Modified Thrombin Generation Assay: Application To The Analysis Of Immunoglobulin Concentrates

Corresponding Author:
Dr. Herbert Koenig,
Scientist, Paul Ehrlich Institute, Paul Ehrlich Str. 51-59, 63225 - Germany

Submitting Author:
Dr. Herbert Koenig,
Scientist, Paul Ehrlich Institute, Paul Ehrlich Str. 51-59, 63225 - Germany

How to cite the article:

Competition Interests:
The authors declare they have no competing interest
Modified Thrombin Generation Assay: Application To The Analysis Of Immunoglobulin Concentrates

Author(s): Grundmann C , Kusch M , Keitel S , Hunfeld A , Breitner- Ruddock S , Seitz R , Koenig H

Abstract

Intravenous immunoglobulins cause thromboembolic events at a frequency of 2-3%. Complications of this nature can be life threatening. Nevertheless, a test for thrombogenicity such as non activated partial thromboplastin time is not mandatory for these drugs. As a result of recent incidents we have performed experiments to evaluate a modified thrombin generation test for its ability to detect minute amounts of thrombogenic impurities in intravenous immunoglobulin preparations. We were able to show that this modified thrombin generation test is more sensitive than the Ph. Eur. assay for activated coagulation factors.

Introduction

Human plasma derived medicinal products are essential for the treatment of many disorders, hereditary or acquired. Examples are coagulation factor VIII and factor IX (FVIII, FIX) concentrates for the treatment of haemophilia A and B (1), prothrombin complex concentrates (PCC) for the reversal of oral anticoagulation (2), intra venous immunoglobulins (IVIG) for the treatment of immune deficiencies (3), or albumin for the treatment of e.g. severe burns or liver failure (4).

In the past, certain products have been found to be associated with thromboembolic events (TEE) of which FIX concentrates and PCC have been prominent examples (5-8). Therefore, a thrombogenicity test (non activated partial thromboplastin time, NAPTT) must be passed before batch release of these products is granted. In general, this test is performed by adding the substance to be investigated to normal citrated plasma, followed by addition of phospholipids and calcium, and determining the time clotting occurs. An alternative in vitro test, the thrombin generation test (TGT) is increasingly being explored for detecting thrombogenic agents in plasma derived products. Typically, the TGT is performed by mixing citrated plasma with the substance to be investigated, adding phospholipids, minute amounts of tissue factor, and a specific, slow reacting fluorogenic peptide substrate for thrombin (9-11). Thrombin formation is then initiated by the addition of calcium and monitored with a fluorescence photometer. This test has the advantage of giving information on the amount of thrombin formed, and the time it takes to reach peak levels of thrombin. These events continue after the time of clotting, the result obtained by NAPTT. Under suitable conditions, the effect of inhibitors or imbalances in clotting factors on the clotting cascade may also be determined (12, 13). Thus, it appears the TGT is able to detect modulators and activators of the coagulation cascade.

In plasma used for NAPTT and TGT, preactivated factors (FXIIa and FXIa) of the coagulation cascade are already present in conventional citrated plasma before start of the reaction due to inevitable events during blood collection. In the NAPTT this results in typical clotting times of the citrated plasma of 200-350 seconds. This clotting normally occurs at a time when only ~5% of the prothrombin is converted to thrombin, while the main thrombin burst is yet to come. In the TGT, the moment of clotting is normally invisible, but the time to the corresponding thrombin concentration may be derived from the TGT curve when suitable calibrators are used (14, 15). The clotting time in NAPTT, and the time to maximum thrombin in a thrombin generation experiment are also influenced by the initiator concentration employed. To obtain maximum sensitivity, tissue factor concentrations in a typical TGT have been reduced to the low to sub picomolar range (16, 17).

Corn trypsin inhibitor (CTI) may be added to blood already during collection in order to inactivate FXIIa specifically (18). This is to prevent FXI activation and results in FXIa free or at least FXIa poor plasma, as this factor is known to play an important role in the amplification of the clotting cascade (19).

Recently, we were confronted with the need to detect trace amounts of coagulation activators in IVIG preparations as some of these preparations had apparently caused thromboembolic complications in patients. It was thought that a system based on the TGT, which we have applied in the past for the analysis of certain coagulation factor concentrates (10, 13) might be suitable. IVIGs normally do not contain high concentrations of coagulation factors due to their manufacturing procedure, but eventually contain traces of FXI and/or its activated form FXIIa (20).
decided therefore to modify our established TGT with the intention to detect even minor quantities of potential activators such as FXIa in these preparations. Since traces of FXIa are expected to be present in conventionally prepared citrated plasma, we considered it prudent to use plasma free of FXIa. In addition, we considered it useful not to add any exogenous activator to this system but make the initiation of coagulation absolutely dependent upon the substance to be investigated. We hereby report the outcome of our study and possible implications on the evaluation of IVIGs (and perhaps other biomedicines).

Materials & Methods

Materials
Blood collection tubes were from Sarstedt (Nuembrecht, Germany). CTI and FXIa were purchased from Calbiochem (Merck Biosciences, Darmstadt, Germany). Cephalin (phospholipid component of PTT reagent) was from Roche Diagnostics (Mannheim, Germany). Phospholipids containing tissue factor (Innovin) was from Siemens Healthcare Diagnostics (Eschborn, Germany). The fluorogenic thrombin specific peptide substrate Boc-Val- Arg- AMC (B-VR-AMC) was obtained from Bachem (Weil am Rhein, Germany). All other chemicals were of the highest grade purity available from different suppliers. Microplates were from Nunc (Wiesbaden, Germany), the fluorescence photometer (Fluoroscan Ascent FL) was from Thermo electron (Dreieich, Germany). The BCS-XP coagulation analyzer was from Siemens Healthcare Diagnostics (Eschborn, Germany). IVIGs and albumin- solutions represent the spectrum of preparations submitted to our institute for batch release control.

Methods
Plasma was obtained from healthy individuals by clear venepuncture and carefully drawing nine volumes blood into one volume 106 mM sodium citrate supplemented with CTI in a final concentration of 100µg/ml. After centrifugation for 20 minutes at 800xg at room temperature, the upper half of the supernatant was withdrawn and centrifuged again at 4000xg for 20 minutes at room temperature. Again, the upper half of the supernatant was collected and frozen in small aliquots at -80°C. TGT was performed in triplicates according to published procedures (10, 15) with modifications as follows: 6.25 µl of a 5% solution of the IVIG preparation to be investigated was added to 25 µl Plasma obtained as described above. Human albumin solutions were included in some experiments as control, because one IVIG- preparation is on the market which contains high concentrations of albumin. The reaction mixture was made up to 50 µl by adding buffer containing Cephalin and the substrate B-VR-AMC. After 5 minutes preincubation at 37°C, the reaction was started by adding 50 µl prewarmed CaCl2- solution in buffer. Thrombin generation was monitored for 1 hour by measuring the developing fluorescence every 20 seconds. A blank curve (containing all ingredients plus EDTA in excess to calcium) was subtracted from all measurements. A dilution series of FXIa (and in some experiments tissue factor) served as calibrator.

NAPTT was essentially performed as described in the general chapter “2.6.22. Activated coagulation factors” of the Pharmacopoeia Europaea (Ph Eur). Briefly, 30 µl human virus inactivated pooled plasma (Octaplas LG, Octapharma, Dessau, Germany) were mixed with an equal volume of Cephalin. After 60 s at 37°C, 30 µl of 1:10 and 1:100 sample dilutions in Tris buffer pH 7.5, and 30 µl of 25 mM CaCl2 were added and the clotting time measured. Assays were performed using the BCS-XP coagulation analyzer (Siemens Healthcare Diagnostics).

Results

Thrombin generation in plasma prepared with and without CTI
As plasma collected in presence of CTI is not commercially available, we compared the influence of CTI on thrombin generation in an experiment in plasma obtained from a healthy donor. The plasma was prepared in presence and absence of CTI (100µg/ml), respectively. The reaction was triggered by addition of tissue factor (0- 5 pM final concentration). The result is presented in illustration 1. With increasing tissue factor concentrations the time to peak shortens in plasma prepared without CTI. However, the amount of thrombin generated is not significantly influenced in these plasmas. As has been reported already by other groups, at high tissue factor concentrations there is only a limited difference in TGT between plasmas prepared with and without CTI, whereas at lower tissue factor concentrations this difference becomes more pronounced (22, 23). This effect is most likely explained by the inhibition of activated FXII by CTI, and thus avoidance of FXI activation during blood collection.

Thrombin generation in plasma with FXIa as the trigger
The result above suggests that plasma prepared in absence of CTI contains enough FXIa to trigger the coagulation cascade. This prompted us to examine the
effect of FXIa as initiator of thrombin generation in plasma prepared in presence of CTI without tissue factor addition. The result of this experiment is shown in illustration 2. It appears that triggering the reaction by FXIa results in generation of a constant amount of thrombin when a certain threshold level of FXIa (~1 pM) is exceeded. On the other hand, the time to peak (TTP) varies with FXIa concentration over the entire range tested. This range was determined in preliminary range finding experiments with selected IVIG- concentrates (including the product which caused TEE as well as products from different suppliers which did not cause complications). Illustration 3 shows a good correlation between FXIa-concentration and TTP.

**Influence of IVIGs and albumins on thrombin generation**

Using CTI treated plasma we have attempted to answer the question: Do IVIGs or some albumin solutions available on the German market contain factor(s) able to trigger the coagulation cascade? Solutions to be investigated were adjusted to a concentration of 5% (based on the labelling). These samples were then added to CTI- plasma and the thrombin generation experiment started. As shown with purified FXIa as the trigger (illustration 2), we observed differences in the time to peak in the experiments with IVIG concentrates and a decrease of peak height only in the IVIG and albumin preparations showing very long lag times (~20 minutes). A comparison of IVIGs from different manufacturers is presented in illustration 4. All IVIGs from the manufacturer who produced the preparation which caused TEE (manufacturer A) show a similar behaviour of pronounced peaks. However, the batches in this group differ significantly with respect to the lag time. In contrast, all products tested from the other manufacturers show strikingly longer time to peak and a remarkably reduced peak height.

**Influence of IVIGs and albumin on NAPTT**

To compare the results obtained by the modified TGT with the established NAPTT, we tested the IVIGs investigated above and albumins by NAPTT. These results are summarized in illustration 5 together with results obtained by TGT (expressed as TTP). Three groups can be identified from this analysis: One group (albumins and IVIGs from competitors) is clustered around 250 seconds as determined by NAPTT or 40 minutes by TTP. A second group (all from manufacturer A) shows clotting times between 220 and 270 seconds and 15 to 25 minutes in TTP. The third group (also from this manufacturer) fails the test for activated clotting factors NAPTT (less than 150 seconds). This clotting time corresponds to 10 minutes or less in TTP.

**Discussion**

In a conventional clotting experiment such as partial thromboplastin time (PTT) or NAPTT one measures the time a sample takes to clot. This is only part of the relevant information as several events triggered by initiation of the clotting cascade are still to follow. During the last decade, thrombin generation measurements have become more and more attractive, as it could be shown that in the course of a TGT, information is gathered of events even after clotting has occurred. Some examples are reviewed by Hemker et al. (9). Despite many merits TGT has earned, this test is not employed as a routine clinical test, probably because of poor standardization (24, 25).

Since we wanted to preclude any preactivation, we avoid using vacuum containers for blood collection and prefer cautious manual aspiration (15). The risk of activation of clotting factors during blood harvesting is further reduced by including CTI as inhibitor of FXIa. Principally, the use of FXI depleted plasma could provide a means to prevent FXIa formation. However, as long as the initiator of coagulation in the sample to be tested is unknown, we consider it more appropriate not to exclude molecules from the analysis which may be needed for downstream events. FXI is considered to play an essential role during amplification mechanisms in the clotting cascade which we do not believe should be left out when assessing the whole process of coagulation. These thoughts led us to a modification of the TGT based on plasma prepared in presence of CTI and the TGT performed without tissue factor addition. For the special purpose of detecting thrombogenic components in IVIGs or albumins, we omitted tissue factor as initiating reagent, since this molecule could mask triggering effects caused by components in the sample under investigation. This assumption seems justified when CTI inhibited plasma is compared to uninhibited plasma (illustration 1). CTI uninhibited plasma may contain enough trigger molecules (presumably FXIa) to initiate the coagulation cascade.

Although the agent in IVIGs responsible for triggering the coagulation cascade in affected patients and in our in vitro model is not known as at now, we have chosen FXIa as reference molecule for two reasons: i) FXI and presumably FXIa are known contaminants in IVIG-preparations (20). ii) FXIa shares a remarkable similarity with the unknown component in IVIGs in our modified TGT: TTP of 20 minutes or less are not
accompanied by increasing amounts of thrombin with either FXIa or IVIGs as initiator. This corresponds to \( \sim 1 \) pM FXIa and discriminates it from tissue factor (illustrations 1, 2, 3). As long as the exact nature of this molecule is not clarified we prefer to use the term “FXIa equivalents”.

What part do IVIGs play in our modified TGT? The answer to this question is given in illustration 4, where three representative batches produced by manufacturer A are compared to four batches from other manufacturers. The three batches from manufacturer A are selected from a total of 19 batches tested so far: the first batch represents the highest initiator concentration, followed by one with intermediate concentration, and the batch with the lowest concentration of initiator.

The NAPTT is mandatory for e.g. FIX- concentrates and PCC but not for IVIGs. This test was performed according to Ph. Eur. procedures with conventionally prepared citrated plasma without CTI (see methods). The comparison of the NAPTT and the TGT is shown in illustration 5. A clear contrast between samples from manufacturer A and samples produced by other manufacturers (IVIGs and albumins) is obvious only in TGT: One group (from manufacturer A) shows clotting times of 150 seconds or less compared to TTP of around 10 minutes. Another group (also from manufacturer A as well as from others) shows NAPTT clotting times of between 220 seconds and 270 seconds (the reference plasma clotted after 275 seconds). These samples are poorly differentiated by NAPTT but very well separated by TGT into two groups: one group with TTP of \(~20\) minutes (from manufacturer A) and the other with TTP of \(~40\) minutes (other manufacturers and blank plasma as the reference). It is evident that all products from manufacturer A contain measurable amounts (by TGT) of thrombogenic agents although at different concentrations.

Our results show that both tests, NAPTT and the modified TGT, are able to detect thrombogenic substances when a certain concentration is exceeded. This concentration is defined in the NAPTT as clotting times less than 150 seconds for PCC (corresponding to \(~50\) pM FXIa equivalents). Although TGT is not an official test for thrombogenicity, it is able to detect even lesser amounts of thrombogenic agents. Its kinetic evaluation allows a finely graded estimation of thrombogenic agents down to below 1 pM FXIa equivalents (illustration 3).

It would be of interest to study the effect of using CTI inhibited plasma in the NAPTT and find out if this test can be improved with respect to sensitivity analogous to TGT. This assumption appears justified as NAPTT does not involve trigger molecules for coagulation other than those contained in the sample under investigation. In this respect it is directly comparable to the modified TGT. We could well envisage that such a modification of NAPTT or a derivative of our modified TGT may become a substitute to the approved NAPTT in the future and may be applied to the analysis of other medicines such as IVIGs as demonstrated in this communication.

Abbreviation(s)

- TEE: thromboembolic events
- NAPPT: non activated partial thromboplastin time
- TGT: thrombin generation test
- FXI(a): (activated) factor XI
- CTI: corn trypsin inhibitor
- IVIG: intravenous immunoglobulins
- TTP: time to peak
- TTC: time to clot

Acknowledgement(s)

We thank Andrea Schroda and Sylvia Rosenkranz for expert technical assistance.

Authors Contribution(s)

Claudia Grundmann, Manuela Kusch, Stefanie Keitel performed the experiments.
Andreas Hunfeld, Susanne Breitner Ruddock, Rainer Seitz, Herbert König designed the research, analyzed and interpreted the results, and wrote the paper.

References

3. Knight AK, Cunningham-Rundles C. Inflammatory and autoimmune complications of common variable immune deficiency. Autoimmunity reviews 2006; 5: 156-159
16. Mann KG, Brummel- Ziedins K, Undas A, Butenas S. Does the genotype predict the phenotype?
Illustrations

Illustration 1

Influence of tissue factor and CTI on thrombin generation

Plasma prepared in presence of CTI (symbols) and plasma prepared in the absence of CTI (lines) was triggered by increasing amounts of tissue factor (TF) and TGT was performed as described in methods.

Continuous line, squares: 5 pM TF, disjointed line, diamonds: 1.25 pM TF, dotted line, triangles: 0.3125 pM TF, disjointed line with dots, circles: 0 pM TF.
Illustration 2

Influence of varying concentrations of FXIa on TGT

TGT was performed as described in methods with CTI treated plasma. FXIa (1000-0 pM) served as trigger. For clarity, only every second concentration is illustrated here (TTPs of the complete experiment are shown in Illustration 3).

- 500 pM FXIa: continuous line with triangles;
- 125 pM FXIa: continuous line with squares;
- 31.25 pM FXIa: continuous line with diamonds;
- 7.8 pM FXIa: continuous line with crosses;
- 1.95 pM FXIa: continuous line;
- 0.49 pM FXIa: disjointed line;
- 0.12 pM FXIa: dotted line;
- 0 pM FXIa: discontinuous line with dots.
Illustration 3

Influence of FXIa on TTP

TGT was triggered with FXIa (1000-0 pM) as in illustration 2. All tested FXIa concentrations are plotted vs. TTP in a double logarithmic plot.
Illustration 4

IVIGs as initiators of IIa generation

Selected IVIGs from manufacturer A and IVIGs from other manufacturers are compared with respect to their influence on TGT.

Continuous line with squares: IVIG from manufacturer A ("high activity"); continuous line with triangles: IVIG from manufacturer A ("low activity"); lines without symbols: IVIGs from competitors.
Illustration 5

Comparison of NAPTT and TGT

NAPTT was done according to Eur. Pharm. TGT was performed as described in methods. The times to clotting (TTC) obtained by NAPTT with the respective concentrates were plotted against their corresponding times to peak (TTP) obtained by TGT. Triangles: IVIGs from manufacturer A. Squares: IVIGs from several other manufacturers. Diamonds: albumin concentrates.
Disclaimer

This article has been downloaded from WebmedCentral. With our unique author driven post publication peer review, contents posted on this web portal do not undergo any prepublication peer or editorial review. It is completely the responsibility of the authors to ensure not only scientific and ethical standards of the manuscript but also its grammatical accuracy. Authors must ensure that they obtain all the necessary permissions before submitting any information that requires obtaining a consent or approval from a third party. Authors should also ensure not to submit any information which they do not have the copyright of or of which they have transferred the copyrights to a third party.

Contents on WebmedCentral are purely for biomedical researchers and scientists. They are not meant to cater to the needs of an individual patient. The web portal or any content(s) therein is neither designed to support, nor replace, the relationship that exists between a patient/site visitor and his/her physician. Your use of the WebmedCentral site and its contents is entirely at your own risk. We do not take any responsibility for any harm that you may suffer or inflict on a third person by following the contents of this website.
Reviews

Review 1

Review Title: Modified Thrombin Generation Assay: Application To The Analysis Of Immunoglobulin Concentrates

Posted by Dr. Peter Turecek on 22 Nov 2010 04:51:28 PM GMT

<table>
<thead>
<tr>
<th></th>
<th>Question</th>
<th>Rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Is the subject of the article within the scope of the subject category?</td>
<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>Are the interpretations / conclusions sound and justified by the data?</td>
<td>Partly</td>
</tr>
<tr>
<td>3</td>
<td>Is this a new and original contribution?</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>Does this paper exemplify an awareness of other research on the topic?</td>
<td>Yes</td>
</tr>
<tr>
<td>5</td>
<td>Are structure and length satisfactory?</td>
<td>Yes</td>
</tr>
<tr>
<td>6</td>
<td>Can you suggest brief additions or amendments or an introductory statement that will increase the value of this paper for an international audience?</td>
<td>Yes</td>
</tr>
<tr>
<td>7</td>
<td>Can you suggest any reductions in the paper, or deletions of parts?</td>
<td>No</td>
</tr>
<tr>
<td>8</td>
<td>Is the quality of the diction satisfactory?</td>
<td>Yes</td>
</tr>
<tr>
<td>9</td>
<td>Are the illustrations and tables necessary and acceptable?</td>
<td>Yes</td>
</tr>
<tr>
<td>10</td>
<td>Are the references adequate and are they all necessary?</td>
<td>Yes</td>
</tr>
<tr>
<td>11</td>
<td>Are the keywords and abstract or summary informative?</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Rating: 5

Comment:

General comments

The manuscript “Modified Thrombin Generation Assay: Application To The Analysis Of Immunoglobulin Concentrates” by Koenig et al describes the application of a standard laboratory test for the potential use to differentiate IVIG products that cause thromboembolic events from those which do not. The paper is of scientific relevance, and is well written. The methods are sufficiently described and the discussion and the conclusion are sound, but need to be partly re-written to better address the complexity of the topic.

The applied method is simple and clear. Apparently no calibrator was used nor was any effort undertaken to standardize the method by use of a standard reference plasma as a control. It is known that thrombin generation (TG) very much depends on the plasma that is used. Therefore if no calibration is performed there is not any information how reproducible the proposed assay is. The authors try to standardize the assay by subtracting a blank curve containing all ingredients but also EDTA in excess of calcium, thus, inhibiting any thrombin generation. This may be considered as a kind of calibration but is only relevant for one individual plasma sample. Therefore the method as described can only be used with the one normal plasma sample that is available to the laboratory that tries to reproduce the test. This test is, however, suggested for use of an objective comparison of products. Given the dependence on the availability and nature of a “normal” plasma sample it is impossible to transfer results from one laboratory to the other. This, by the way, had been recognized as a general issue of thrombin generation assays. The Thrombin Generation Assay Working Party of the ISTH SSC has taken up this issue but has not come to a conclusion yet although the effort is ongoing for years. While the internal as well as the laboratory standardization of the TG assay had always been a hurdle as the authors themselves acknowledge in the sentence on page 4, right column, paragraph 2: “Despite many merits TGT ....”, this kind of standardization for TG assays has not yet been properly addressed and it is still not foreseeable that reference values for these assays for any use will be ever available. The authors should include more thoughts around this in the discussion.

Adding corn trypsin inhibitor (CTI) is also an approach that is controversially discussed. This topic had been strongly debated during the last SSC meetings. CTI specifically inhibits FXIIa, and therefore was introduced into
TG assays to avoid an “artificial” contact activation during the blood taking that may falsify the results. As a result of CTI addition, peak thrombin is lower and TTP (time to peak) is longer in such plasma samples, especially when coagulation is triggered with very low (or no) TF (tissue factor). It is well known, that the lower the basic TG is, the higher is the sensitivity of the assay. This then allows detection of minor differences between samples. As it was shown in Illustration 5, there was a group of IVIGs, having a normal NAPTT, but slightly shortened TTP. The question is, whether these lots should also be considered as potentially thrombogenic or not? A major disadvantage of the TG assay is that, based on the current knowledge, it is impossible to define a threshold in thrombin generation that could be defined as an alert level for pharmaceutical products to cause thrombosis. This is again a matter of standardization that, provided the dependence on the normal plasma used, is currently not possible. The authors should address this adequately in the discussion.

The authors are against the use of FXI depleted plasma, because they do not want to exclude any factors that may play an essential role during amplification mechanisms in the clotting cascade and as such being potentially causative for the thromboembolic events seen with IVIGs (Page 4, right column, paragraph 3: “However, as long as the initiator of coagulation in the sample to be tested is unknown, we consider it more appropriate not to exclude molecules from the analysis which may be needed for downstream events”). However, when CTI is added this will exclude the interaction of plasma FXII with IVIG that contains substances such as prekallikrein, kininogen, complement factors, and others, which may potentially contribute to the mechanism of thrombogenicity of IVIGs. Addition of CTI as an inhibitor makes also impossible to directly detect FXIIa which may also be contained in IVIG products as a trace impurity. This is a contradiction and the authors should include a clear statement on their position and thinking on these considerations.

The authors also believe that even a very low amount of FXIa or FXIa-equivalent components in the low pM range could be considered as thrombogenic agents. Do the authors have any results on FXIa concentrations in IVIG products included in the study? It would be particularly interesting to know this for the samples shown in Illustration 5, where TTP in thrombin generation was affected, but the NAPTT was only very little. The authors should provide additional data to help interpretation of the results and should address this adequately in the discussion.

Specific comments

1) Title
The title should be changed. The authors do not only show thrombin generation and NAPTT results of immunoglobulin concentrates but also of commercial albumin products. Therefore I would suggest to modify the title as follows: “Modified Thrombin Generation Assay: Application To The Analysis Of Immunoglobulin And Albumin Concentrates”.

2) Abstract
The statement that “Intravenous immunoglobulins cause thromboembolic events (TE) at a frequency of 2-3%” is not referenced. This claimed frequency is higher than written in a recent publication from the Paul-Ehrlich-Institute where the authors are employed. This publication issued on September 20, 2010, upon suspension of the licenses of the immunoglobulin products Octagam 5% and Octagam 50 mg/ml describes the frequency of thromboembolic complications as “very rare events with a frequency of < 1 per 10 000 treated patients” (http://www.pei.de/nn_163024/DE/infos/fachkreise/am-infos-ablage/sik/2010-09-20-octagam.html). There have been no studies published that were designed to determine the incidence of TEs following IVIG, and/or to determine the root causes. (e.g., two-armed study with one arm w/o predisposing risks and one arm with predisposing risks or any similar strategy in a specific indication like PID).

The authors should either support this statement with appropriate literature references or replace it with a more
general statement such as:

"Thromboembolic adverse events are a known, infrequent complication associated with the administration of intravenous immunoglobulins, yet the incidence and cause of these events remains unclear."

3) Introduction

The last sentence of the introduction in brackets contains the extension “and perhaps other biomedicines”. This should be removed for the following reason. The assays used for measurement of IVIG and albumin had been previously used for other biomedicinal products. For example the use for the TG assay to determine the potency as well as to measure clinical samples, can be considered as an established method for clotting factor concentrates like FVIIa and FVIII but also for heparin only to name a few examples. Therefore I would suggest modifying the last sentence as follows: “We hereby report the outcome of our study and possible implications of the evaluation of IVIGs and albumin”.

4) Materials & Methods

Paragraph: “Materials”:

I understand that the authors working for a regulatory agency cannot disclose product names that directly relate to their results. Nevertheless it would be of relevance for the reader to understand which IVIG products had been included in the study. Therefore I would suggest to include a list of the brand names of all IVIG and albumin products that had been tested. Of course there is no need to specify which is the product the authors call “from Manufacturer A”.

5) Discussion

Page 5, left column, paragraph 4:

Sentence “A clear contrast between samples from manufacturer A …” should be changed to “A clear difference between samples from …”.

Page 5, left column, paragraph 4:

The authors say that there is a difference in clotting times between 220 and 270 seconds for one group of IVIG products for manufacturer A that were tested and they also show with their results in Illustration 5 that there is more pronounced difference in TPP than in TTC. However, there is a difference in the NAPTT time to clotting that will be recognized by the assay. Therefore it cannot be concluded that NAPTT cannot be used at all and I would therefore suggest to weaken the statement. The authors themselves speculate around improvements of the NAPTT assay which makes it more sensitive towards FXIa or related coagulation activators. Therefore the use of the NAPTT should not be precluded for the future. The big advantage of the NAPTT over the thrombin generation assay is that it gives a clear readout that can be easily compared to a control figure which is the time to clotting.

Page 5, right column, paragraph 3:

As already indicated above the last sentence should be modified as follows, due to the very speculative comment on “other medicines”: “We could well envisage that such a modification of NAPTT or a derivative of our modified TGT may become a substitute to the approved NAPTT in the future and may be applied to the analysis of IVIG and albumin products as demonstrated in this communication.”

6) Illustrations

Illustration 3:

A coefficient of correlation should be given to better understand the value of the results. This needs to be included in the legend to illustration 3 as well as on page 4, paragraph 2.
Competing interests: Full time employee of Baxter Innovations GmbH, Vienna, Austria; Baxter is producing and marketing IVIG products

Invited by the author to make a review on this article? : No

Experience and credentials in the specific area of science:
Professional experience with development and use of assays to measure thrombogenicity of pharmaceutical products,

Own publications on thrombin generation assays used to assess hematological disorders.

Long term experience with plasma fractionation and plasma product development including IVIG products.

Publications in the same or a related area of science: Yes


Disclaimer

This article has been downloaded from WebmedCentral. With our unique author driven post publication peer review, contents posted on this web portal do not undergo any prepublication peer or editorial review. It is completely the responsibility of the authors to ensure not only scientific and ethical standards of the manuscript but also its grammatical accuracy. Authors must ensure that they obtain all the necessary permissions before submitting any information that requires obtaining a consent or approval from a third party. Authors should also ensure not to submit any information which they do not have the copyright of or of which they have transferred the copyrights to a third party.

Contents on WebmedCentral are purely for biomedical researchers and scientists. They are not meant to cater to the needs of an individual patient. The web portal or any content(s) therein is neither designed to support, nor replace, the relationship that exists between a patient/site visitor and his/her physician. Your use of the WebmedCentral site and its contents is entirely at your own risk. We do not take any responsibility for any harm that you may suffer or inflict on a third person by following the contents of this website.