Thank you very much.
Good morning ladies and gentlemen. I would like to focus your interest within the next ten minutes on a new method for the removal of the protein-bound uremic toxins that Professor Van Holder mentioned some minutes ago.

Slide 3

Introduction

- Increased cardiovascular mortality and morbidity of CKD patients
- Hydrophobic uremic toxins involved
- Less efficiency in removal
- Accumulation in blood of CKD-patients

Professor Van Holder has already talked about the impact of these protein-bound uremic toxins and we all know that there's an increased cardiovascular mortality and morbidity in our CKD patients. As already mentioned some minutes ago, the hydrophobic uremic toxins, which are bound to proteins in our patients, are obviously involved in this process. The current problem with our therapy is, as already mentioned by Raymond Van Holder a few minutes ago, that we are not able to remove the large amount of these uremic toxins by our conventional hemodialation or hemofiltration methods. Therefore, these uremic-bound proteins accumulate in our patients dramatically. This is one of the molecular structures of these substances and in these cases this is the molecular structure of phenylacetic acid.
The alchemist already knew that "similia similibus solvuntur" means 'like dissolves like'.

However, we still try to remove hydrophobic uremic toxins by a physiologic salt solution and this does not work very well as we know from our different studies.
Therefore, we set up the hypothesis that on the one hand the protein-bound hydrophobic uremic toxins have a strong impact on the progression of cardiovascular diseases in our patients. We set up the hypothesis in this study that adsorbents are more effective for the removal of these protein-bound uremic toxins than our hemofiltration solution.

Characteristic protein-bound uremic toxins

These are some examples of protein-bound uremic toxins and Professor Van Holder has already showed the molecular structure of indoxyl sulfate shown here and para-cresyl sulfate shown here. This is the third characteristic protein-bound uremic toxin, this was already mentioned, phenylacetic acid.
This part of these uremic toxins is hydrophobic, a phenol group and a methyl group are the hydrophobic parts of these protein-bound uremic toxins and because of this hydrophobic part, the proteins are bound slightly in our patients to albumin.

However, the second part of the molecule is hydrophobic and this is an anionic group for example in cases of indoxyl sulfate, the sulfate group, the sulfate group for cresyl sulfate and the acid group in the case of phenylacetic acid. That means our protein-bound uremic toxins have two different groups: a hydrophobic part, a hydrophilic part and the hydrophilic part is most unlikely anionic.
Because of these chemical characteristics of the uremic toxins, we set up a new method for the fractionated plasma separation and adsorption device shown here. This device works with a hydrophobic adsorber and a cationic adsorber. The hydrophobic adsorber interacts with hydrophobic groups and the cationic adsorber interacts with anionic groups. The plasma from the patient is separated by a polysulfone filter first of all and the plasma then interacts first of all with the hydrophobic adsorber and then with the anionic changer and after this the cells in the plasma are mixed again.
and afterwards, the blood of the patient is filtrated by a hemofiltration dialysed unit.

**Slide 12**

**Design of *in-vivo* study**

**Separation of protein-bound uremic toxins by adsorptive techniques**

- **Study design:**
  - *study group*: 5 CKD stage 5D patients; single FPSA treatment; 14 days follow up
  - *control group*: 5 CKD stage 5D patients; regular HD treatment; 14 days follow up

We set up recently a small study for the separation of these protein-bound uremic toxins by this technique.

**Slide 13**

**Design of *in-vivo* study**

**Separation of protein-bound uremic toxins by adsorptive techniques**

- **Study design:**
  - *study group*: 5 CKD stage 5D patients; single FPSA treatment; 14 days follow up
  - *control group*: 5 CKD stage 5D patients; regular HD treatment; 14 days follow up

We included in the study group 5 CKD patients stage 5 and treated these patients with this adsorption unit and in the control group used again 5 CKD patients and treated these patients with regular HD. The follow-up time was up to 14 days.
These are the clinical and biochemical characteristics of the patients included in the two different groups and you can see that there were no real significant differences in clinical and biochemical parameters in these patients.

### Slide 15

**Fractionated plasma separation and adsorption device**

This is a characteristic -gram of the plasma before and after the treatment with this adsorption unit. You can see here on the x-axis the retention time and on the y-axis the UV absorption. I have labelled the UV absorption of the phenylacetic acid, indoxyl sulfate and para-cresyl sulfate and used an internal standard. You can see here that the concentration of these 3 characteristic uremic-bound proteins significantly decreased after the treatment shown here.
We quantified the concentration of these different uremic toxins within the dialysis session and you can see here the concentration for example of phenylacetic acid during the treatment. You can see here the curve for the HD group and here the curves of the group treated with a new adsorber technique. You can see that we were able to increase the removal rate, in this case for phenylacetic acid, within these 240 minutes significantly.

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**Indoxyl sulfate and cresylsulfate concentrations**

These are the results of indoxyl sulfate and cresyl sulfate and the results are comparable. We dramatically increased the removal rate for indoxyl sulfate and cresyl sulfate during this treatment period.
The different uremic toxins bind to different adsorbers. One of the adsorbers was a hydrophobic adsorber and phenylacetic acid obviously binds to this hydrophobic adsorber more than to the anionic changer. Indoxyl sulfate binds to both adsorbers, to the hydrophobic adsorber and to the cationic adsorber and para-cresyl sulfate mostly binds to the cationic adsorber. The different adsorber characteristics of these different uremic toxins are caused by two different points. On the one hand, it is caused by the affinity of the different uremic toxins to the adsorber that means the affinity of, for example, phenylacetic acid to the hydrophobic adsorber or to the cationic adsorber or the same for indoxyl sulfate. This is on the one hand. On the other hand the adsorption characteristics are caused by the plasma concentration and the plasma concentrations of phenylacetic acid, indoxyl sulfate and para-cresyl sulfate are shown here and you can see that the concentration of these different uremic toxins are dramatically different. The concentration of phenylacetic acid is in the millimolar range and the concentration of for example, indoxyl sulfate is in the micromolar range. That means the removal rate of these uremic toxins by the different adsorbers depends on the one hand on the affinity of the uremic toxins and on the other hand on the plasma concentration of the uremic toxins.

### Efficiency of HD vs. FPSA

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<th>HD</th>
<th>FPSA</th>
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<tbody>
<tr>
<td>Δc\text{PAA} (mmol·L\textsuperscript{-1})</td>
<td>0.48 ± 0.20</td>
<td>0.99 ± 0.16</td>
</tr>
<tr>
<td>Δc\text{IDS} (mmol·L\textsuperscript{-1})</td>
<td>0.09 ± 0.03</td>
<td>0.21 ± 0.05</td>
</tr>
<tr>
<td>Δc\text{pCR} (mmol·L\textsuperscript{-1})</td>
<td>0.13 ± 0.06</td>
<td>0.23 ± 0.07</td>
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We quantified overall and compared the concentration of these toxins and you can see here the results. You see here the results of the removal rate of phenylacetic acid, indoxyl sulfate and para-cresyl sulfate for the HD group and here for the group treated with the adsorber. More or less, we were able to demonstrate that we could increase the removal rate for these toxins of about 100%.

On this slide and the next slide, you can see the concentration curves during the treatment and after the treatment. This first part represents a concentration during the treatment and you can see there is a strong decrease of the concentration, for example, of phenylacetic acid in the adsorber group compared to the conventionally treated group. However, in the following days, up to 14 days, we were not able to detect a significant difference in the concentration of phenylacetic acid in the plasma.
These are the result of indoxyl sulfate and para-cresyl sulfate and the results are comparable. We see a stronger removal rate for these uremic toxins during the session shown here but after the session, we were not able to detect any concentration differences in the different study groups. That means the rebound rate of the toxins is too high to see a long-term effect of the removal by this adsorber technique if you use the method only once.

Clinical chemistry

We analysed different clinical parameters and compared the clinical parameters of the different groups, for example, we quantified the concentration of thyronine and different
concentration of different plasma proteins and we didn't detect any significant differences in these clinical parameters within the different groups.

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**Results**

- HD removes protein-bound, hydrophobic uremic retention solutes insufficiently
- FPSA efficiency treatment is higher than of conventional hemodialysis
- adsorber part of FPSA device has stronger impact on removal than hemofiltration part
- plasma concentrations reach initial levels within 3 days

To summarise, HD obviously removes the protein-bound hydrophobic uremic toxins not sufficiently, the treatment caused a higher removal of these uremic toxins than the HD method. We were able to show in this study that the adsorber part has a stronger impact on the removal than the HD part of this technique. However, the plasma concentrations reach initial levels within 3 days.

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**Conclusion**

- FPSA is suitable for treatment of CKD patients
- plasma concentrations reach initial levels within 3 days
- additional clinical studies are essential to validate the long-term effects of FPSA and impact on outcome
We concluded that the method is suitable for the treatment of CKD patients. The plasma concentrations however, reach initial levels within 3 days and therefore, additional clinical studies are essential to validate the impact of these methods by using this method more than once. We currently are performing a clinical study and within this clinical study, the patients are treated for 6 weeks, once per week with this adsorber technique.
Chairman: Thank you Professor Jankowski and now the paper is open for discussion.

Question: Thank you Joachim for your nice presentation, I really like your results. A couple of years we did the same type of experiments using the same device and one big difference between your results and our results is that we saw repeated occlusive thrombosis of the AV fistula in those patients. When looking for mechanisms, we observed a high percentage of loss of several pro- and anti-coagulant factors such as in protein C adsorbed on the anionic changer adsorbent. We don't have data on other glutamine containing domains, such as matrix-Gla protein but can you discuss something about the biocompatibility of this device?

Prof. Jankowski: Thank you very much for this question. I have already read your letter to the editor of the journal we published in this study and there are strong differences in your clinical study and in our clinical study and the difference is that we use heparin for the anti-coagulation of the patients and citrate and you only use citrate for the anti-coagulation. Perhaps this causes the differences because we don't see any or we don't have any of the problems that you described. There was no clotting on the adsorber, no clotting on the arms of the patient and so on and I have the impression that the reason for these differences between your mentioned experiment and our clinical study is the additional use of heparin during this treatment. The only problem we detected in the patient, in the first patient was that the patient was suffering from nausea and this was most likely produced by the strong decrease in the concentration of these uremic toxins during the session. Therefore, at that moment, in the second part of this clinical study, we decreased the velocity of the pump of the adsorbers and now the patient does not suffer from nausea at the moment. The second thing is at the moment we are analysing which components of the plasma binds to the different adsorbers, to the cationic and anionic adsorbers and as already discussed yesterday I guess, we are interested in which of the substances absorb to these different adsorbers. For example, the substances you mentioned. But this analysis is ongoing. But I think the difference between your experiment and our study was the additional use of heparin before that.

Question: I think you're right that you have to look at long-term data because it could be possible that if you do this several times consecutively, you still get a decrease but the other question is, what are the kinetics of what's happening there? Is this really a rebound phenomenon or is this a change in generation rate? So these are things that could be easily studied I think.

Prof. Jankowski: We don't know at the moment. We only know that we see this rebound effect within the three days after the treatment. The second step is that now we have to analyse what the kinetics are, it's only an idea that it's because of the rebound of the substances but we calculated the amount of uremic toxins in the patient. In common publications the amount of toxins, which are described in the literature, are in the range of 1 mg. The capacity of the adsorbers are not sufficient to really decrease these large amounts of toxins in the organs. It's only 1 mg of substances described in the literature and therefore, we have to use this adsorber technique more than once.

Chairman: Just one more question.

Question: Short question. Intestinal adsorption failed, could you comment on the difference
between this extracorporeal adsorption and intestinal adsorption which firstly gave us more hope because works in a preventive way?

Prof. Jankowski: As already mentioned by Ray some minutes ago, we don't know really why this AST 120 study failed. There could be differences, perhaps the amount of adsorbers used in the clinical study was not enough. As already mentioned the amount of the substances is very, very high and perhaps the amount used in this American clinical study using these adsorbers was not high enough and Raymond Van Holder already mentioned that there was a difference in the clinical studies performed in Japan and in the US. I don't know but as I heard this, I had the idea that perhaps the volume of the patient is different. The patients in Japan are smaller therefore, the overall concentration and the overall amount of uremic toxins are lower than in the US. Perhaps this is a problem because perhaps you have the capacity in the clinical study of Japan it's enough because of the lower volume of the patient and the lower amount of these substances. But this is just an idea but we know from our own calculation that there's a huge amount of uremic toxins we have to remove within these adsorbers and I have the impression that perhaps the amount used in the different studies was not different.

Chairman: Ok Professor Jankowski, thank you very much for your very interesting talk.