Markers and disease mechanisms in chronic kidney disease, transplantation and hypertension

Chairman: Henrik Birn

10:45-10:53

1. Urinary and serum calprotectin (S100A8/A9) levels after kidney transplantation.

Christoffer Borst, Lise Pedersen, Lars Meltholt Rasmussen, Martin Tepel.
Department of Nephrology, Odense University Hospital, Odense Denmark

Introduction: Calprotectin is an acute kidney injury marker, mainly secreted from neutrophils and monocytes as part of an innate immune response. Delayed graft function (DGF), defined as more than two times hemodialysis, is a major risk factor for long term renal outcome. We tested the hypothesis that serum or urine calprotectin levels may predict DGF after transplantation.

Methods: 158 patients were enrolled in the prospective study. Calprotectin was analyzed in 115 serum samples and 158 urine samples using fluorescent enzyme sandwich immuno assay. Urinary calprotectin-to-creatinine ratio and estimated glomerular filtration rates were calculated.

Results: 17 patients (11%) developed DGF. The urinary calprotectin-creatinine-ratio was significantly higher in recipients from deceased donor kidneys versus living donor kidneys (5,080 ± 919 versus 1,948 ± 288, p<0.0005). Recipients from deceased donor kidneys who developed DGF had highest calprotectin-creatinine-ratio (10,810±3,109). Serum calprotectin levels were similar in recipients from deceased donor kidneys versus living donor kidneys (8,374±942 versus 6,889±554). Recipients from deceased donor kidneys who developed DGF had highest serum calprotectin levels (16,180±3,442). Serum calprotectin levels were significantly associated with estimated glomerular filtration rate after 1, 2, 3, and 4 weeks (Spearman correlation, r=-0.335, r=-0.382, r=-0.379, and r=-0.375; respectively, each p<0.0001).

Conclusion: Elevated calprotectin levels are associated with DGF and worse renal allograft function within the first month after transplantation.
2. Comparison of an implantable Doppler probe and microdialysis for detection of gradual renal vein occlusion.

Chris Amdisen,1,2, Bente Jespersen,2 Ulla Møldrup,3 Anna Keller3

1. Department of Clinical Medicine, 2. Department of Renal Medicine, 3. Department of Urology Aarhus University Hospital, Denmark.

Introduction Vascular occlusion is a serious complication after kidney transplantation, often resulting in graft loss. This experimental study developed a model for stepwise reduction of renal venous blood flow. An implantable Doppler probe and microdialysis were evaluated for detection of vascular occlusion.

Methods In 20 pigs, implantable Doppler probes were placed on the renal artery and vein and a microdialysis catheter was placed in the renal cortex. An arterial flowprobe served as gold standard. Following two-hour baseline measurements, the pigs were randomised to stepwise venous occlusion, complete venous occlusion, complete arterial occlusion or controls.

Results All parameters were stable through baseline measurements. Glutamate and lactate measured by microdialysis increased significantly 30 minutes after a 2/3 reduction in renal blood flow. The implantable Doppler probe was not able to detect flow changes until there was total venous occlusion. Microdialysis detected changes in local metabolism after both arterial and venous occlusion, while the implantable Doppler probe could only detect vascular occlusions on the vessel on which it was placed.

Conclusions We developed a new model for stepwise renal venous blood flow occlusion. Furthermore, the first comparison of the implantable Doppler probe and microdialysis for detection of renal vascular occlusions was made. The implantable Doppler probe, would only detect flow changes after a complete occlusion, whereas microdialysis detected flow changes earlier, and could detect both arterial and venous occlusion. Based on these results, the implantable Doppler probe for detection of vascular occlusions cannot be recommended.

3. Renal arterial blood flow and glomerular filtration rate in chronic kidney disease

1Dinah Sherzad Khatir, 2Michael Pedersen, 1Bente Jespersen, 1Niels Henrik Buus

1Department of Renal Medicine and 2MR Research Centre Aarhus University Hospital, Aarhus, Denmark

Background: In chronic kidney disease (CKD) little is known about the relation between renal blood supply and glomerular filtration rate (GFR). We aimed to compare the relationship between renal blood supply determined by magnetic resonance imaging (MRI) in stable CKD patients and healthy controls.

Methods: 64 stable CKD patients (61±13 years) were compared with 24 age and sex-matched controls. GFR was measured as a standard 51Cr-EDTA clearance. RABF was estimated using MRI by a phase-contrast velocity-sensitive sequence and RPF was calculated as RABF x (1-hematocrit).

Results: GFR was 36±15 ml/min/1.73 m² in CKD patients and 97±23 ml/min/1.73 m² in the controls (P<0.001). Single-kidney RABF was lower in patients than controls (319 vs. 443 ml/min, P<0.001), however, when correcting for kidney volume, RABF was similar in the two groups (3.4 vs. 3.6 ml/min/cm³). Filtration fraction was 9% in patients and 18% in controls (P<0.001). GFR and total RPF correlated negatively in patients (β = -0.030, P = 0.005) but did not correlate in controls (β = 0.062, P = NS). The relation between RPF and GFR was significantly different for the two groups (P<0.05).
Conclusion: In CKD renal blood supply is considerably better preserved than GFR.

11:09-11:17

4. T-type channels Ca, 3.1 do not affect angiotensin II induced hypertension in mice.
Anne D. Thuesen¹, Boye L. Jensen¹, Pernille B. L. Hansen¹.
¹Institute of Molecular Medicine, Department of Cardiovascular and Renal Research, University of Southern Denmark, Odense, Denmark.

Background: Angiotensin and aldosterone are crucial mediators in the development of hypertension. T-type channels affect aldosterone production and clinical studies show that T-type calcium channel blockers lower aldosterone levels. Furthermore, angiotensin II is suggested to increase the expression of T-type channels and combined T- and L-type channel blockers lowers blood pressure to a greater extent that L-type blockers in hypertension. It was hypothesized that angiotensin II infusion led to an attenuated blood pressure increase and aldosterone response in mice deficient of T-type Ca, 3.1 channels.

Methods: Mean arterial pressure was measured with chronically indwelling catheters in response to a 1-week infusion of angiotensin II (30ng/kg/min) or vehicle. Renin and aldosterone plasma levels were measured with radioimmunoassay.

Results: Angiotensin II significantly increased in mean arterial pressure from 101±2 mmHg to 119±4 mmHg and 106±3 mmHg to 118.5±4 mmHg; Ca, 3.1 KO and Wt mice respectively with no differences between genotypes. Separating the sexes we found that the mean arterial pressure response to angiotensin II was attenuated in females compared with male mice in both groups. A tendency to an aldosterone level increase in response to angiotensin II was observed in both groups (Cav 3.1 KO from 68±22 pg/mL to 107±31 pg/mL and Wt 142±72 pg/mL to 235±60 pg/mL) with no difference between the groups. Renin was significantly lower in both groups (Ca, 3.1 278±54 10⁻⁵ GU/mL to 85±58 10⁻⁵ GU/mL and Wt 457±97 10⁻⁵ GU/mL to 69±2310⁻⁵ GU/mL) after angiotensin II infusion with no difference between the groups.

Conclusion: In summary, no difference in blood pressure response to angiotensin II infusion between Wt and Ca, 3.1 KO mice was observed. However, we saw an expected gender difference in response to angiotensin II. T-type Ca, 3.1 channels do not seem to play a role in angiotensin II mediated hypertension.
Glomerulonephritis and renal vasculitis - diagnosis, treatment and complications

Chairman: Jan Carstens

11:17-11:25


Wladimir M. Szpirt¹, MD, Elizabeth Krarup², MD, Martin Egfjord, MD, PhD¹.

¹Nephrology, Rigshospitalet, Denmark; ²Nephrology, Herlev Hospital, Univ. of Copenhagen, Copenhagen, Denmark

Background: Salama et al. published in cJASN (2013,Nov.15) results on 41 ANCA Associated Vasculitis (AAV) patients (pts) requiring dialysis on admission, who were treated with Plasma Exchange (PLEX), standard prednisolone 1 mg/kg/day (STOCS), I.V. CYC (IVCYC) (6-10 pulses given over 13 weeks; 7.5 - 12.5 mg/kg depending on age). Their mortality and dialysis dependency at 3 and 12 months were superior to and compared with MEPEX oral CYC (ORCYC) arm (2.5 mg/kg daily). The total doses of IVCYC given were 2.96-7.38 g, whereas MEPEX pts. received up to 15-16 g in cumulative CYC dose.

Methods: We analysed this report in connection to a subgroup of 39 HD pts -out of- 132 AAV pts referred to our centre between 2000-2010 and treated in 105 pts by 7 PLEX sessions, low ORCYC regimen (1.5 mg/kg/day < 65 years, 0.75 mg/kg/day > 65 years) and STOCS. Totally our pts received a estimated cumulative CYC dose of 4.75 g for older and 9.5 g for younger pts. Azathioprine or mycophenolate mofetil was given for maintenance of remission after 4 months.

Results: At 3 months 3 of IVCYC treated and 3 pts in our low ORCYC cohort died, 12 pts were dialysis dependent compared to our 8 low ORCYC pts. At 1 year 13 IVCYC pts remained dialysis dependent, whereas 13 of 39 our pts were on dialysis at 12 months. Patient survival in ORCYC cohort at 3 years was 72% (97% in no HD group) and 5 years 56% (87% in no HD group). Kidney survival in ORCYC cohort was 53% at 3 years (95% in no HD) and 40% (80% in no HD group) at 5 years. 11 of ICCYC pts had leukopenic episodes, 17 experienced infectious complications during 3 months of IVCYC therapy. This compared to 10 of our pts being leukopenic and 16 having infections during follow up. 5 relapses in IV CYC pts were observed compared to 12 relapses occurred during the 12 years of follow up in our pts. Furthermore no difference in observed maligncy can be reported (4 cases in ORCYC pts).

Conclusions: In our opinion low oral CYC induction regimen is not inferior to IV CYC in HD dependent AAV pts, when combined with PLEX and STOCS.

11:25-11:33

6. The Effects of Prophylactic Anticoagulation in Nephrotic Syndrome – a Retrospective Analysis.

Karen Marie Nykjær¹, Sarah Kelldal², Jon Waarst Gregersen³, and Henrik Birn⁴

¹Department of Medicine, Holstebro Regional Hospital; ²Department of Medicine, Viborg Regional Hospital; ³Department of Nephrology, Herlev University Hospital; ⁴Department of Nephrology, Aarhus University Hospital, Skejby

Background: Nephrotic syndrome (NS) is associated with an increased risk of thromboembolic complications. No randomized studies have evaluated the effect of prophylactic anticoagulation (P-AC) and the associated risks. Local guidelines at the Department of Nephrology, Aarhus University Hospital, recommend P-AC for all patients with NS and very low plasma albumin (<20g/l) using low molecular-weight heparin and/or warfarin.
The aim of this study was to examine bleeding episodes and thromboembolic events associated with P-AC, and to compare this with patients with NS not receiving prophylactic anticoagulation.

**Methods:** A retrospective follow-up study including patients with NS at the Department of Nephrology, Aarhus University Hospital and Department of Medicine, Viborg Regional Hospital from September 2006 to January 2012. Exclusion criteria were age < 16 years, anticoagulation for other reasons than NS, diabetic kidney disease, and renal replacement therapy including transplantation. Demographic and biochemical data as well as thromboembolic events and bleeding episodes were obtained by review of patient files.

**Results:** Seventy nine episodes of NS were included. Forty four received P-AC for a median of 26 days. Median follow-up time was 71 weeks. Four patients had a thromboembolic event (3 venous, 1 arterial). None of these patients received P-AC. Seven bleeding episodes were recorded. Five of these were in patients receiving P-AC, two of which required transfusion and one died in relation to the bleeding. Bleeding episodes were more likely to occur in membranous nephropathy.

<table>
<thead>
<tr>
<th></th>
<th>Anticoagulation</th>
<th>No-anticoagulation</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td>Episodes</td>
<td>44</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Sex, n (%) Male</td>
<td>26 (59)</td>
<td>13 (37)</td>
<td>NS</td>
</tr>
<tr>
<td>Age (years)</td>
<td>43,2 (16,7-78,4)</td>
<td>52 (22,3-83,9)</td>
<td>NS</td>
</tr>
<tr>
<td>Lowest plasma albumin (g/l)</td>
<td>15 (10-23)</td>
<td>20 (11-29)</td>
<td>0,0001</td>
</tr>
<tr>
<td>Plasma creatinine (μmol/l)</td>
<td>83 (37-547)</td>
<td>107 (43-208)</td>
<td>NS</td>
</tr>
<tr>
<td>eGFR (ml/min/1,73 m2)</td>
<td>91 (11-248)</td>
<td>61 (24-153)</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of treatment (days)</td>
<td>26 (1-350)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Anti-platelet treatment, n (%)</td>
<td>4 (9)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Bleeding episodes</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>• Major*, n (%)</td>
<td>2 (5)</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>• Minor, n (%)</td>
<td>3 (7)</td>
<td>2 (6)</td>
<td>NS</td>
</tr>
<tr>
<td>Thromboembolic events, n (%)</td>
<td>0</td>
<td>4 (11)</td>
<td>0,035</td>
</tr>
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</table>

Data represent number (percentage of total) or median (range). Groups were compared using Chi-square (categorical variables) or Mann Whitney U-test (continuous variables). *Major bleeding = requiring transfusion.

**Conclusion:** P-AC was associated with a significantly lower incidence of thromboembolic episodes; however, also a trend towards a greater incidence of potential fatal bleedings. A randomized study is needed to evaluate the benefits and risks of P-AC in NS.

11:33-11:41

7. **Anti-phospholipase a2 receptor antibodies in Danish patients with membranous nephropathy**

Trine Korsholm¹, Henrik Birn², Jon Gregersen³,⁵, Per Ivarsen²

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**Background:** Membranous nephropathy (MN) is a common cause of the nephrotic syndrome in adults. Circulating M-type phospholipase A2 receptor antibodies (anti-PLA2R) have been associated with idiopathic
MN (IMN) being present in 50-80% of patients with high specificity for this disease. This analysis evaluates the presence and changes in anti-PLA2R in Danish patients with MN.

**Methods:** The analysis included 29 prevalent and incident patients with biopsy-proven MN (21 (72%) IMN; 5 (17%) secondary MN (4 with autoimmune disease, 1 with cancer); 3 (10%) post-transplant), as well as 14 controls with proteinuria, and 25 healthy blood donors. All were analysed by indirect immunofluorescence (Anti-PLA2R IIFT, Euroimmun AG, Germany) for qualitative and semi-quantitative detection of anti-PLA2R.

**Results:** Among IMN patients 13/21 (62%) were anti-PLA2R positive (12 with active disease, 1 in complete remission). Eight patients were anti-PLA2R negative (4 with active disease, 4 in partial or complete remission). Anti-PLA2R was not detected in patients with secondary MN, in proteinuric controls, or in healthy blood donors. In 8 of 9 anti-PLA2R positive patients treated with calcineurin inhibitors or rituximab, consecutive testing revealed a decrease in anti-PLA2R titer which correlated with complete or partial clinical remission (proteinuria). The frequency of anti-PLA2R measurements was insufficient to determine whether serological remission occurred prior to clinical remission. In one patient anti-PLA2R became undetectable without clinical remission at 8 months follow-up.

**Conclusion:** In this small cohort of Danish patients with MN at various stages (active/remission, treated/untreated) we identified anti-PLA2R in 62% of IMN patients similar to previously reported observations (52-78%). Clinical remission was associated with a reduction in anti-PLA2R titer.

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11:41-11:49

8. **Rituximab treatment for children and adolescents with steroid- or cyclosporine-dependent nephrotic syndrome; 6 year single center experience**

Raymond Suffolk, Hanne Noergaard, Ida Maria Schmidt

*Department of Pediatrics and Adolescent Medicine, Copenhagen University Hospital, Rigshospitalet, Denmark*

**Background:** Rituximab (RTX) show promising results in pediatric steroid dependent nephrotic syndrome, however treatment protocols vary and there is still need for long-term follow-up data on safety and efficacy.

**Methods:** We performed a single centre retrospective study on 8 steroid dependent children/adolescents receiving Rituximab. All patients had minimal change disease on renal biopsy. The indications for Rituximab were relapses despite intensive immunosuppressive therapy, unacceptable adverse effects, lack of compliance, or to reduce sterioide/calcineurine inhibitor toxicity. Rituximab was initially given ones weekly 2-4 times. Subsequent infusions of Rituximab were given on CD20-cell recovery during 18 months. The median follow-up time post-RTX was 16.4 months (range: 7.8-58.2).

**Results:** Five patients remained in remission after Rituximab, three had in total 5 relapses. Before Rituximab (pre-RTX) there was a total of 104 relapses during 808 months of observation versus 5 relapses during 201 months of follow-up post-RTX (p<0.001). The median duration between relapses post-RTX was markedly longer than pre-RTX (22.4 months (range: 18.9-58.2) vs 10.2 months (range: 3.2-20.3)). All patients had their immunosuppressants reduced. At follow-up 3 patients still received some immunosuppressants. We found a significant average improvement of eGFR pre-RTX 82.5ml/min/1.73 m² to post-RTX 92.1ml/min/1.73 m² (p=0.001). No serious adverse events occurred.

**Conclusion:** Rituximab treatment for steroid dependent nephrotic syndrome in children and adolescents is effectively reducing the number of relapses, reducing the amount of immunosuppressive agents and in some patients induce long-term remission.
Dialysis and metabolism
Chairman: Bente Jespersen

11:49-11:57

9. Clearance of glucoregulatory peptide hormones in non-diabetic patients during haemodialysis and haemodiafiltration
Morten B. Jørgensen, Filip K. Knop, Thomas Idorn, Jens J. Holst, Mads Hornum, Bo Feldt-Rasmussen

Department of Nephrology, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark, Diabetes Research Division, Department of Internal Medicine, Gentofte Hospital, University of Copenhagen, Hellerup, Denmark, The NNF Center for Basic Metabolic Research, Department of Biomedical Sciences, the Panum Institute, University of Copenhagen, Copenhagen, Denmark

Background: Patients with end-stage renal disease have increased fasting concentrations and disturbed postprandial responses of several glucoregulatory hormones. These findings constitute current or potential targets in the treatment of diabetes. The aim of the present study was to evaluate removal of glucoregulatory peptide hormones during high-flux haemodialysis (HD) and high-volume haemodiafiltration (HDF).

Methods: 10 non-diabetic patients receiving chronic haemodialysis were examined with a standardised liquid mixed meal test one hour into an HD and an HDF. On a third, optional, examination day, the meal test was performed without concurrent dialysis treatment. Blood and dialysate samples for measurement of C-peptide, insulin, glucagon and the two incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) were collected repeatedly during the examinations.

Results: 10 participants completed the meal test during HD, 8 completed the meal test during HDF and 4 the optional meal test without dialysis. All plasma hormone concentrations declined during the first fasting hour for both dialysis modalities ranging from 24.8 to 47.0% (P<0.043). All hormones had a significant clearance ranging from 44.3 to 136.5 ml/min (P<0.013). The fractional appearance of hormone entering the utilised dialysate ranged from 16.6 to 60.0%. A tendency towards higher clearance during HDF was observed for all peptides, significant for C-peptide (P<0.001), insulin (P<0.001) and GIP (P<0.022). The average hormone concentrations following the meal test were lower during dialysis treatments (P<0.038).

Conclusions: Haemodialysis and haemodiafiltration significantly remove glucoregulatory peptide hormones in non-diabetic dialysis patients. These findings may affect the prescribed dose of present and future antidiabetic treatments in dialysis patients.

11:57-12:05

10. The effect of meals and insulin on fibroblast growth factor 21 in maintenance hemodialysis patients
Mark Reinhard, Jan Frystyk, Bente Jespersen, Else Randers, and Per Ivarsen

Department of Renal Medicine, Aarhus University Hospital, Aarhus, Denmark; Medical Research Laboratory and Department of Endocrinology and Internal Medicine, Aarhus University Hospital, Aarhus, Denmark; Department of Internal Medicine, Viborg Regional Hospital, Viborg, Denmark

Background: Fibroblast growth factor 21 (FGF21) regulates carbohydrate and lipid metabolism. In a recent trial, an FGF21 analog improved the lipid profile in patients with type 2 diabetes. We investigated the effect of 1) meal intake and 2) insulin infusion on serum FGF21 in maintenance hemodialysis (HD) patients.
Methods: In the meal study, 12 non-diabetic HD patients were randomly assigned to three 10-h lasting study days: 1) a non-HD day with one meal served at baseline, 2) a HD day with one meal served during HD, and 3) a HD day with two meals served during and after HD, respectively. Twelve healthy controls conducted an experiment identical to the non-HD day.

In the insulin infusion study, 11 non-diabetic HD patients were randomly assigned to receive a HD session with either: 1) no treatment, 2) glucose infusion, or 3) glucose-insulin infusion. Each experiment consisted of three periods: pre-HD (-120 to 0 min), HD (0 to 240 min), and post-HD (240 to 360 min). A meal was served at -120 min and infusions were administered from -120 to 240 min.

Results: Meal study: Fasting FGF21 levels were 23-fold higher in HD patients than in controls ($P < 0.001$). Postprandial FGF21 declined below baseline levels at all four study days ($P \leq 0.005$), but the reductions from baseline were significantly greater in controls ($P < 0.008$). Postprandial changes in FGF21 were inversely related with triglycerides ($P = 0.042$) and positively related with insulin-like growth factor binding protein-1 (IGFBP-1) ($P < 0.001$).

Insulin infusion study: Compared with no treatment days, glucose and glucose-insulin infusion prevented the postprandial reduction in FGF21 and resulted in higher FGF21 levels by up to $+25\%$ ($[+8; +45\%]$), $P = 0.003$ at 360 min.

Conclusion: FGF21 levels declined in the postprandial period and changes were opposite to those of insulin, glucose, and triglycerides and resembled those of IGFBP-1. Continuous hyperinsulinemia prevented the postprandial reduction in FGF21 in HD patients.

12:05-12:13

11. Safety and efficacy of liraglutide in patients with type 2 diabetes and end-stage renal disease: an investigator-initiated randomised, placebo-controlled, double-blinded, parallel trial

Thomas Idorn$^1$, Filip K. Knop$^{2,3}$, Morten Jørgensen$^1$, Tonny Jensen$^4$, Marsela Resuli$^5$, Pernille M. Hansen$^5$, Karl B. Christensen$^6$, Jens J. Holst$^3$, Mads Hornum$^1$ and Bo Feldt-Rasmussen$^1$

$^1$Department of Nephrology, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark; $^2$Diabetes Research Division, Department of Internal Medicine, Gentofte Hospital, University of Copenhagen, Hellerup, Denmark; $^3$The NNF Center for Basic Metabolic Research, Department of Biomedical Sciences, Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark; $^4$Department of Endocrinology, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark; $^5$Department of Internal Medicine, Hillerød Hospital, Hillerød, Denmark; $^6$Department of Biostatistics, Institute of Public Health, University of Copenhagen, Denmark.

Background: Diabetes is the leading cause of end-stage renal disease (ESRD), however, few antidiabetic agents are available to these patients. We evaluated safety and efficacy of liraglutide treatment in patients with type 2 diabetes (T2D) and dialysis-dependent ESRD.

Methods: 24 patients with T2D and ESRD and 23 patients with T2D and normal kidney function (71 and 74% treated with insulin, respectively) were randomly allocated to 12 weeks of double-blinded liraglutide (titrated dose of 0.6 mg, 1.2 mg or 1.8 mg) or placebo (1:1) treatment injected subcutaneously once-daily. 20 participants (1:1) in each group completed the study period. Dose-corrected plasma trough liraglutide concentration was evaluated at the final trial visit as the primary outcome measure. Additional safety and efficacy parameters were assessed.

Results: Liraglutide-treated ESRD patients were titrated slower than liraglutide-treated controls, but ended at comparable doses at 12 weeks (1.33±0.13 and 1.26±0.06 mg/day, $P = 0.61$). Dose-corrected plasma trough
Liraglutide concentrations at the final trial visit were increased by 49% (confidence interval: 6–109%, \( P = 0.02 \)) in liraglutide-treated ESRD patients compared with liraglutide-treated controls (plasma concentrations 11,975 [9,379–15,289] and 8,057 [6,306–10,293] pmol/L/mg). Nausea and vomiting were more frequent in liraglutide-treated ESRD patients compared with liraglutide-treated controls. There were more severe adverse events in the ESRD group (\( N = 7 \), including \( N = 6 \) during liraglutide treatment) compared with controls (\( N = 1 \) during placebo treatment), although none were assessed to be related to trial medication. Glycaemic control was improved in all treatment arms assessed by HbA1c and blood glucose measurements, and baseline insulin treatment was significantly reduced in both liraglutide-treated groups (\( P < 0.02 \)).

**Conclusions:** Liraglutide treatment appears to be applicable in patients with ESRD and T2D. Slower dose titration and reduced treatment doses may be advisable.

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**Hemodialysis - treatment, outcome and complications**

*Chairman: Susanne Bro*

13:15-13:23

12. *Angiotensin II receptor blockade does not protect against progressive loss of residual renal function in hemodialysis patients: A randomized controlled trial (SAFIR study)*

Krista Dybtved Kjaergaard\(^1,2\); Christian Daugaard Peters\(^1,2\); Bente Jespersen\(^1,2\); Ida Nørager Tietze\(^3\); Jens Kristian Madsen\(^1\); Birgitte Bang Pedersen\(^4\); Marija Kristina Novosel\(^5\); Kathrine Skaaning Laursen\(^4\); Bo Martin Bibby\(^6\); Charlotte Strandhave\(^4\); Jens Dam Jensen\(^1,2\)

\(^1\) Department of Renal Medicine, Aarhus University Hospital, Denmark, \(^2\) Institute of Clinical Medicine, Aarhus University, Denmark, \(^3\) Department of Internal Medicine, Regional Hospital Viborg, Denmark; \(^4\) Department of Nephrology, Aalborg University Hospital, Denmark; \(^5\) Department of Medicine, Fredericia Hospital, Denmark; \(^6\) Department of Public Health, Institute of Biostatistics, Aarhus University, Denmark

**Background:** Glomerular filtration rate (GFR) declines during chronic dialysis treatment. In predialysis patients and in peritoneal dialysis patients, blockade of the renin-angiotensin-aldosterone system reduces GFR decline. Observational studies suggest that similar treatment may preserve renal function in hemodialysis (HD) patients.

**Methods:** The SAFIR study is a multicenter randomized placebo-controlled double-blinded trial initiated by the investigators with one-year follow-up. Inclusion criteria were adult HD patient with urine output > 300 mL/24h, HD vintage < 1 year, and cardiac ejection fraction > 30%. Patients were included from six hospitals and randomized to placebo or the angiotensin II receptor blocker irbesartan 300 mg daily. Target systolic blood pressure (BP) was 140 mmHg. Outcomes were GFR measured as the mean of creatinine and urea renal clearance, urine volume and time to anuria.

**Results:** Of the 82 patients randomized (placebo \( n=41 \)/irbesartan \( n=41 \)), 56 completed one year of treatment. The groups were comparable at baseline (means): males 26/30, age 62/61 years, HD vintage 137/148 days
The target BP level was reached in both groups and BP did not differ significantly between groups over time. Adverse event rates were similar. GFR declined by 1.7 (1.2 to 2.3) (mean (95% CI)) mL/min/1.73m²/year in the placebo group and by 1.8 (1.1 to 2.4) mL/min/1.73m²/year in the irbesartan-treated group. Mean difference (baseline-12 months) between groups was -0.0 (-0.8 to 0.8) mL/min/1.73m². In each group, four patients became anuric.

**Conclusions:** At equal BP-levels, we found that irbesartan treatment did not affect the decline in GFR or urine volume significantly during one-year treatment in HD patients. Irbesartan treatment was safely used in the studied population.

13:23-13:31

13. Short and long-term effects of irbesartan on intradialytic central haemodynamics: A randomised double-blind placebo-controlled one-year intervention trial (the SAFIR study)

**Christian D. Peters**¹², Krista D. Kjærgaard¹², Jens D. Jensen¹², Kent L. Christensen³, Charlotte Strandhave⁴, Ida N. Tietze⁵, Marija K. Novosel⁶, Bo M. Bibby⁷, Bente Jespersen¹²

¹) Dept. of Renal Medicine, Aarhus University Hospital, Denmark, ²) Institute of Clinical Medicine, Aarhus University, Denmark, ³) Dept. of Cardiology, Aarhus University Hospital, Denmark, ⁴) Dept. of Nephrology, Aalborg University Hospital, Denmark, ⁵) Dept. of Medicine, Viborg Regional Hospital, Denmark, ⁶) Dept. of Medicine, Fredericia Hospital, Denmark, ⁷) Dept. of Biostatistics, Aarhus University, Denmark

**Background** Haemodynamic instability is a frequent complication during haemodialysis (HD) treatment. Whether intradialytic haemodynamics is affected by specific antihypertensive treatment is unknown. We hypothesised that angiotensin II receptor blocker (ARB) therapy may affect intradialytic haemodynamic stability and this study describes short and long-term effects of ARB treatment vs. placebo on intradialytic haemodynamic parameters in a cohort of Danish HD patients.

**Methods** Adult HD patients were randomised for double-blind treatment with the ARB irbesartan or placebo using a predialytic systolic blood pressure (BP) target of 140 mmHg. Intradialytic hypotension (IDH) was defined as symptomatic hypotension requiring intravenous fluid administration or preterm ending of the HD session and was recorded for all HD treatments. At baseline, 1 week, 3, 6, 9, and 12 months cardiac output (CO), stroke volume (SV), central blood volume (CBV), total peripheral resistance (TPR), mean arterial BP (MAP), and heart rate (HR) were measured within the first (HD START) and last (HD END) 30 minutes during HD using the Transonic saline dilution technique.

**Results** Eighty-two patients were randomised (placebo/ARB: 41/41). Predialytic systolic BP decreased significantly, but similarly in both groups during the study period. The total number of IDH episodes was (placebo/ARB) 22/25 (P=0.7). Mean HD START and mean HD END CO, SV, TPR, HR, and MAP were stable and similar in the two groups, whereas CBV increased equally and significantly over time. The mean intradialytic haemodynamic response showed decreased CO, SV, MAP, and CBV, whereas HR increased from HD START to HD END. TPR did not change significantly. Overall, this pattern remained stable over time in both groups and there was no significant impact of ARB treatment.

**Conclusions** At equal BP-levels, central haemodynamic parameters during HD were not significantly affected by ARB and IDH episodes were not more prevalent in ARB treated patients.
14. Lower mortality in ICU patients on chronic dialysis than for those with dialysis requiring acute kidney injury

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Background: The mortality of patients requiring acute hemodialysis (HD) in the intensive care unit (ICU) is high. In this study we aim to investigate to what extent this may rely upon the lack of renal function. Thus the mortality of ICU patients requiring acute HD and ICU patients on chronic dialysis before ICU admission are compared.

Methods: All adult patients admitted to the multidisciplinary ICU, Rigshospitalet, from Jan 1st 2005 to Dec 31st 2012 were identified through the ICU database, excluding repeat admittances. Chronic dialysis patients were identified from the Danish Society of Nephrology database. Kaplan-Meier plots were calculated for the mortality of the first 30-days after ICU admission.

Results: In the study 6076 patients were included. Of these, 1030 (17.0 %) received acute HD and 154 (2.5 %) were on chronic dialysis before admission. The age was 56.3 years (±17.8) (mean (SD)) for non-dialysis patients. The mean age was 60.7 years (±14.2) for acute HD patients and 60.0 years (±14.5) for chronic dialysis patients (p=0.576). The mean APACHE II score was 28.3 (±7.8) and 28.8 (±8.1), respectively (p=0.497). The mean SAPS II score was 58.6 (±17.2) for acute HD patients and 56.0 (±16.5) for chronic dialysis patients (p=0.126). The mean of the highest SOFA score during admission was 14.4 (±3.8) for acute HD patients and 12 (±3.5) for chronic dialysis patients (p<0.001). Thirty-day mortality (95 % CI) was 41.4 % (38.3 % - 44.5 %) for acute HD patients and 29.5 % (22.1 % - 36.9 %) for chronic dialysis patients (p=0.026). For non-dialysis patients thirty-day mortality was 15.5 % (14.4 % - 16.5 %).

Conclusion: Patients with acute HD in the ICU had a significantly higher mortality compared to patients on chronic dialysis. This may have reflected acute HD patients being more severely ill, particularly during the course of their ICU stay. There was no evidence that lack of renal function alone could explain the high mortality amongst patients requiring acute HD in the ICU.

15. Impact of blood flow rate on blood pressure, heart rate and cardiac output in haemodialysis patients

Philip A. Schytz, Maria L. Mace, Nikolaos Karamperis, Søren D. Ladefoged* and Henrik P. Hansen

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Objective Recent data by Trivedi et al. (2007) suggests that an increase in dialysis blood flow rate (pump flow rate) from 200 to 400 ml/min results in an increase in blood pressure of 3-4 mm Hg. The underline mechanism is unknown. The aim of our study was to evaluate the potential mechanism(s) involved, by investigating the impact of changes in blood flow rate on blood pressure, heart rate and cardiac output in haemodialysis patients with AV-fistulas.

Design and Methods We performed a randomised, crossover trial in 22 haemodialysis patients. After a conventionel haemodialysis session, each patient was examined during blood flow rates of 200, 300 and 400 ml/min in random order. At the end of each examination period (15 min), blood pressure, heart rate (AK200)
and cardiac output (Transonic Flow-QC; Krivitski 1999) were measured. AV-fistulas were evaluated prior to examination and ensured that no recirculation was present. No ultrafiltration was performed during the examination periods.

**Results** Mean age of enrolled patients was 71 (10) years (mean (SD)). Systolic blood pressure was significantly higher at a blood flow rate of 200 ml/min as compared with 300 ml/min (5.0 (0.6 to 9.4) mm Hg; p<0.03). Compared with a blood flow rate of 400 ml/min, systolic blood pressure at 200 ml/min was also higher, although the difference was not significant (3.9 (-0.4 to 8.3); p=0.08)(mean difference (95 % CI)). At blood flow rates of 200, 300 and 400 ml/min, respectively, diastolic blood pressure (67 vs 66 vs 66 mm Hg; NS), heart rate (75 vs 75 vs 75 beats/min; NS) and cardiac output (5.2 vs 5.2 vs 5.1 L/min; NS) remained unchanged.

**Conclusions** In contrast to previous data, our study suggests that systolic blood pressure decreases when the blood flow rate exceeds 200 ml/min. We speculate that this can be attributed to a decline in total peripheral resistance.

**Kidney disease and calcium-phosphate metabolism**

*Chairman: Lisbet Brandi*

13:47-13:55

16. **Existence of a rapid kidney - bone regulatory axis in the secretion of FGF23**

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**Background:** The phosphaturic hormone, fibroblast growth factor 23 (FGF23), plays a principal role in phosphate (P) and calcium (Ca) homeostasis, which is progressively disturbed as kidney function declines. Chronic uremia is characterized by very high circulating levels of FGF23.

**Objective:** The purpose of the present investigation was to study the impact of the presence of kidneys in the very early regulation of FGF23, as well as the impact of P, Ca, and PTH on this very early FGF23 regulation.

**Methods:** Adult male Wistar rats were randomized to either acute bilateral or unilateral nephrectomy (NX, UNX), parathyroidectomy (PTX), or sham surgery. Acute hyperphosphatemia and hypercalcemia were induced by an intravenous bolus of Addiphos or Calcium Sandoz, respectively. The observation period was up to 60 minutes. FGF23 levels were measured using the intact FGF23 assay from Kainos Lab, Japan.

**Results:** NX resulted in an immediate, within 15 minutes, increase in FGF23 levels from 84±14 to 211±16 pg/ml (p < 0.001) which then remained stable. UNX generated as well a prompt rise in FGF23 to 147±20 pg/ml, reaching a level in between NX and control group. Stable FGF23 levels were measured in control group. A comparison of FGF23 levels in NX and PTX+NX groups showed similar rapid increases. Addiphos infusions increased significantly plasma P to 5.50 mmol/l in normal and NX rats (p<0.01). High P levels had no impact on the rapid regulation of FGF23 secretion (ns). Similarly, an elevation of plasma Ca²⁺ to 2.30 mmol/l had no effect on FGF23 levels (ns).

**Conclusion:** The present results clearly demonstrate, for the first time, a key role of kidneys in FGF23 regulation, as absence of kidney tissue resulted in a very rapid and significant increase in FGF23 by 3 fold. This may suggest the existence of a kidney- bone regulatory axis, independent of calcium, phosphate and PTH.
Effect of the new Nordic diet on phosphate homeostasis - a post hoc analysis of a 6 months randomized controlled trial

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Background: Recent observations have linked phosphate intake and serum phosphate levels within the normal range to cardiovascular mortality and morbidity in populations with normal kidney function. Modern Western diet contains high loads of phosphate due to animal based proteins and inorganic phosphate (phosphate containing food additives). It has been suggested that the food composition databases are insufficient and underestimate the phosphate content in food.

The New Nordic Diet (NND) is designed as a healthy diet (mainly organic food items, large amounts of fruit and vegetables, wholegrain and fish) and has shown to reduce weight and blood pressure in centrally obese subjects, compared to an average Danish diet (ADD).

The hypothesis tested in the present study was that the NND reduced phosphate load and thus be suitable for patient with chronic kidney disease.

Material and Methods: Serum and urine samples from 147 centrally obese subjects were analysed for phosphate intake, the fractional excretion of phosphate (FEP) and urinary phosphate excretion at baseline, week 12 and week 26.

Results: Dietary phosphate intake, calculated by using food tables, was at baseline for NND =1910mg/day, ADD =2000 mg/day. Week 26 NND =1645 and ADD =1641. Both diets resulted in a significant reduction in phosphate intake during the study period (p<0.001).

Mean FEP was at baseline for NND =22%, ADD =22% and at week 26 NND =18 and ADD =19% respectively. Mean urinary phosphate excretion was at baseline for NND=1220 mg/day, ADD=1289 mg/day and at week 26 NND=1087 mg/day and ADD=1056 mg/day.

Conclusion: In centrally obese subjects with normal renal function we found no difference in phosphate intake, FEP or urinary phosphate excretion despite profound differences in dietary composition. The food composition database calculated phosphate load correlated well with the other parameters of phosphate homeostasis and thus provided a realistic estimate of the phosphate content.

An experimental animal model on uremic vascular calcifications

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Background. Vascular calcification (VC) is accelerated in patients with chronic renal failure (CRF) and associated with significantly increased mortality. The mechanism of VC is linked to disturbed mineral homeostasis resulting in transformation of the phenotype of vascular smooth muscle cells (VSMC) to osteoblast-like cells with increased phosphate (P) and uremic osteodystrophy as important causative factors. As this transformation
is due to highly regulated cell-mediated processes, it may be possible to inhibit progression or even reverse established VC.

Our aim was to produce an animal model of uremic VC in order to be able to examine the reversibility of VC.

**Methods.** CRF was induced in inbred Dark Aguti (DA) rats by 5/6-nephrectomy. Sham operated rats were used as controls. In order to induce severe VC, CRF rats were fed a high P diet and treated with intraperitoneal alfacalcidol 3 times weekly. The sham group was kept on a standard diet and randomly assigned either to alfacalcidol or vehicle. The rats were sacrificed after 14 weeks of uremia. Uremia and associated mineral metabolism disturbances were evaluated by measurements of plasma creatinine, urea, P, total Ca, Ca2+, PTH and FGF23. The abdominal aorta and the aortic root were examined for calcification by von Kossa staining.

**Results.** CRF rats became severely uremic with significantly increased p-urea from 6±0.2 to 15±1.5 mmol/L, creatinine from 28±1 to 51±2 µmol/L and P from 1.38±0.07 to 2.32±0.09 mmol/L (p<0.0001). Severe increase in FGF23 was induced from 133±15 to 4133±121 pg/mL. Von Kossa staining of the aorta from the CRF rats showed severe media calcifications and in some areas VSMC with signs of chondrogenic transformation.

**In conclusion.** In the present study we have created an animal model of severe uremic VC, where CRF rats showed hallmarks of biochemical abnormalities inherent to chronic uremia. This experimental model in inbred DA rats is suitable for studying the potential reversibility of uremic VC by kidney transplantation.

14:11-14:19

19. Calcium regulation in acute experimental nephrectomy

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**Introduction:** Plasma Ca\(^{2+}\) is kept very stable. A ‘labile’ calcium storage pool, in equilibrium with blood calcium, exists on bone surface. The exact exchange mechanism of Ca\(^{2+}\) between plasma and bone with the purpose of keeping p-Ca\(^{2+}\) stable, still needs to be determined. Our purpose was to evaluate the role of the kidney, with special focus on the rapid minute-to-minute regulation of p-Ca\(^{2+}\) after acute nephrectomy (NX).

**Methods:** Rapid regulation of p-Ca\(^{2+}\) was examined in sham operated rats, acute NX rats, acute thyroparathyroidectomized (TPTX) rats and in TPTX NX rats. Acute hypocalcaemia was induced by intravenous EGTA infusion for 30 min, and then the rapid recovery of p-Ca\(^{2+}\) was followed for 80 min.

**Results:** NX resulted in a significant reduction of p-Ca\(^{2+}\) from 1.22±0.02 to 1.06±0.03 (P < 0.05) in control rats. In EGTA rats, nadir of hypocalcaemia was lower in NX rats than in sham rats: 0.50±0.2 vs 0.80±0.02 (P < 0.01). After discontinuing EGTA a rapid increase of p-Ca\(^{2+}\) took place but was significantly lower in NX than in sham operated rats: 0.96±0.01 vs 1.09±0.01(P < 0.05). Acute TPTX resulted in hypercalcemia, within 60 min p-Ca\(^{2+}\) increased from 1.25±0.01 to 1.43±0.02 and further to 1.50±0.02 (P < 0.01) after another 80 min. The increase in p-Ca\(^{2+}\) was less in TPTX/NX rats, to 1.29±0.02 and further to 1.41±0.02 (P < 0.05). After the EGTA infusion, nadir of hypocalcaemia was lower in TPTX/NX than in TPTX rats: 0.86±0.03 vs. 1.01±0.03 (P < 0.01). The recovery of p-Ca\(^{2+}\) from EGTA induced hypocalcaemia resulted in lower levels in TPTX/NX than in TPTX rats: 1.20±0.02 vs 1.30±0.02 (P < 0.05).

**Conclusion:** The present results clearly showed that p-Ca\(^{2+}\) levels on a minute-to-minute basis are influenced by the presence of kidneys. Hypocalcaemia developed rapidly after acute NX, and NX changed the level of p-Ca\(^{2+}\), which was obtained during recovery from EGTA induced hypocalcemia in normal and TPTX rats. These results
indicate that the kidney is of significant importance for the set-point of p-Ca\textsuperscript{2+} on the bone surface and point towards existence of an as yet unknown factor or mechanism involved in the kidney-bone-axis.