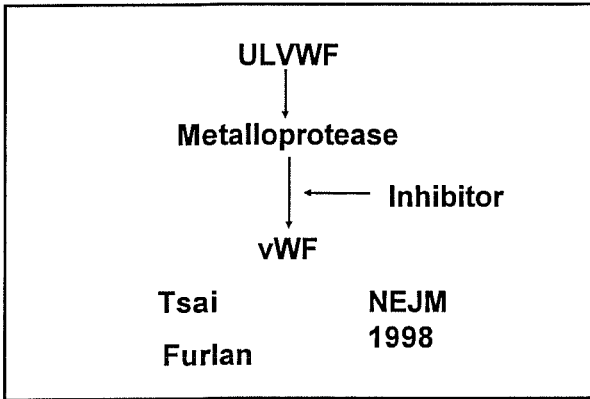
Moake 1982

CONGENITAL

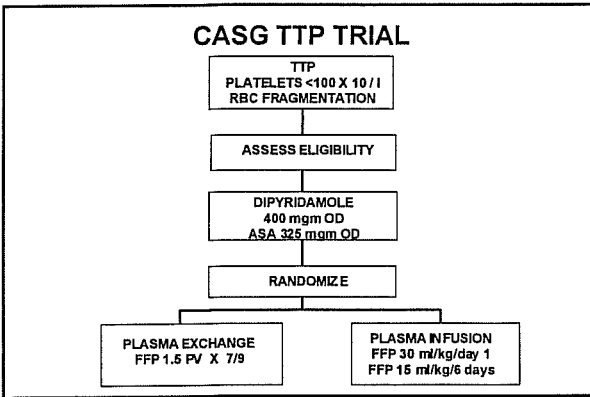
**Deficiency of metalloprotease
which reduces VWF**

Furlan 1998

Respond Plasma Infusion



- 1925 Moschowitz
- 1925 Lederer - BT
- 1959 Rubenstein - exchange FB
- 1977 Bukowski - exchange FFP
- 1990 Byrnes - CSP
- 1991 CAG - PE > PI
- 1991 Bell - PE
- 1996 CAG - CSP



OUTCOME MEASURES

Responders	>150 x 10 ⁹ /L
Partial responders	>100 x 10 ⁹ /L but <150 x 10 ⁹ /L
Failures	<100 x 10 ⁹ /L or incr <100%

TTP: DEATHS SIX MONTHS

GROUP	SURVIVED	DIED	TOTAL
EXCHANGE	40 (78%)	11	51
INFUSION	32 (63%)	19	51
No crossover	10 (50%)	10	20
Crossover to PE	22 (71%)	9	31

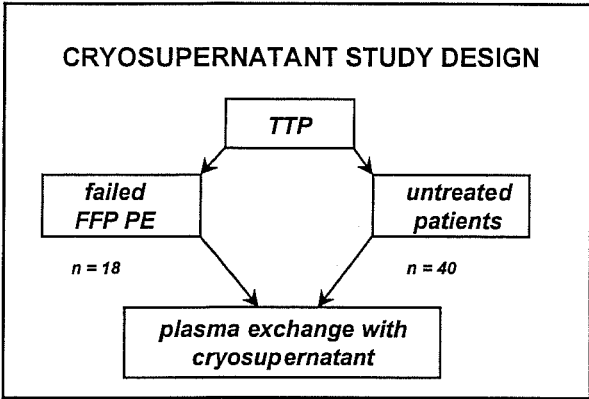
**RESPONSE TO PLASMA EXCHANGE IN 24 PATIENTS
WITH TTP, OLIGURIA & AZOTEMIA**

HUS

	Day 7	Eventual
Increased platelet count	15	21
Survival	23	20

CONCLUSION

**More than one third of patients
who survive an acute episode of
TTP
will have at least one relapse during
the following ten years.**



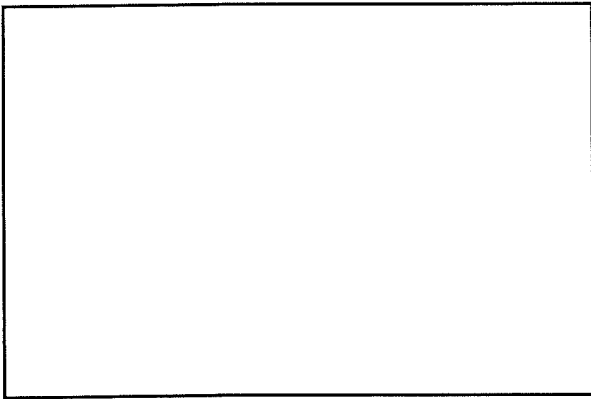
- RATIONALE FOR POSSIBLE SUPERIORITY OF CRYOSUPERNATANT**
- Unusually large vWF multimers in plasma of some patients with chronic relapsing TTP (Moake et al. 1982)
 - Aggregation of platelets in TTP plasma enhanced by the addition of cryoprecipitate (Kelton et al. 1985)
 - vWF demonstrated histochemically along with platelets in the microthrombi in TTP (Asada et al. 1985)

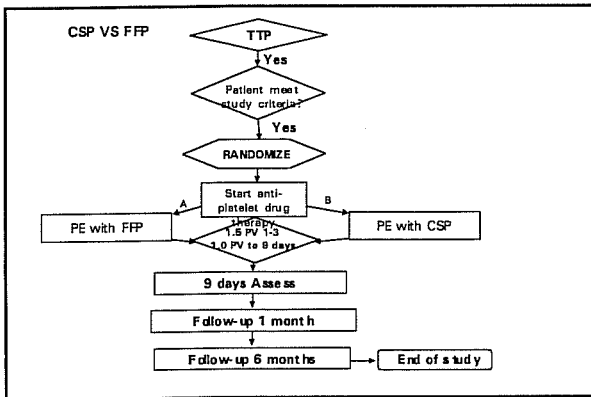
- RATIONALE FOR POSSIBLE SUPERIORITY OF CRYOSUPERNATANT**
- Cryosupernatant is depleted of large vWF multimers but is still active in converting large vWF multimers to smaller vWF forms (Frangos et al. 1989)
 - vWF and factor VIII elevated in all TTP patients (Rock et al. 1991)

VWF (1 U/ml)

Stored in endothelium:
Weibel - Palade Bodies, platelets
as UL forms

Plasma: polymer of polypeptides
- cleaved in circulation
200 kD metalloprotease
176 kD and 140 kD
(300 and 250 kD on gels)
series of multimers



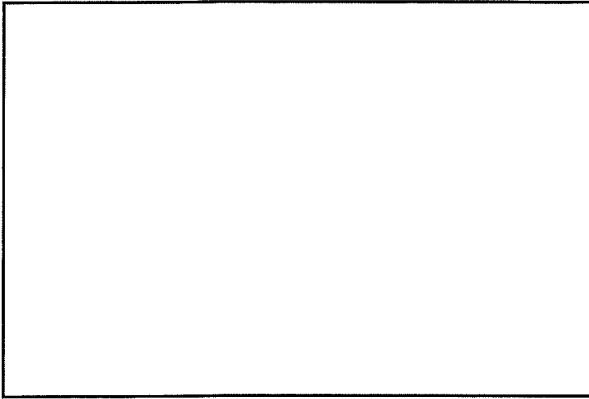


CONCENTRATION OF VWF IN PLASMA DURING PE THERAPY

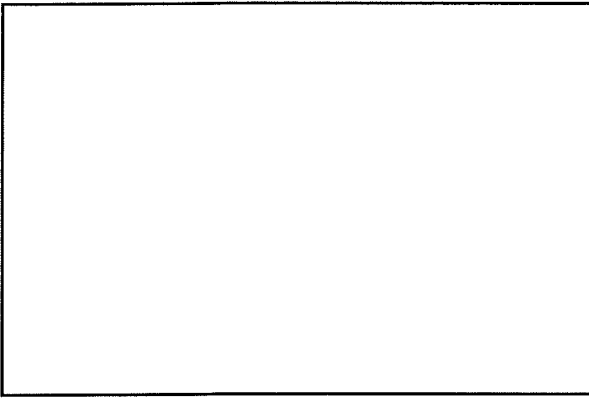


VWF MULTIMERS

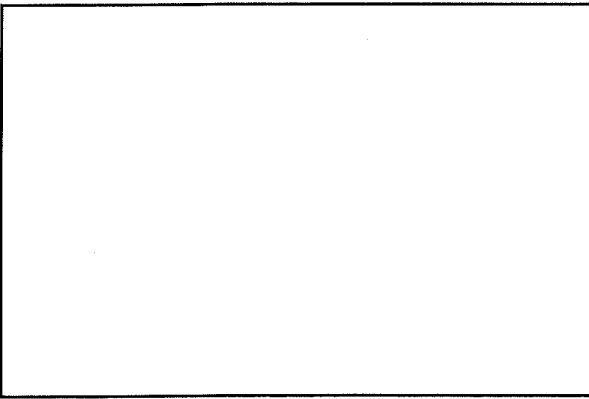
- at entry, pattern variable
few with ULVWF
- protease not always reduced,
inhibitor varies with platelet count



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Seven horizontal lines for writing, aligned to the right of the third box.

**ROLE OF PLATELET ANTIBODIES
IN TTP**

- PRESENCE
- SPECIFICITY
- SIGNIFICANCE

**MULTIPLE ANTIBODIES
IN TTP
WESTERN BLOT**

Empty rectangular box for notes or diagrams.

GPIV (CD36; Mr 88,000)

- Occurs in platelets and endothelial cells (Tandon et al., 1989)
- Coupled to protein tyrosine kinases (Huang et al., 1991)
- Mediates initial stages of platelet-collagen interaction (Tandon et al., 1989)

GPIV (CD36; Mr 88,000)

- Elevated in myeloproliferative disorders (Bolin et al., 1977)
- Reacts with platelet agglutinating protein p37 present in a subset of TTP patients (Lian et al., 1991)





CANADA

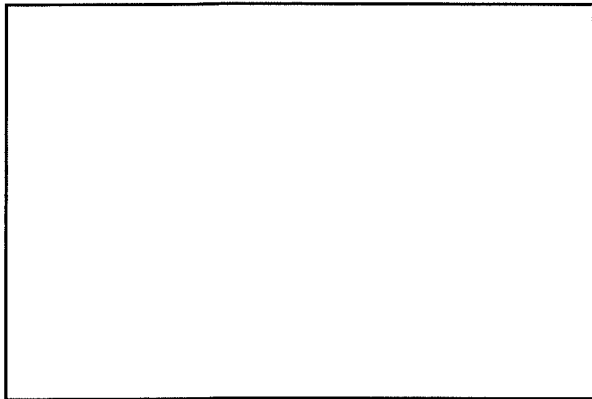
TTP/HUS

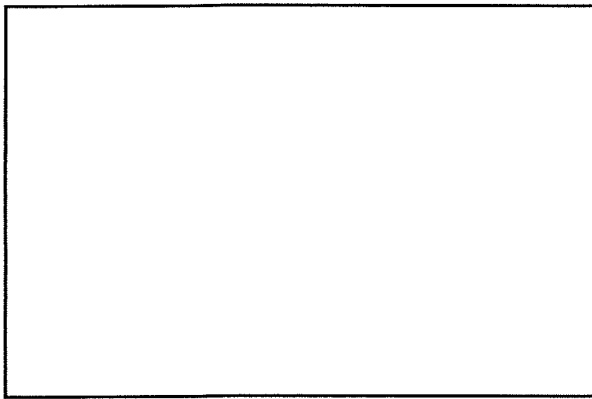
SDP

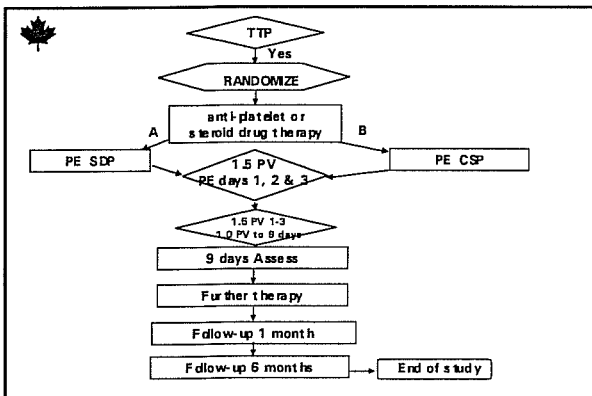
- **Screened, tested**
- **Pooled (2500 units)**
- **Treated**
- **200 ml**

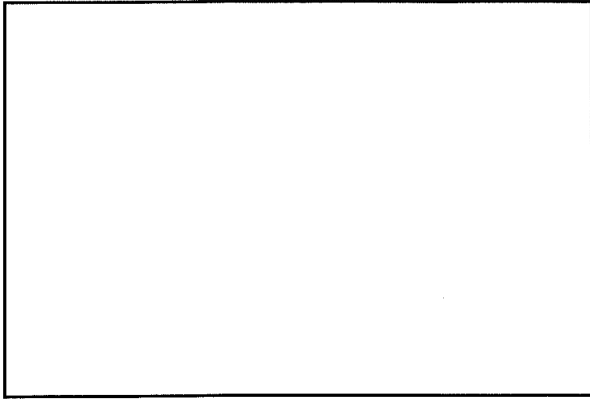
PLAS+SD features

- **Inactivation of lipid-enveloped viruses (HIV, HBV, HCB, HTLV, HGV, ...)**
- **Consistent levels of coagulation factors**
- **Leukocyte and bacteria-free**
- **Largest von Willebrand multimers absent**
- **Neutralizing antibodies**
- **Standardized 200 ml unit**

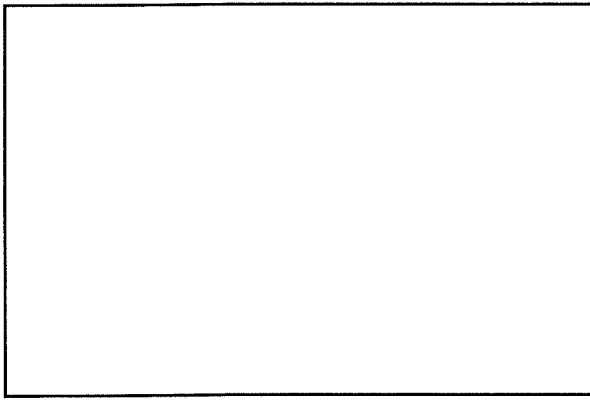




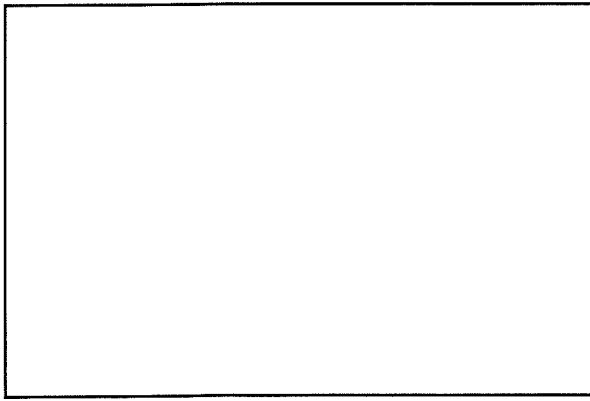




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Empty rectangular box for notes.

Seven horizontal lines for notes.


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SAFETY ISSUES

- Reactions
- Viral Transmission
- Thrombosis

Seven horizontal lines for notes.



THIS STUDY WILL:

- Resolve best therapy
- Determine availability of blood products
- Assist in resolution of disease mechanisms

CONCLUSIONS

1. Continues to have significant mortality
2. Relapse occurs in >35% of patients
3. Relapse occurrence and interval is variable

Slide 1: PLASMA EXCHANGE IN THE TREATMENT OF TTP/HUS WITH LONG TERM FOLLOW UP AND PROGNOSIS

Transcripts

MS. GIRAND:

Well, when you want to build something really strong you want to build something on rock and I have to say that our next speaker has been incredibly gracious.

Two years ago when we started thinking about this conference, originally we knew that we very much wanted to have a hematologist and practically the first physician we contacted was Dr. Gail Rock, based out of Canada.

Dr. Rock agreed to speak. We then moved the date of the conference at least twice on her and she continued to agree to speak. And then I'm sure that her schedule has been beyond control and she continued to agree to speak. So we are very, very pleased and incredibly grateful to her coming today to give this presentation.

I would like you to please join me in welcoming Dr. Rock.

DR. ROCK:

Ladies and gentlemen, it's a pleasure for me to be here today and I would like to begin by thanking the organizing committee for inviting me here to Seattle. It gives me a wonderful opportunity to go up to Vancouver and see my daughters, as well as hearing all of the presentations today.

What I would like to speak with you about this morning is the therapy that we have applied to adult cases of TTP and Hemolytic Uremic Syndrome.

The major therapy that we have found over the past 10 years to be efficacious is the use of plasma exchange therapy. So I would like to spend a few minutes just simply describing what it is I mean by plasma exchange.

Plasma exchange has now been in existence in common use only for the last 25 years. Prior to that, it was possible to remove plasma from patients, most certainly, but in not large enough quantities to be truly effective.

Now, with the development of new, large machines, which are rather like the dialysis machine in principle, we're able to use these machines to remove large volumes of blood and plasma from a patient during a very short period of time.

A typical plasma exchange procedure in which 1.5 to two plasma volumes of a patient are removed and exchanged are completed in two to four hours depending on the patient's size.

Essentially what is involved is the use of the machine to draw blood from the patient through a single venipuncture. The whole blood comes out and is separated, generally by centrifugal techniques with a bowl inside that device I showed you earlier, which separates the plasma into its various components, meaning red cells, white cells and plasma.

Then in Apheresis we are able to extract any one of these layers we want so that we can use this procedure for donor collection. Mainly, we can collect platelets from a random donor to use in a bone marrow transplantation setting, for example or, alternatively, we can remove the plasma from the patient if we, in fact, consider there to be something harmful in that plasma.

Now, that could be the presence of a toxin, it could be the presence of a variety of antibodies and/or it could be that the plasma is lacking something which the patient requires but that can not be replaced by use of a blood concentrate.

So now it is about 25 years since we've had the capability in North America to use large volume plasma exchange to treat a variety of patients.

At the present time about 55 percent of the cases treated in Canada are treated for immunological disorders. About 35 percent are treated for neurological disorders, such as acute uremic syndrome where we know there's an antibody, at least one in the plasma that is harmful to the patient.

And then there are a variety of other diseases, including collagen vascular rheumatological, etcetera, disorders for which we carry out plasma exchange.

Canada is one of the few countries in the world that has a complete registry of every plasma exchange procedure carried out in the country. As such, we can tell you that over the past few years we have done about 10,000 plasma exchange procedures in all of our patients in 35 major medical centers across the country.

And in putting together this data and establishing the Canadian Apheresis group, which we did 20 years ago, we have been able to carry out a number of randomized clinical trials in a variety of disorders. Some of those involve the therapy and the treatment of adult patients with TTP/HUS.

And if I could go onto the other set of slides, now, what I would like to talk about is the results of some of our and other people's clinical trials in the therapy of these disorders.

Slide 2: TTP MAHA

DR. ROCK:

Now, one of the first things I would say to you is that in the adult setting it is extremely difficult to separate out in a specific fashion the disorders of Thrombotic Thrombocytopenic Purpura and Hemolytic Uremic Syndrome. **DR. ROCK:**

Now, TTP is what we call a micro angiopathy and was first described in 1924, by Moschowitz, who had a 16-year-old patient who went into coma and then died from the deposition wide-spread in the body of platelet microthrombi.

And while the disease has been around for a very long period of time, it really wasn't until 1998, when two different groups those led by Dr. Tsai, in New York, and Furlan in Switzerland demonstrated the presence in the patient's plasmids of an antibody directed against an enzyme and associated it with the pathophysiology of the disease.

Slide 3: TYPICAL LESION

DR. ROCK:

Now, the typical disease lesion is a hyaline microthrombus; in other words, an amorphous staining protein deposit in the blood vessels, particularly in the small blood vessels, with platelet aggregates and fiber. As such, these lesions and clots are unlike those that we see in disseminated intravascular coagulation.

They are not enriched in fibrinogen, one of the clotting proteins, but rather are highly enriched in von Willebrand factor, the protein which is deficient in patients with von Willebrand's disease. The other thing we didn't see is endothelial cell proliferation or certainly a reaction.

Slide 4: TTP PENTAD

DR. ROCK:

TTP has classically been categorized as having a pentad presentation with thrombocytopenia or a low platelet count, anemia, fever, neurological signs and renal abnormalities.

Now, in the so-called adult form of TTP the emphasis is largely on the neurological signs. Nonetheless, the platelet microthrombi in fulminant disease can be widespread. And it is by no means unusual to see renal abnormalities and, in fact, in our first series of a hundred patients that we randomized, as you will see in a few minutes, an additional 35 patients or 25 percent of those patients, and these are in the adult group of age above 18, were anuric or oliguric at the time of presentation and thereby would be considered and were considered to have Hemolytic Uremic Syndrome.

This is what the textbooks used to say about TTP and this is what you do see, even today, when the disease has run its course and is truly well developed. Fortunately, we're detecting this disorder earlier and earlier, possibly because the awareness which has come about through the number of clinical trials we have developed, and also, I think, because physicians have a true appreciation of the fact that one can intervene, particularly if you do so earlier in the course of the disease, with very good outcome.

Slide 5: CAG

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n = 135

DR. ROCK:

In fact, in a recent publication that we had in the British Journal of Hematology, and this is a publication by all the members of the Canadian Apheresis group, we found that the pentad did not present in entirety.

All of our patients had hemolytic anemia with the odd looking red cells that you just saw in the slide from Dr. Lingwood. They all had thrombocytopenia or low platelet counts. They did not all have CNS symptoms. However, those that we saw rather later in the course of the disease, more often had CNS symptoms. Only a third of our patients had fever.

And, again, 25 percent presented with impaired renal function and, again, could be classified and in the standard course of events in most hospitals would be considered to be patients with adult HUS.

Slide 6: PATHOPHYSIOLOGY

DR. ROCK:

Now, over the years, since 1924, there have been a large number of agents, reagents, that have been considered to be responsible for the disease.

A variety of platelet aggregating factors have been identified; these include the 37 Kd protein of Rinad (phonetic) and his group from Miami.

Several years ago John Calpain and his group from McMaster University in Hamilton identified an enzyme, calpain, which is active against von Willebrand. And we know that von Willebrand factor is a very critical element, first of all, in the clots that are formed in these disorders, and secondly, von Willebrand factor has an important role in platelet aggregation.

Joel Moake out of Texas has reported in four cases of chronic TTP the presence of very large, unusually large, von Willebrand factor forms in the plasma of these patients. And while von Willebrand factor normally is responsible for causing platelets to stick to the subendothelium, these unusually large forms are even more active and have a propensity for accumulating proteins or platelets far more quickly and effectively than does the normal protein.

For many years other investigators have reported the presence of antibodies in these patients' serum, antibodies directed against platelets, and also antibodies against endothelial cells.

Then more recently the works of Tsai and Furlan have shown an antibody against the metalloprotease. And what this is, is an antibody that -- I see what happens, it does work on the wall and on the floor, but not on the

screen, in particular, okay -- these antibodies inhibit the normal breakdown of von Willebrand factor resulting in the fact that the plasma has more of the larger forms of von Willebrand factor present in the plasma.

Dr. Tsai visited us recently in Ottawa at a meeting educating the Apheresis group and claims that he finds these antibodies in the majority of patients presenting with TTP. As I will show you later in my presentation, we have found this to be variably so.

Slide 7: TTP **Multiple forms**

DR. ROCK:

Nonetheless, we have come a long way to define what is going on in TTP. And one thing we know for certain is that this disorder does present in multiple forms. It will present as an acute form in which a previously well 35-year-old woman suddenly comes down with thrombocytopenia and schistocytic hemolytic anemia and if not treated, will go on to develop those other signs in the pentad.

There is a chronic relapsing form. I will give you a bit of information on the number of our patients that relapse, shortly, but there's also a form that appears to be congenital in which individuals are born with a particular propensity to have recurrent episodes of TTP and who can be treated, particularly when they are young simply by infusing one to two units of plasma.

Slide 8: CHRONIC RELAPSING

DR. ROCK:

Now, the chronic relapsing form was first described by Joel Moake in 1982, and it seemed kind of biblical that these forms depended on these unusual forms of von Willebrand factor multimers which enhanced the binding.

There still are not a very large number of these patients in the literature, reported in the literature. And in our investigation of relapsing patients, we have not found the von Willebrand factor multimers nor, in fact, anything else to be predictive other than a low platelet count.

Slide 9: CONGENITAL

DR. ROCK:

Most recently, Furlan has looked at the congenital forms and been able to find that in some small children who have recurrent forms of TTP there is a deficiency in the metalloprotease which reduces von Willebrand factor.

Now, what this is all about is you have normally secreted from the endothelium von Willebrand factor -- the amounts will increase when the endothelium is insulted, such as by exposure to verotoxin. In fact, data from my lab about 10 years ago showed when we exposed normal cultured endothelial cells to purified verotoxin, we got the release of von Willebrand factor and unusually large forms of von Willebrand factor from the endothelium.

Slide 10: Untitled

DR. ROCK:

Normally, an enzyme, a metalloprotease, acts on those forms to form the normal kind of von Willebrand factor that goes around in the circulation. But in the presence of some patients, including the congenital ones, and in the series of Tsai and Furlan, they have found an inhibitor present in the plasma that blocks this conversion leaving them with these more active forms of von Willebrand factor circulating in the plasma.

Slide 11: Untitled

DR. ROCK:

Now, there's been a long history of attempts of therapy of this or these disorders. In 1925, Moschowitz first described the disorder and attempted treatment by a variety of methods which led, in 1924, Lederer to use simple blood transfusion and, in fact, achieve some response with an elevation of platelet counts. This led Rubenstein, in 1959, to carry out exchange procedures with whole blood.

Now you can just imagine that a normal whole blood collection at that time would be about 400 hundred ml's, and so in order to replace a blood volume of let us say four liters or five liters, this would be an extremely time consuming, tedious and difficult procedure, but nonetheless, it was tried, and with some reasonable success leading Bukowski, in 1977, to consider carrying out exchanges but now using fresh frozen plasma.

Then, of course, the machines came along. And while the machines were originally developed and certainly the first use I ever had for them was for collecting white blood cells to transfuse to septic patients, it was very, very early in the game we recognized that if we could take and separate the cells from Apheresis procedure we could just as well remove the plasma so the field of plasma Apheresis or plasma exchange began.

So Bukowski's work, in 1977, led to the consideration of the use of fresh frozen plasma. And we began the study in the mid '80s, in Canada, in which we compared plasma exchange to plasma infusion in the treatment of

adult patients with acute TTP.

It took us a number of years to get this data published because it took us a long while to really make sure that we could recruit all the patients, get the information across what is essentially a long skinny country. But in 1991, we published data in the New England Journal of Medicine indicating that plasma exchange was preferable to plasma infusion in the treatment of these patients.

Now we acknowledged the fact that we did not use as much plasma in the plasma infusion arm as we did in the exchange arm, but that's simply because we used the maximum dose that could be tolerated by the patients. We were not so much interested in looking at volumes of plasma as a therapy but modes of treatment.

Then, in 1991, Byrnes published a case report in which he used a different kind of replacement fluid, cryosupernatant plasma, to treat these patients leading us then, after we finished our first study, to then look at the use of cryosupernatant plasma versus fresh frozen plasma in plasma exchange and found, again, a superiority of the latter treatment.

Slide 12: CASG TTP TRIAL

DR. ROCK:

Now, just to describe to you our first trial in a little more detail. Patients were entered from all the centers across the country and had to, in order to be considered for the trial, meet very strict criteria which meant, in some cases, we were certainly not treating the majority of patients in some centers who either presented very late in the disease or had a lot of other complications.

Of particular note, we did not treat the patients who had, at that point, impaired renal function. The reason for that is they could not be randomized to this trial because they had to be able to accept plasma infusion. And, of course, if their kidneys were shut down, this was not a reasonable thing to do.

Slide 13: OUTCOME MEASURES

DR. ROCK:

So this was a restricted trial, in the first place, but nonetheless, ended up with our ability to very cleanly, if you will, to compare plasma infusion to plasma exchange, which categorized the patients according to their outcome as responders, partial responders, and failures based primarily on platelet count. There are a number of other factors involved but I won't go into all of those at this time.

Slide 14: TTP: DEATHS SIX MONTHS

DR. ROCK:

Which is to say that if you go back into the 1960 literature, 85 percent of all patients died who had TTP. If you are now looking at the outcome from these studies, you will see that in both arms the majority of patients survived and at six months we had a 78 percent survival with plasma exchange, which is statistically significant and improved over the infusion arm.

I should note to you that patients did crossover to the trial, but during this crossover, if anything, they prejudiced the exchange arm in a negative way so that these results would be considered the worst case scenario that we would get with plasma exchange. And in actual fact, if you don't consider the crossover, the results are likely to have been better, but statistically, of course, we have to look at crossovers.

Slide 15: This slide intentionally left blank

DR. ROCK:

These are the plots that show the relative effectiveness of plasma exchange versus plasma infusion from time of initiation of treatment. And you can see that the difference between exchange in the infusion arms certainly holds up with time.

Now, particularly in this audience, I would like to emphasize the effect of plasma exchange and plasma infusion on patients who have impaired renal function because, once again, our group struggled with this definition of TTP vs. HUS only three weeks ago when we're trying to get ready to submit another grant.

At this point in time we feel it is extremely difficult, except in those very overt, strong cases, where you've got a known verotoxin bloody diarrhea associated in an adult that you can call that truly HUS and not TTP with renal manifestations.

Slide 16: RESPONSE TO PLASMA EXCHANGE IN 24 PATIENTS WITH TTP, OLIGURIA & AZOTEMIA

DR. ROCK:

So we have reported on 24 cases, and this was reported in the British Journal of Hematology a couple of years ago, in which these patients received plasma exchange in the same -- according to the same formulation as the patients who did not have renal impaired function. And, in fact, we found essentially the same survivals. So these patients do respond to treatment with plasma exchange therapy.

Slide 17: This slide intentionally left blank

DR. ROCK:

I would like to say a few words now about the relapses in our patients. We've been following some of our patients through up to 10 years, of course those who entered the studies near the end we only have a few years' data on, but basically overall at this point in time we are seeing a 35 percent relapse over this 10-year period.

The interesting thing from my perspective on this is that, as I told you before, there's nothing particularly predictive, according to the laboratory markers, but there's also no pattern for the relapses. You can see that some patients relapse early in the course of disease; others very late.

This patient was perfectly fine for years and years and then relapsed. But we're at the point now where the patients themselves are all being taught that such relapses are possible. And I have had patients represent to me simply by phoning up and saying I've got a few small blue spots on my arm, do you think I should come in, and they came in and they were right. So we were able to get them very early in the disease and there's no question at all that this therapy, which is removing material and replacing material, is absolutely best done if done early in the course of the disease.

Slide 18: CONCLUSION

DR. ROCK:

So I would remind you that more than one third of patients who have survived an acute episode will have at least one relapse. So we tell our patients very clearly that this is a possibility and something to be watched for.

Slide 19: CRYOSUPERNATANT STUDY DESIGN

DR. ROCK:

The first study and the report from Byrnes led us to then carry out another study. First of all, we took a TTP patient who had failed the treatment with plasma exchange, and as you will recall about 20 percent don't respond, and we carried out plasma exchange with cryosupernatant. This is a pilot study.

We had to start with a patient who had failed because on the other side of the coin we knew for sure that the fresh frozen plasma was effective in 80 percent of cases. We had no idea if this would be effective in more than the few cases reported in the literature.

But because of the success that we had in our early failed patients, we then went on to treat untreated patients with cryosupernatant. Both of these, I must tell you, are pilot studies for which we are simply looking for an indication of benefit to do the larger randomized prospective clinical trials in sufficient numbers to statistically prove that benefit.

Slide 20: RATIONALE FOR POSSIBLE SUPERIORITY OF CRYOSUPERNATANT

DR. ROCK:

What is the rationale for using cryosupernatant? Well, the large molecular weight multimers of von Willebrand factor which are in plasma have been associated, as I mentioned earlier, certainly with the chronic relapsing forms of TTP and more recently Furlan and Tsai have indicated that they have a role in the commoner acute presenting form.

We know for sure that aggregation of platelets in TTP plasma is enhanced by the use of cryosupernatant rather than plasma. And von Willebrand factor has been demonstrated to actually be present in the clot.

Slide 21: RATIONALE FOR POSSIBLE SUPERIORITY OF CRYOSUPERNATANT

DR. ROCK:

Now, cryosupernatant has the advantage over fresh frozen plasma in that it doesn't contain much von Willebrand factor to begin with and selectively it contains fewer of the larger molecular weight forms than does plasma. And, of course, we found consistently that while we don't see a particular pattern to the multimers we do find that both von Willebrand factor and factor eight are elevated in all TTP patients and all HUS patients in the adult series.

Slide 22: VWF
(1 U/ml)

DR. ROCK:

What is this von Willebrand factor? Well, it's a glycoprotein which is stored in the vascular endothelium in granules called Weibel - Palade Bodies. It's also found in platelets, but it does not appear to be particularly released from the platelets in these high forms during stimulation.

In the plasma it consists of lower molecular weight forms which even after immediate release into the circulation are further cleaved into a variety of subunits of multimers.

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DR. ROCK:

This is what they look like. If you put the plasmas or the cryosupernatant onto a gel and you run them down through an electrical field to separate them, and then you probe them or identify them with an antibody to von Willebrand factor, you see these kinds of patterns.

This is a normal plasma control in which you see repeating bands of von Willebrand factor up to and around this range. This is a platelet control where you see the same kind of bands, but you will notice that there are more down here in this area which is the area where the larger molecular weight forms of von Willebrand factor are present.

If you look at the plasma, you will see that this is pretty much like our control, which it should be, but if you look at cryoprecipitate, you will see that it's darker and appears to have more of the bigger forms.

What cryoprecipitate is, is a precipitate which is formed when plasma is frozen at very low temperatures and then slowly thawed. It is highly enriched in three percent of all the plasma proteins and particularly contains factor eight and von Willebrand factor. It was the 1960 discovery of cryoprecipitate by Judith Prewell (phonetic) which made possible the treatment of hemophiliacs by any form of concentrate rather than using whole plasma. It was the first time hemophiliacs had a therapeutic option.

But now we find that the other side of that product, the cryodeflated plasma appears to be of benefit in our patients because as you can see from the supernatant plasma, first of all, there's obviously much less von Willebrand factor present than there is in any of the presentations plus, certainly, it doesn't have the larger forms of von Willebrand factor.

Slide 24: CSP VS FFP

DR. ROCK:

Now, we then set out in Canada to undertake a randomized controlled study looking at cryosupernatant plasma versus fresh frozen plasma. The rationale to us was clear. Our pilot studies indicated benefit but the gold standard is always the randomized controlled clinical trial.

I will just walk you briefly through this to show you that patients with TTP if they give informed consent and meet all our study criteria are randomized. They start antiplatelet drug therapy, which is simply aspirin, and then what we see is fresh plasma exchange, either with fresh frozen plasma or with the cryosupernatant. We always carry out the exchanges for seven of the first nine days, at which point the patients are assessed, but therapy continues depending on the degree of response with follow-up at one month and then at six months, which is the end of the study.

Slide 25: CONCENTRATION OF VWF IN PLASMA DURING PE THERAPY

DR. ROCK:

Let me show you some data from that study so far. You can see here if von Willebrand factor is an important element in all of this that clearly you achieve much better reduction in von Willebrand factor if you exchange with cryosupernatant plasma than with FFP.

These are values taken from the patients' plasma, from blood taken before the procedure starts, immediately after the first procedure, and day three and then again on day five. So we're certainly able to effectively decrease von Willebrand factor by this technique.

Slide 26: VWF MULTIMERS

DR. ROCK:

But it's interesting for us because we've now done hundreds of patients, and in entry, we find that the von Willebrand factor pattern is multimer. Multimer is variable. We find, in fact, very few patients with very large molecular weight von Willebrand factor multimers. And in our subsequent assays carried out with Dr. Tsai in New York, we find that the metalloprotease, this enzyme that is inhibited, is not always reduced. And when it is, the inhibitor count appears to die with the platelet counts.

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DR. ROCK:

Now, this, again, is a slide showing multimer patterns. And what we have here are the patterns from six different patients with the normal plasma control here and the platelet control with the larger forms of the multimers shown here.

But what you see is that really, with the possible exception of this patient, they are not identical to the platelet control with the larger forms of the protein present. And there is no particular overall increase in these multimers.

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DR. ROCK:

In fact, overall, if we look at the pattern of multimers and the responders, the patients who respond to plasma exchange, those that relapse and those patients that die, in terms of the absolute pattern we don't see anything that is predictive; if anything, you would see the patient that relapsed has fewer of the higher molecular weight forms than does the one that responds.

So in our studies we've not found any multimer patterns have been particularly predictive or, for that matter, useful.

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DR. ROCK:

This shows you now the protease assay on a series of TTP patients. In this column, you have normal von Willebrand factor, which is not digested; in the other columns you have von Willebrand factor which has been exposed to the patient's plasma, in other words, with the enzyme or the inhibited enzyme present to see what the effect on reducing the von Willebrand factor is.

And I think it's clear here that there's a great pattern and that they are certainly not all the same. And yet in this series the majority of patients were seen within a few days of presentation of disease at the most.

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DR. ROCK:

This is an assay for protease activity in cryosupernatant plasma. And I have it here simply to show you that using cryosupernatant plasma would be of benefit from the point of view that it contains the same amount of protease activity as does plasma. So there's no reduction in the concentration of the protease in the cryosupernatant plasma.

Slide 31: ROLE OF PLATELET ANTIBODIES IN TTP

CANNOT FIND 31

Slide 32: MULTIPLE ANTIBODIES IN TTP WESTERN BLOT

DR. ROCK:

What we have found in our series of 135 patients is that there are multiple antibodies in patients with TTP by protein blotting. And this is a technique in which the patient's plasma is run out and then tested to see whether antibodies are present or not. And these antibodies would be directed against different parts of the platelets because we use platelet lysate to run these protein blocks.

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CANNOT FIND 33

Slide 34: GPIV (CD36; Mr 88,000)

DR. ROCK:

Now, glycoprotein four is found both on platelets and the endothelial cells as an antigen on the surface of both of these cells. It's coupled with protein tyrosine kinases; in other words, it has something to do with signaling within these cells. And it is thought to be the collagen receptor on platelets.

Slide 35: GPIV (CD36; Mr 88,000)

CANNOT FIND 35

Slide 36: Untitled

DR. ROCK:

Most recently, in considering directions for therapy in Canada, the Canadian Apheresis group is considering carrying out a study in which we would look at cryosupernatant plasma versus solvent detergent treated plasma rather than fresh frozen plasma.

**Slide 37: CANADA
TTP/HUS**

CANNOT FIND 37

Slide 38: SDP

DR. ROCK:

Solvent detergent treated plasma is licensed in the United States and Canada. It is a screened tested pooled plasma product. It is pooled from 2,500 donors, on average. It is then treated with a combination of solvents and detergents with the purpose of inactivating lipid envelope viruses. It's then recycled off into 200 ml volumes and made available, then, for transfusion purposes.

Slide 39: PLAS+SD features

DR. ROCK:

Now, as I said, it inactivates lipid enveloped viruses. Now, this includes the AIDS virus, Hepatitis B, HTLV and Hepatitis G virus. The product is pooled and as a result gives us consistent levels of coagulation factors. It is filtered so it's leukocyte and bacteria free.

It has an interesting advantage or disadvantage, depending on your point of view, in that it does not contain the higher molecular rate forms of von Willebrand factor. These appear to be removed by the filter during the processes.

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DR. ROCK:

And in looking at these in our lab what we have is a solvent detergent treated plasma from a group AOB and AB, compared to our normal plasma pool which shows you the higher molecular rate forms of von Willebrand factor multimer. And you will notice that there is a consistent decrease in these in the solvent detergent treated plasma. So, again, a reasonably good rationale for the use in TTP/HUS.

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DR. ROCK:

Our study end point, I will just briefly go through the study now so you have some idea of what it is we intend to do. Our primary end point is survival at six months with a number of secondary considerations, complete response rate and disease free survival.

Slide 42: Untitled

DR. ROCK:

This is the algorithm which you will recognize as pretty similar as the one of the study that is currently ongoing.

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DR. ROCK:

Our subsequent therapy at nine days depends on the initial response and I will just quickly go through this.

Slide 44: This slide intentionally left blank

DR. ROCK:

We will have complete responders,...

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DR. ROCK:

... partial responders, ...

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DR. ROCK:

... non-responders, and all of those patients will receive some form of tapering or other therapy.

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DR. ROCK:

In considering the statistics on the study and the number of patients that we need to have, it was based on the fact that fresh frozen plasma, solvent detergent treated plasma, are considered to be equivalent in the therapy of liver disease.

So, in fact, it actually could have the same mortality of fresh frozen plasma and TTP, which is 20 percent if, in fact, those multimers -- the lack of the molecular weight multimers are not a critical part and the critical part of treatment is simply replacing protease.

Slide 48: SAFETY ISSUES

DR. ROCK:

And the simple fact of the matter is we don't have answers to those questions, that's clearly why we're doing the study, but there are a number of safety issues around all of this. Just think of this, we're putting liters and liters and liters of fluid in the people. So, of course, we want to know, overall, what are the relevant rates of reaction. Are we transmitting any viruses?

Slide 49: This slide intentionally left blank

DR. ROCK:

This solvent detergent treated plasma has an ability to eliminate the lipid envelope viruses but not those that don't have a lipid envelope. And there is some suggestion that because it's relatively low in one plasma protein, protein S, which is an anticoagulant protein that there could be an added risk of thrombosis.

All of those factors we will be looking at in our study. This is the protein incubator and it simply shows here that the protein S is relatively lower in the solvent detergent treated plasma.

Slide 50: THIS STUDY WILL:

DR. ROCK:

So hopefully, this next study we're going to do will resolve the best therapy, determine availability of the blood products (in Canada, there are problems with our blood product supply) and hopefully assist us in the resolution of disease factors because we will continue to look at von Willebrand factor levels, multimer patterns, and protease activity.

Slide 51: CONCLUSIONS

DR. ROCK:

In concluding my talk this morning I would like to remind you that we have quite effective therapies now in TTP/HUS, but it's necessary to start those therapies very early, the minute we hear of the patient with this disorder.

Nonetheless, it continues to have significant mortality, a death rate of 20 or 10 percent in people who are previously quite healthy, suffer acute insult, and develop a microangiopathy is not acceptable in today's world. We have to work very hard at that to resolve this. I will remind you that relapse occurs in 35 percent of the cases and the occurrence of that relapse interval is variable. So patients have to be involved in self-monitoring and work with their doctor to let them know of changing signs. Thank you.