




3rd International Conference

HUS & MPGN & related diseases

Current diagnosis and therapy of
hemolytic uremic syndrome (HUS)
membranoproliferative glomerulonephritis (MPGN)
and related diseases

In Memoriam: Univ.-Prof. Dr. Lothar Bernd Zimmerhackl



Hotel „Grauer Bär“
Innsbruck, Austria

22-24 May 2011

www.hus-online.at

INTRODUCTION



Dear colleague,

This is the third time in three years, a meeting on HUS and related diseases will be held in Innsbruck. This series was inaugurated by the unforgettable Lothar Bernd Zimmerhackl, who was the main organiser of the first two meetings, but was unable to fully take part in the second one, due to a massive heart attack he had suffered shortly before. Those who attended that meeting remembered well that Bernd was already markedly handicapped and really only a shadow of his former self, a vivid, enthusiastic, highly educated, social and above all internationally well recognised and respected physician and scientist.

After a second fatal heart attack Prof. Zimmerhackl died on August, 27 last year. This meeting will be held in his honour.

As, apart from the captain, all co-pilots, navigators, stewards and skippers, now even more experienced, were still part of the organisation committee, we put our best foot forward trying to continue captain's idea and organise an inspiring meeting for scientists and physicians. Concerning the contents we put more emphasis on the expanded spectrum of complement associated disorders, including DDD, TTP and AMD, which show comparable pathophysiologicals. Due to that we expect vivid discussions on disease classifications. Typical HUS will play a greater role than previously, as it may be more related to the other diseases than previously anticipated. We are also looking forward to the session on diagnostic approaches and on treatment. This includes Eculizumab, in my view a very promising specific drug - however, I am biased, having characterised its murine prototype N19-8 in Göttingen, more than 20 years ago. To allow a pleasant atmosphere and enough time, we will have two parallel poster sessions.

Innsbruck is the capital of the alps - as Bernd has mentioned in his former foreword, a twice Olympic city (1964, 1976) and medieval Habsburg dynasty town, so we hope that you have planned some extra time outside the program.

The venue will be the Hotel Grauer Bär, by which we have reduced the short distances between bed, venue and town even further - enjoy our Tyrolian gala night with local esprit!

Next year the meeting will move to Amsterdam, as one of two satellite symposia to VTEC2012: <http://www.vtec2012.org/scientific-program/satellite-symposia/>. The main congress will be organised by Nicole van de Kar and the satellite symposium by Jean-Claude Davin, both present (<http://www.vtec2012.org/welcome/meet-the-organizers/>). In 2013 we may be back here!?

Enjoy the scientific presentations, the friendly atmosphere and the city!
Cheers,

Reinhard Würzner

also on behalf of

Dr. Thomas Giner, Dr. Johannes Hofer, Dr. Therese Jungrathmayr,
Dr. Magdalena Riedl, Dr. Alejandra Rosales & congress assistant Claudia Triendl

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PROGRAM

Sunday, May 22

- 18:00 - 20:00 Start Registration Hotel "Grauer Bär"
18:00 - 19:00 Postermounting Hotel "Grauer Bär"
19:00 - 20:30 Welcome Reception Hotel "Grauer Bär"

Monday, May 23

08:00 - 18:30 Registration open

08:15 Welcome from Innsbruck Medical University
Lochs/Würzner

I. Classical HUS *Chairs: Bielaszewska/Orth*

- 08:30 L1 Escherichia coli and the hemolytic uremic syndrome:
the microbiological view
Karch
- 09:00 L2 Escherichia coli and the hemolytic uremic syndrome:
the critical "pre-renal" phase
Tarr
- 09:30 FC1 Typical HUS - Long term follow up experience from the highest
endemic region in 2009
Exeni
- 09:45 FC2 HUS in developing countries: diagnostic and therapeutic
difficulties
Safouh
- 10:00 FC3 Shiga-like toxin upregulates production of C3 mRNA and protein
in human endothelial cells
Volokhina
- 10:15 FC4 The antimicrobial peptide cathelicidin completely protects
mice from E.Coli O157:H7-mediated disease
Chromek

10:30 - 11:00
Coffee break

II. Atypical HUS *Chairs: Simonetti/Steichen-Gersdorf*

- 11:00 L3 aHUS/DDD/AMD - lessons from the functional characterization of disease-associated genetic variants
Rodriguez de Cordoba
- 11:30 FC5 Mutations in the CFHR5 gene and in genes encoding components of the terminal complement pathway in patients with atypical hemolytic uremic syndrome
Westra
- 11:45 L4 Acquired forms of atypical HUS
Hofer
- 12:15 FC6 Factor H mutations and autoantibodies associated with atypical hemolytic uremic syndrome impair factor H binding to pentraxin 3
Jozsi

12:30 - 14:00
Lunch

III. HUS-aHUS *Chairs: Tönshoff/Lass-Flörl*

- 14:00 L5 *In Memoriam: Lothar Bernd Zimmerhackl*
Typical HUS and complement
Würzner
- 14:30 L6 Complement activation and endothelial dysfunction in typical and atypical HUS
Noris
- 15:00 L7 Complement deposition on blood cells during HUS
Karpman
- 15:30 L8 Hemolytic uremic syndrome: Observations from a 5-year prospective multicenter study
Rosales

16:00-16:30
Coffee break

16:30 - 18:30 Postersession Wine & Cheese
Chairs (Posters # 1-9): Cohney/Brunner
Chairs (Posters # 10-18): Langman/Rudnicki

Tuesday, May 24

08:00 - 12:30 Registration open

IV. Diagnostic approaches

Chairs: Van de Kar/Griesmacher

- 08:30 L9 Analysis of complement-mediated nephropathy
Kirschfink
- 09:00 L10 Analysis of thrombocytopathies in HUS and related diseases
Streif
- 09:30 FC7 Complement activation in thrombocytopenic purpura
Prohaska
- 09:45 FC8 Familial MPGN
Soylemezoglu

10:00-10:30
Coffee break

V. MPGN-DDD-AMD

Chairs: Wühl/Janecke

- 10:30 L11 New genetic scenarios in membranoproliferative glomerulonephritis: lessons from the European cohort
Zipfel
- 11:00 L12 Therapy of MPGN - a case for complement
Licht
- 11:30 L13 DDD - An update on genetics, nephritic factors and treatment options
Smith
- 12:00 L14 Role of CFHR proteins in AMD and kidney diseases
Skerka

12:30 - 14:00
Lunch

VI. MPGN-aHUS, Part I: Classification*Chairs: Dragon-Durey/Mayer*

- 14:00 L15 C3 glomerulopathy and atypical hemolytic uremic syndrome: risk haplotypes and mutations in factor H gene
Fremeaux-Bacchi
- 14:30 L16 An update on animal models of atypical HUS
Pickering
- 15:00 L17 Membranoproliferative glomerulonephritis: pathogenetic heterogeneity and proposal for a new classification
Sethi
- 15:30 L18 Histological aspects of HUS/TTP and MPGN and their differential diagnoses
Amann

16:00-16:30
Coffee break

VII. MPGN-aHUS, Part II: Treatment*Chairs: Arbeiter/Jungraithmayr*

- 16:30 L19 Complement inhibition strategies - targeting C5
Riedl
- 17:00 FC9 Prophylactic plasma exchange (PE) and Eculizumab (E) allow long term renal function preservation in CFH related atypical hemolytic uremic syndrome (aHUS)
Davin
- 17:15 L20 Transplantation in MPGN & atypical HUS
Saland
- 17:45 FC10 Successful long term outcome after renal transplantation for atypical HUS (aHUS) with combined MCP and complement-factor I (CFI) mutation
Sparta

19:00
Gala Dinner

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- Shlomo Cohney, Dept. of Nephrology, Royal Melbourne Hospital
- Craig Langman, Children's Memorial Hospital Chicago
- Michael Rudnicki, Dept. of Nephrology, Innsbruck Medical University
- Reinhard Würzner, Dept. of Hygiene, Innsbruck Medical University

The poster price will be awarded on Tuesday evening during the Gala Dinner.

ABSTRACTS

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SHIGA-LIKE TOXIN UPREGULATES PRODUCTION OF C3 MRNA AND PROTEIN IN HUMAN ENDOTHELIAL CELLS

E.B. Volokhina¹, A. Vos¹, T. van der Velden¹, D. Westra¹, N.C. van de Kar¹, L.P. van den Heuvel^{1,2,3}.

¹Department of Pediatric Nephrology, ²Department of Laboratory Medicine, Radboud University Nijmegen Medical Centre (RUNMC), ³Department of Pediatrics, University Hospital Leuven.

Aim of the study Most of the HUS cases are preceded by infection with Shiga-like toxin (Stx) producing *Escherichia coli* (STEC). Although Stx inhibits protein synthesis, the exact mechanism of how Stx exposure leads to HUS is poorly understood. Complement dysregulation is a hallmark of less common non-STEC HUS. Therefore, we studied whether Stx1 is able to alter complement gene expression in human endothelial cells.

Methods HUVEC from 9 donors and conditionally immortalized and primary human glomerular microvascular endothelial cells (GMVEC) were treated for 24 hours with Stx1 with or without prestimulation with TNF α or LPS. The mRNA levels were analyzed by qPCR and protein levels were measured using ELISA. Protein synthesis inhibition was assessed by testing ³H-leucine incorporation.

Results C3 mRNA levels in HUVEC and GMVEC were elevated 3 \pm 1 and 6 \pm 1 fold, respectively, after Stx1 treatment. This effect was greatly enhanced by prestimulation with TNF α or LPS to 45 \pm 14 and 1698 \pm 672 fold. C3 protein levels were upregulated up to 6 fold and 8 fold for prestimulated HUVEC and GMVEC. Protein synthesis inhibition only partially explains the difference between C3 mRNA and protein levels. No major impact was observed on CFI, CFH and MCP mRNA expression.

Conclusion Our results suggest that exposure of HUVEC and GMVEC cells to Stx1 leads to an increased production of C3 especially on the mRNA level and marginally changed levels of CFI, CFH and MCP complement inhibitors, that might lead to local inefficient inactivation of C3b and damage of glomerular endothelial cells in STEC-HUS through improper complement activation.

THE ANTIMICROBIAL PEPTIDE CATHELICIDIN COMPLETELY PROTECTS MICE FROM *E. coli* O157:H7-mediated disease

Milan Chromek, Ida Arvidsson, and Diana Karpman

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Introduction and aim: The antimicrobial and immunomodulatory peptide cathelicidin protects epithelial surfaces including gut against invasive infections. We sought to investigate the role of cathelicidin in the pathogenesis of *E. coli* O157:H7 infection and subsequent hemolytic uremic syndrome (HUS).

Methods: Wild-type (129/SvJ) and cathelicidin knock-out (CRAMP^{-/-}) mice were inoculated with Shiga toxin 2 (Stx2)-producing and non-producing *E. coli* O157:H7. The course of the disease was followed by visual assessment of the animals, microbiological, hematological analyses, as well as histological and immunofluorescent examination of the tissues.

Results: All CRAMP^{-/-} mice inoculated with Stx2-positive *E. coli* O157:H7 developed signs of the disease and histopathology showed renal damage. In the presence of cathelicidin or in the absence of Stx2 all mice remained asymptomatic. Cathelicidin did not influence the number of bacteria in feces but CRAMP^{-/-} animals had more attaching and effacing lesions in the colon. Cathelicidin killed *E. coli* O157:H7 *in vitro* and in very low concentrations also inhibited formation of bacterial biofilm. Moreover, cathelicidin-deficient mice had a thinner mucus layer in the colon as compared with wild-type animals.

Conclusion: Cathelicidin substantially influences mechanical and chemical antimicrobial barrier in the colon mucosa. Accordingly, lack of cathelicidin leads to high susceptibility to the *E. coli* O157:H7 infection. The presented results open new possibilities for treatment of the *E. coli* O157:H7-mediated diseases.

MUTATIONS IN THE *CFHR5* GENE AND IN GENES ENCODING COMPONENTS OF THE TERMINAL COMPLEMENT PATHWAY IN PATIENTS WITH ATYPICAL HEMOLYTIC UREMIC SYNDROME

D. Westra¹, E.B. Volokhina¹, L.M. Geerdink¹, A. Klaasen¹, N.M. Held¹, C.D. Huigen², N.C. van de Kar¹, L.P. van den Heuvel^{1,3}.

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³*Department of Pediatrics, University Hospital Leuven, Leuven, Belgium.*

Introduction Atypical HUS (aHUS) is a rare and severe renal disorder that is thought to be caused by predisposing aberrations in complement proteins of the alternative pathway. Previously, a cohort of Dutch and Belgian aHUS patients was screened for mutations in *CFH*, *CFI*, *CD46*, and *CFB*, and for the presence of α FH. In 31.9% of the patients, complement deficiencies were found. More than 4% of the patients carried an alteration in more than one gene. Very recently, a mutation has been identified in a regulator of the terminal complement pathway, but no mutations have been identified in components of the membrane attack complex (MAC), yet.

Aim of the study Identify potentially pathogenic aberrations in the alternative pathway regulator *CFHR5* (*CFHR5*) and the MAC proteins C8 α (*C8A*), C8B (*C8B*), and C9 (*C9*) in 66 aHUS patients.

Methods In a research population of 66 aHUS patients, mutational screening was performed in *CFHR5*, *C8A*, *C8B*, and *C9*, by means of PCR on genomic DNA and sequence analysis. Potential pathogenicity of genetic alterations was checked in literature, evolutionary conservation, and *in silico* mutation prediction programs. Influence of mutations in *C8A* on protein structure was analyzed with respect to available structural data. No structural models were available for *CFHR5*, *C8B* or *C9*.

Results In seventeen patients a genetic aberration, resulting in a change at amino acid level, was found in at least one of the above mentioned genes. After evaluation of potential pathogenicity, we state that in twelve patients (12/66; 18.2%), a potentially pathogenic sequence variation was found in one of the terminal complement pathway components or in *CFHR5*. Analysis of available structural data indicates that one of the amino acids altered in C8 α might be located in the proximity of the C8 α interface with C8 γ . Prediction models for interaction between C8 α and C8 γ will be displayed.

In six patients, a genetic defect was previously found in at least one of the previously screened genes (*CFH*, *C8A* and *CFHR5*: 2x; *C8A* and *CFH*: 1x; *C8B* and *CFI*: 1x; *CFHR5* and *CFH*: 1x; *CFHR5* and *C9*: 1x).

Conclusion A potentially pathogenic genetic abnormality in one of the components of the membrane attack complex or in *CFHR5* was observed in 18.2% of the patients. In total, in 45.5% (30/66) of the patients, at least one potentially pathogenic defect was identified in one of the complement genes that were screened in this cohort. The combined genetic defects identified in 13.6% (9/66), might partly explain the incomplete penetrance described in the disease.

FACTOR H MUTATIONS AND AUTOANTIBODIES ASSOCIATED WITH ATYPICAL HEMOLYTIC UREMIC SYNDROME IMPAIR FACTOR H BINDING TO PENTRAXIN 3

Anne Braunschweig, Stefanie Strobel, Mihály Józsi

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Endothelial injury is involved in the pathophysiology of atypical hemolytic uremic syndrome (aHUS). Pentraxin 3 (PTX3) is a soluble recognition molecule produced and rapidly released by several cell types, including endothelial cells, in response to inflammatory stimuli. PTX3 can initiate complement activation by its interactions with C1q, mannose binding lectin and ficolin-2. However, PTX3 also binds factor H (FH), the major regulator of the alternative complement pathway. The aim of this study was to analyze the FH-PTX3 interaction in more detail and to investigate whether FH mutations and anti-FH autoantibodies associated with aHUS influence the interaction between FH and PTX3.

PTX3 did not interfere with the cofactor and convertase decay accelerating activities of FH in the fluid phase, and FH maintained its complement regulatory activity when bound to immobilized PTX3. Using a peptide array, a PTX3 binding site within the SCR20 domain of FH was identified. The identified residues involved in PTX3 binding are surface exposed. FH mutations within this binding site that are associated with aHUS caused a reduced FH binding to PTX3. Likewise, anti-FH autoantibodies isolated from the plasma of aHUS patients inhibited the FH-PTX3 interaction. PTX3 produced by endothelial cells bound locally to the underlying extracellular matrix *in vitro*, and recruited functionally active FH. Whereas C3 deposition from plasma was enhanced by PTX3 due to complement activation, the levels of C5a and deposited C5b-9 were not increased.

In conclusion, PTX3 recruits the complement regulator FH to the exposed subendothelial extracellular matrix, which limits PTX3-mediated local complement activation to the early components, thus preventing inflammatory effects and host cell damage. The FH-PTX3 interaction, however, is impaired by certain disease-associated mutations and autoantibodies against SCR20 of FH. This could amplify local complement-mediated inflammation as well as endothelial cell activation and damage in aHUS.

COMPLEMENT ACTIVATION IN THROMBOTIC THROMBOCYTOPENIC PURPURA

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³*Department of Hematology and Stem Cell Transplantation, St László and St István Hospital, Budapest*

Background: Endothelial injury is the central factor in the events leading to thrombotic microangiopathy, however, mechanisms involved are poorly understood. Whereas pathological activation of the alternative complement pathway is linked to atypical hemolytic uremic syndrome, the role of complement activation in thrombotic thrombocytopenic purpura (TTP) is unknown.

The aim of this study was to investigate whether signs of complement activation are characteristic to TTP.

Patients and methods: Thirty-eight patients with TTP were included (31 women, mean age 41 years, SD 12 years); 14 patients had 1 sample, taken in complete remission (CR), 24 patients had samples taken in acute episode, among them 13 patients had samples taken before the initiation of plasma exchange (PEX), during PEX and in CR. Complement parameters (C3, Factors H, I, B and total alternative pathway activity) together with complement activation fragments (Bb, C3a) or complexes (sC5b9) were measured by ELISA or RID. ADAMTS13 activity and anti-ADAMTS13 inhibitory antibodies were measured by the VWF-FRET73 assay. Fourteen healthy controls (10 women, age 36±20 years) were also studied.

Results: Complement C3, FH, FI, FB levels and alternative pathway activity was not different between TTP patients in acute episode and CR, and low levels, indicative for complement consumption, did not occur. However, increased levels of C3a, Bb or sC5b9 were observed in TTP during acute episodes, as compared to TTP CR samples or healthy controls. Significant decrease of C3a, Bb and sC5b9 was observed during PEX. Higher sC5b9 levels in samples taken before PEX were associated with obesity. The presence of anti-ADAMTS13 inhibitory antibodies was not associated with increased complement activation.

Conclusion: These data document for the first time in a clinical study the potential contribution of complement activation to the pathogenesis of TTP.

PROPHYLACTIC PLASMA EXCHANGE (PE) AND ECULIZUMAB (E) ALLOW LONG TERM RENAL FUNCTION PRESERVATION IN *CFH* RELATED HEMOLYTIC UREMIC ATYPICAL HEMOLYTIC UREMIC SYNDROME (AHUS)

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Background: In patients with atypical hemolytic uremic syndrome (aHUS) known to have a mutation in the complement factor H gene (*CFH*), there is a 75% risk of ESRF, a 80% risk of a renal allograft lost to recurrent disease within 2 years of transplant and a 100% risk of graft loss after recurrence.

Patients:We have had the unique opportunity to follow over a 18 y period 3 sisters with aHUS associated with a *CFH* mutation. Two of the sisters are monozygotic twins. A similar natural evolution and response to treatment would be expected for the three patients, as they all presented with the same at risk polymorphisms for *CFH* and *CD46* and no identifiable mutation in either *CD46* or *CFI*.

Method: Because of previous experience of immediate ESRF at the first aHUS episode and loss of 2Tx by recurrence under no or insufficient plasma therapy in the older sister, intensive PE was used at first episode or recurrence: daily sessions until pl.creat normalisation followed by progressive tapering and indefinitely prolongation at a frequency depending on patient necessity; in case of Tx, one pre-Tx session followed by daily PE for one week and progressive tapering and indefinitely prolongation.

Results: a/ older sister: after 14 y dialysis and 2 immediate Tx losses by recurrence, successful third Tx (Tx3) despite recurrence at month 8. Severe cerebral artery stenosis present after 12 y dialysis. At month 12 post-Tx3, PE was stopped and patient was shift to EC because of allergy to plasma. Pl.creat: 125 µmol/L 3 years post Tx3, at 20 y age; b/ twin 1 : after 2 years dialysis, successful first Tx (Tx1) despite several CMV infection-associated recurrences. Pl. creat: 150 µmol/L at age 17 y, 8 y after Tx1. c/ twin 2: complete recovery after first episode. Pl.creat. 58 µmol/L at age 17 y, 10 y after first HUS episode. No cerebral artery stenosis demonstrated in both twins after 10 y evolution.

Conclusions: Intensive en prophylactic PE, eventually shifted to EC allow long term preservation of renal function of native kidney and of transplant and might prevent large vessels stenosis in *CFH* mutation aHUS.

SUCCESSFUL LONG-TERM OUTCOME AFTER RENAL TRANSPLANTATION FOR ATYPICAL HUS (aHUS) WITH COMBINED MCP AND COMPLEMENT-FACTOR I (CFI) MUTATION

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²*Children's Hospital Lucerne, Switzerland*

Background:

The risk of post-transplant recurrence of aHUS in children is high. Guidelines for a mutation-associated management after renal transplantation are not yet established. The risk of recurrent aHUS depends on the type of genetic abnormality and is higher in mutations of genes encoding circulating complement regulators and lower in those encoding membrane cofactor protein (MCP). We describe the successful post-transplant follow-up of a child having aHUS based on the combination of MCP and complement factor I (CFI) mutation.

Patient:

An 8 ½ year-old boy with aHUS based on an MCP and CFI mutation (both parents are healthy carriers of each heterozygous mutation) was on peritoneal dialysis during 4 years followed by renal transplantation at the age of 5 years (deceased donor). Initial immunosuppression consisted of cyclosporine A (CyA), mycophenolate mofetil and prednisone. After biopsy proven steroid-resistant rejection (BANFF IIA), CyA was switched to tacrolimus 7 days after transplantation. Interventions to prevent aHUS recurrence included regular administration of fresh frozen plasma (FFP, Octaplas®), initially daily, gradually tapered to a maximum of every 2 months and finally stopped 3 years after renal transplantation.

Despite recurrent viral infections (as possible triggers of aHUS recurrence) during the last 8 months since FFP had been stopped, there were no clinical or laboratory signs of recurrent aHUS. The patient is doing well and glomerular filtration rate is stable (82ml/min / 1.73 m²).

Conclusion:

We describe the successful post-transplant follow-up of a child with combined MCP and CFI mutation. Even after stopping the FFP administration, there were no signs of recurrence. The combination of MCP and CFI mutations in children suffering aHUS seems to have a positive impact on post-transplant follow-up possibly predicting a low risk of aHUS recurrence.

INCIDENCE, ETIOLOGY AND OUTCOME OF EHEC-HUS IN NORWAY, 1999-2008

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Short introduction. There has been considerable focus on hemolytic-uremic syndrome (HUS) and enterohemorrhagic Escherichia Coli (EHEC) is increasing in Norway after an outbreak of O103H25 in 2006, where 10 of the affected children developed HUS, one with fatal outcome. There is scarce knowledge concerning incidence, epidemiology and clinical presentation, treatment and outcome.

Aim of the study. Our aim is to identify age-specific incidence and mortality rate, gender and etiologic distribution, and describe clinical aspects of overall HUS and specifically EHEC-HUS, among children (0-16 years) in Norway, 1999-2008.

Methods. This is a retrospective, descriptive study, based on data from patient journals from pediatric departments in Norwegian hospitals. Search criteria were ICD-10 codes D59.3 (HUS) and N17 (ARF). Inclusion criterion was the characteristic clinical triad. In complicated cases, reported schizocytes in blood smear and hemolysis, with elevated LD, were diagnostic.

Results. In total, 49 HUS cases were identified, 24 (49,0%) of these confirmed EHEC-HUS (11 (45,8%) O103, 5 (20,8%) O157). Estimated overall yearly incidence rate is 0,51 per 100000 children/year, peaking at 0-4 years with 1,29 per 100000 children/year. For EHEC-HUS the estimated rate is 0,24 per 100000 children/year, peaking at the same age group (0,68 per 100000 children/year). 17 (70,8%) of EHEC cases were female. Patients in this group spent an average 24 days in hospital, during which 17 (70,8%) developed anuria, and 18 (75,0%) needed dialysis (average 14,4 days). 1 year after hospital admittance, 12 (25,5%) still had proteinuria and 9 (19,1%) moderate hypertension. One required a kidney transplant. Mortality rate for EHEC-HUS was 8,7%.

Conclusion. Incidence rate of HUS and EHEC-HUS is low in Norway. Both groups show peak incidence in age group 0-4 years. Clinically, developing dialysis-dependent anuria is common. Percentage of mentioned sequelae is consistent with reports. Mortality rate for EHEC-HUS is high compared to other reports.

HUMAN SERUM PROTECTS HUMAN PODOCYTES FROM SHIGATOXIN INDUCED INCREASE IN CASPASE 3 ACTIVITY

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Introduction. Hemolytic uremic syndrome (HUS) is one of the most frequent causes for renal insufficiency in children. Typical HUS is induced by infection with Shigatoxin (Stx)- producing E. coli. Podocytes are an important part of the glomerular filtration barrier, that express high numbers of Stx-binding GB3-receptors and thus are targets in typical HUS.

Aim of the study. The aim of the study was to evaluate the effect of Stx on cultured human podocytes and to identify influencing factors *in vitro*, such as components of the complement system (e. g. factor H).

Methods. Human podocytes in tissue culture were incubated with Stx. Medium for incubation was supplemented with either fetal bovine serum (FBS) or normal human serum (NHS). For complement inactivation, NHS was heated to 56°C. To test the influence of factor H, polyclonal antibodies against factor H, pure factor H, or serum with a known factor H (SCR20) mutation were used. Human albumin or immunoglobulin served as controls to assess unspecific binding of Stx. Cell-lysates were tested for caspase 3 activity.

Results. Stx induced an increase in caspase 3 activity in a time and dose dependent manner. The increase was markedly less when NHS was present in the incubation medium compared to FBS and decreasing the content of NHS increased caspase 3 activity. In the search for putitive factors in serum causing this protection no reduction of caspase activity under serum-free conditions was observed by addition of pure factor H (and also not by human albumin or immunoglobulin). Furthermore, the decrease in Stx-mediated caspase 3 induction by NHS was also not neutralized by factor H antibodies or achieved by using mutated factor H or heat inactivated human serum.

Conclusion. Thrombotic microangiopathy contributes to renal insufficiency, but the definitive role of podocytes in HUS is not completely understood. We could show that Stx induces apoptosis in human podocytes *in vitro*, suggesting that they are an important target for Stx *in vivo*. Human serum, seems to protect podocytes from Stx-induced apoptosis. According to the present findings, this protection does not appear to be due to unspecific binding of Stx to abundant serum proteins, including factor H.

BINDING OF SHIGA TOXIN TO FACTOR H-RELATED PROTEIN 1

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Introduction: Typical hemolytic uremic syndrome (HUS), an acute renal disease, is mainly caused by infections with enterohemorrhagic *E.coli* (EHEC) strains. Shiga toxin 2 (Stx2) is a major virulence of EHEC. Recently, an involvement of Stx2 in complement activation and its binding to complement regulatory protein factor H has been described. The aim of our study was to investigate the binding of Stx2 to another member of the FH family, factor H-related Protein 1 (FHR1).

Methods: The experiments were performed by ELISA. Stx2 was immobilized onto microtiter plates. After blocking, FH or FHR1 were introduced, and the bound proteins were detected with polyclonal Factor H antiserum and a secondary anti-sheep IgG conjugated with alkaline phosphatase. The reaction was developed with the chromogen substrate 4-nitrophenylphosphate, and absorbance was measured at dual wavelengths of 415 and 490 nm.

Results: Stx2 does not only bind to FH, but also to FHR1 in a dose dependent manner and, in addition, FHR1 binding appears to be more pronounced than FH binding. FH binding is dose-dependently decreased in the presence of increasing concentrations of FHR1. Stx2 binds to the short consensus repeats (SCRs) 3-5 of FHR1, resembling SCRs 18-20 of FH, while it does not bind to SCRs 1-2. Two allotypes of FHR1 (FHR1*A and FHR1*B) also bind to Stx2 in dose dependent manner and it appears that Stx2 binds better to FHR1*A which is found less frequent in atypical HUS than to FHR1*B. In addition, it is the Stx beta subunit which binds to both FH and FHR1.

Conclusion: In addition to FH, Stx2 can also bind to FHR1 in which SCRs 3-5 represent the binding site. Both proteins compete for binding to Stx2. Stx2 binds to FHR1*A better than FHR1*B, suggesting that this may give some protection against typical HUS. In addition, the Stx beta subunit which is responsible for binding to receptors on host cells, is the region of the toxin that binds to FH and FHR1.

COMPLEMENT-COATED RED BLOOD CELL MICROPARTICLES ARE RELEASED DURING HEMOLYTIC UREMIC SYNDROME

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Background and aim: Shiga toxin-producing enterohemorrhagic *Escherichia coli* are associated with hemolytic uremic syndrome (HUS) characterized by hemolysis, thrombocytopenia and acute renal failure. Shiga toxin binds to globotriaosylceramide, also known as CD77 or the P^k histo-blood group antigen. In this study we investigated plasma samples from HUS patients (n=12) for the presence of C3 and C9-coated microparticles derived from red blood cells (RBCs). Furthermore, we investigated the interaction of Shiga toxin 2 with RBCs regarding C3 deposition and release of RBC-derived microparticles bearing C3.

Methods: Microparticles were isolated from plasma samples of patients and controls and Shiga toxin binding and C3 deposition investigated by flow cytometry. RBCs of common P1PK blood group system phenotypes (i.e. P₁ and P₂) as well as RBCs from individuals with the rare P₁^k and P₂^k phenotypes that express high levels of P^k antigen were included in the study.

Results: Patients exhibited high levels of C3 and C9-coated RBC-derived microparticles during the acute phase of disease but levels decreased after recovery and were then comparable to pediatric controls (n=4). Shiga toxin bound to RBCs of the P₁^k and P₂^k phenotypes but C3 deposition was not detected. Incubation of P₁ and P₁^k RBCs with Shiga toxin 2 led to release of RBC-derived microparticles. These microparticles exhibited minimal Shiga toxin 2 on their surfaces but were coated with C3. This effect was enhanced by co-stimulation with *E. coli* O157-lipopolysaccharide.

Conclusion: C3 and C9-coated RBC microparticles are released during acute phase of HUS. Shiga toxin 2 induced the release of C3-coated microparticles from RBCs. Future studies will investigate if this is a destructive process ultimately leading to cell lysis or a protective mechanism by which complement deposits bud off the cell to prevent lysis.

PHENOTYPIC EXPRESSION OF ADAMTS13 IN GLOMERULAR ENDOTHELIAL CELLS

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Background and aim: ADAMTS13 is the physiological von Willebrand factor (VWF)-cleaving protease. The aim of this study was to examine ADAMTS13 expression in kidneys from ADAMTS13 wild-type (*Adamts13^{+/+}*) and deficient (*Adamts13^{-/-}*) mice and to investigate the expression pattern and bioactivity in human glomerular endothelial cells.

Methods and results: Immunohistochemistry was performed on kidney sections from ADAMTS13 wild-type and ADAMTS13-deficient mice. Phenotypic differences were examined by ultramorphology. ADAMTS13 expression in human glomerular endothelial cells and dermal microvascular endothelial cells was investigated by real-time PCR, flow cytometry, immunofluorescence and immunoblotting. VWF cleavage was demonstrated by multimer structure analysis and immunoblotting. ADAMTS13 was demonstrated in glomerular endothelial cells in *Adamts13^{+/+}* mice but no staining was visible in tissue from *Adamts13^{-/-}* mice. Thickening of glomerular capillaries with platelet deposition on the vessel wall was detected in *Adamts13^{-/-}* mice. ADAMTS13 mRNA and protein were detected in both human endothelial cells, and the protease was secreted. Proteolytic activity was demonstrated in glomerular endothelial cells as cleavage of VWF.

Conclusions: Glomerular endothelial cells express and secrete ADAMTS13. The proteolytic activity could have a protective effect preventing deposition of platelets along capillary lumina under the conditions of high shear stress present in glomerular capillaries.

RECURRENT ATYPICAL HEMOLYTIC UREMIC SYNDROME IN A 13.5-YEAR-OLD BOY

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Recurrence of hemolytic uremic syndrome (HUS) is usually a risk factor for unfavorable course of disease and a sign of poor prognosis for adequate renal function.

The authors describe a case of 13.5-year-old boy with atypical HUS. The first episode was in October 2008, after the upper respiratory tract infection (purulent pharyngitis), the second one in February 2011. Both relapses had almost identical manifestations. In 2008 and 2011, 4 and 5 hemodialysis were performed, respectively before HUS resolved. In 2008 the boy required two blood transfusions, in 2011-none. Initial platelet counts dropped to $7-8 \times 10^9/l$ (each episode), without any bleeding. eGFR ranged 35-40 ml/min. After both episodes eGFR remained normal. No antibodies to erythrocytes were detected at any time. On peripheral blood smear schistocytes were present 4-8/field, with poikilocytosis and anisocytosis of erythrocytes. Reticulocyte counts were slightly elevated. Hiperbilirubinemia was moderate during both episodes. Complement C₃ and C₄ levels were within the normal range. There were no antibodies ANA, dsDNA, pANCA, cANCA detectable, no hypertension. Both times D-dimmers were initially elevated. In 2011 he was treated with low molecular heparin injections. EBV was positive in IgG fraction, negative in IgM from 2008. Past history regarding the family, pregnancy, labor, and neonatal period was unremarkable. He underwent tonsillectomy and adenotomy at the age of nine. Antibodies against factor H have not been detected.

Multiple questions arise about the cause of this recurrent HUS, the mild character of the disease during the follow-up observation, and the possibility of next episodes.

In conclusion, it is difficult to predict further disease course and treatment strategy in such patient. Extended laboratory examinations should enable selection of the optimal management for recurrence of HUS, either dialysis, plasma therapy, intravenous immunoglobulins or low-molecular heparin.

DIAGNOSTIC DIFFICULTIES DURING ASSESSMENT α -HUS-PATIENT FOR RENAL TRANSPLANTATION - A CASE REPORT

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Background: α -HUS is a rare cause of ESRD. The problem arises during selecting patients for renal transplantation, in whom ESRD developed in the course of α -HUS. In such cases, genetic testing should be performed (C3, deficiency of factor H, I, MCP). A risk of recurrence of α -HUS after ktx and graft loss is dependent on the type of defect (the highest risk of recurrence ~ 80% for CFH and CFI mutations, ~ 20% MCP). Poland does not recommend a living donor for patient with α -HUS. Kidney transplantation from a cadaveric donor is acceptable, although there is a high risk of recurrence of the primary disease and graft failure. We present the case of a patient after ktx with ESRD in the course of α -HUS with an obvious family history, in which, due to the lack of real opportunities, genetic testing proving α -HUS was not performed.

Description: The patient, age 25, previously with no signs of a disease, suddenly developed hemolytic anemia, thrombocytopenia and acute renal failure. Two years earlier, in two brothers among nine siblings had similar symptoms (one of the brothers died suddenly with symptoms of haemorrhagic diathesis, the other one requires hemodialysis). The patient was treated with steroids, plasmapheresis, FFP infusions, but renal function did not improved and hemodialysis were continued. The patient was highly motivated to ktx so he was referred to pretransplant assessment. Unfortunately, genetic testing for α -HUS is not available in Poland and testing in a foreign laboratory could not be covered by health care insurance. Due to economical reason we did not perform genetic testing abroad.

Patient was informed about the increased risk of graft loss and underwent ktx from a cadaveric donor with immunosuppressive regimen: GS + TAC + MMF. Three months after ktx protocol biopsy was performed - no signs of TMA. Currently, five month after ktx, graft function is stable, serum creatinine 1.5 mg / dl.

Discussion: The case illustrates the difficulties in application for ktx α -HUS-patient. We did not manage to perform necessary α -HUS genetic testing due to price, what became a serious concern. The highest risk of recurrence of TMA and graft loss occurs in the first 6 months. CNI-avoidance regimen does not reduce the risk of recurrence of TMA.

Uneventful course after ktx might be determined by the presence of mild defects of complement or other cause, what is elusive for the authors.

Conclusion: The lack of widespread access to specialized α -HUS genetic testing impeded qualification for ktx and forces the doctor to make decisions based on clinical experience and personal intuition.

Anti-Factor H autoantibody is present in the peritoneal dialysis effluent of a patient with autoimmune form of hemolytic uremic syndrome

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Background: Peritoneal dialysis (PD) is a method of renal replacement therapy used in ~12% of dialysis patients worldwide to restore the composition of the body's fluid environment back to normal. Previous works analyzing samples from pediatric PD patients have described the representative proteom of PD effluents (PDE), and showed plasma proteins and extracellular matrix proteins as major constituents. Several complement proteins including factor H and CFHR1, and immunoglobulins have been demonstrated in the PDE proteom.

Aim: The aim of the present work was to analyze whether pathogenic anti-actor H autoantibodies are present also in the PDE of an end-stage renal disease patient with autoimmune form of aHUS receiving PD.

Case history: The patient (HUN11) first presented at our clinic with symptoms of icterus, oedema, petechiae and vomiting. The laboratory results showed anaemia, thrombocytopenia, high levels of LDH, serum creatinine and proteinuria with hematuria. Based on these results we started to treat the patient with the diagnosis of HUS. Subsequent investigations suggested factor H deficiency, therefore, plasmapheresis was applied ten times followed by regular fresh frozen plasma substitution. In the next 7 months the patient experienced three acute episode of the disease. After the third episode she developed chronic renal failure, therefore she is on peritoneal dialysis since that time. Further complement investigation confirmed anti-FH autoantibody positive form of aHUS. In the last 2.5 years, since we started the peritoneal dialysis, in spite of anti-FH positivity, the patient did not develop acute episode of HUS without receiving immunosuppressive treatment.

Methods: Our patient is currently on continuous ambulatory dialysis (CAPD), four times per day, 40ml/bwkg with 1.5% glucose and lactate/bicarbonate-buffered solution. The presence of anti-FH IgG autoantibodies in PD effluent and serum was investigated by ELISA and western blot. Factor H antigen levels were measured by sandwich ELISA, and genetic analysis was done by MLPA.

Results: The presence of anti-FH IgG was clearly shown in the PDE fluidum by ELISA and confirmed with western blot analysis. The binding of anti-FH from PDE to purified factor H could be blocked in competitive ELISA. Furthermore, the anti-FH IgG in the PDE also recognized CHR1 alpha and beta isoforms. The amount of Factor H antigen in the PDE was 4 mg/L.

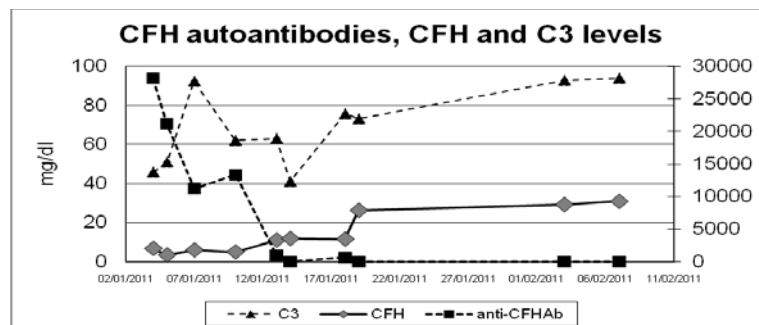
Conclusion: These data indicate that, as components of the total serum IgG pool, the pathogenic anti-FH IgG molecules are also filtered or transported across the peritoneal membrane during PD. Therefore, this mechanism can be considered as a potential therapeutic modality to decrease anti-FH IgG serum levels. It is currently unknown whether the crossing of peritoneal membrane of anti-FH IgG happens as free IgG or as specific immunocomplexes together with factor H.

EARLY DIAGNOSIS AND REMISSION IN A CHILD WITH CFH-AUTOANTIBODIES MEDIATED ATYPICAL HUS

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11% of children with aHUS belong to DEAP-HUS subgroup, defined by CFH acquired dysfunction secondary to CFH-AutoAb, often associated with CFHR1 & CFHR3 genes deletion. Early recognition is crucial as plasma exchange (PEX) and immunosuppressive (IS) treatment achieve remission and prevent disease progression. *Case report:* previously healthy 7 y old girl was admitted for HUS and vomiting, but lack of diarrhea. At admission she was alert but pale, with data of microangiopathic hemolytic anemia (Hg 7.9 g/dl, Hct 22.4%, LDH 4401 U/L, haptoglobin <0.0775 g/L, 2% schistocytes, 49.000/10³ platelets, and renal failure (urea 347 mg/dL, Cr 5.8 mg/dL). She went into spontaneous remission (urea 19 mg/dL, Cr 0.79 mg/dL, platelets 200.000/10³), but suffered a severe relapse 2 weeks later. C3c was low (46 mg/dL; normal 77-210) meaning increased hemolytic activity (53%) of C'alternative pathway. CFH deficiency (6.8 mg/dL; normal 12-56) secondary to positive CFH-autoAb (28160 U/mL) was diagnosed, and CFHR1 & CFHR3 genes deletion demonstrated. Repeated PEX and IS led to CFH-autoAb disappearance, C3c (94 mg/dL) and CHF normalization (31 mg/dL), and HUS remission. 3 HD were needed. 2 months later CFH-autoAb level continues negative despite progressive PEX reduction, and IS tapering, and patient´s renal function is normal (Figure 1). *Comment:* early recognition of DEAP-HUS supports PEX and IS therapy to remove CFH-autoAb and improve outcome.



NOVEL DNA ALTERATIONS IN FCN1 AND FCN2 GENES IN ATYPICAL HUS PATIENTS

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Aim of the study Atypical hemolytic uremic syndrome (aHUS) results from defective complement control on the cell surface and is associated with mutations affecting alternative complement pathway, clusterin, thrombomodulin and with autoantibodies to Factor H. The aHUS etiology is of prognostic value for the patient, particularly for renal transplantation. Nevertheless, in 50% of cases genetic cause is not found. Lectin complement pathway activation is associated with glomerular damage in IgA nephropathy. In this study we screened the aHUS patient cohort for sequence variations in genes encoding ficolins (pattern recognition molecules) of the lectin pathway to explore whether this pathway might be involved in complement damage to the glomeruli in aHUS.

Methods The *FCN1*, *FCN2* and *FCN3* genes were analyzed by means of PCR and DNA sequencing using genomic DNA of 56 aHUS patients and of 141 healthy controls. Impact of the found missense sequence variations on the protein function was analyzed using prediction software and available structural data.

Results One and three sequence variations were found in *FCN1* and *FCN2*, respectively, in four different aHUS patients. These variations resulted in amino acid substitutions. None of these changes were present in dbSNP, described before, or found by us in a group of 141 control individuals. No alterations were found in the *FCN3* gene. The observed sequence variations are predicted to affect protein function.

Conclusion Novel aHUS predisposing alterations in *FCN1* and *FCN2* are described. Our results suggest that the lectin pathway might be involved in the pathogenesis of aHUS.

SEVERAL GENETIC ABERRATIONS IN DIFFERENT COMPLEMENT GENES IN A PATIENT WITH MPGN II

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Introduction Membranoproliferative glomerulonephritis type II or dense deposit disease (MPGN II) is characterized by onset of hematuria and/or proteinuria, acute nephritic or nephrotic syndrome. The exact pathogenesis of MPGN II is still not known, but there is an involvement of the complement system (low C3 levels). In some patients, it is associated with C3-nephritic factor (C3NeF), which stabilizes the C3 convertase C3bBb, with mutations in the alternative complement regulator Factor H (CFH), or with autoantibodies against CFH (αFH).

Aim of the study Identify potentially pathogenic genetic aberrations in genes encoding proteins of the alternative and/or terminal complement pathway in a MPGN II patient with low C3 values.

Methods In one MPGN II patient, mutational screening was performed in alternative pathway genes *CFH*, *CFI*, *MCP*, *CFHR5*, *C3*, *CFB*, and *CFD*, and the membrane attack complex genes *C8A*, *C8B*, and *C9*, by means of PCR on genomic DNA and sequence analysis. Potential pathogenicity of genetic alterations was checked in literature, evolutionary conservation, and *in silico* mutation prediction programs.

Results The male patient presented at six years of age with hematuria without other symptoms. C3 values were low (<0,5 mg/l; normal values: 0.9-1.8 g/l). MPGN II was identified in a renal biopsy. The patient is C3NeF positive, but αFH negative; no drusen are present, yet. C3 values continue to be extremely low, indicating ongoing activation of the alternative complement pathway. A genetic aberration was found in *CFD* (A41P), in *C3* (K155Q), and in *C8A* (A221E). Prediction models for structural influence of the mutations will be displayed. Family screening showed that only the MPGN II patient carried all three genetic variations; family members were not screened for C3NeF, yet.

Conclusion In our MPGN II patient, three potentially pathogenic genetic aberrations are found in complement genes of the alternative pathway and the terminal pathway, which have not been associated with MPGN II, previously. In the patient, a combination of three sequence variations was found, while healthy family members carried only one or two of these variations. This indicates that, next to C3NeF, a combination of genetic defects in the complement system may contribute to the persistent low C3 level and might be needed to display the disease.

MUTATIONS IN COMPONENTS OF COMPLEMENT AND IMPROVED GENETIC TESTING BY NEXT-GENERATION SEQUENCING (NGS) IN PATIENTS WITH AHUS

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Hemolytic uremic syndrome (HUS) is characterized by hemolytic anemia, thrombocytopenia, and impairment of renal function, and is the most common cause of acute renal failure in childhood. Most cases occur secondary to infection with Shiga toxin-producing bacteria. About 10% of HUS patients are classified as atypical (aHUS) with a generally poorer prognosis. Genetic complement abnormalities play a major role in aHUS and lead to uncontrolled overactivation of the alternative complement pathway. While the glomerular vasculature of the kidney is the main target, about 20% of patients additionally show extrarenal involvement (CNS or multivisceral).

There is increasing evidence for a multiple-hit scenario in aHUS with complex inheritance, i. e. mutations in genes encoding complement regulatory proteins or complement activators predispose to aHUS rather than being causative *per se*. According to the guidelines of the European Paediatric Study Group for HUS (Ariceta et al., *Pediatr Nephrol* 2009) genetic testing of *CFH*, *CFI*, *MCP*, *THBD*, *CFB* and *C3* is indicated in all patients with aHUS, even if plasma levels are normal. Genotype-phenotype correlations make genetic testing an essential part of clinical management; the genetic predisposition often determines the prognosis after the initial HUS episode and after renal transplantation. While patients with *CFH* mutations usually have the worst prognosis, the outcome of patients with *MCP* mutations is relatively good. Early intensive plasmatherapy should be started as early as possible and appears to have a beneficial effect, except in *MCP*-mutated patients.

We present our molecular findings in patients with aHUS. Our data corroborate that many patients carry an alteration in more than one gene which may aggravate the clinical phenotype in line with a mutational load model. Thus, genetic testing for all susceptibility factors is necessary. Its complexity makes interpretation challenging and requires an interdisciplinary approach in diagnostics and treatment of patients with aHUS.

We also present a novel genetic testing approach in patients with aHUS based on Next-Generation Sequencing (NGS) that allows simultaneous investigation of multiple genes in a time- and cost-efficient manner. Next-Generation Sequencing (NGS) will considerably improve genetic diagnostics and clinical management of patients with aHUS and is established in our lab.

SUCCESSFUL TREATMENT WITH WEEKLY PLASMA INFUSIONS IN A BOY WITH ATYPICAL HEMOLYTIC SYNDROME

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A previously healthy boy was diagnosed with atypical hemolytic uremic syndrome at the age of 5 years. He had anemia, thrombocytopenia and serum creatinine 150 $\mu\text{mol/L}$. He was hypertensive. Complement analysis was normal. Treatment was started with plasma but was shortly converted to plasma exchange (PE). After 5 sessions of PE serum creatinine was 70 $\mu\text{mol/L}$, hemoglobin 90 g/L and platelet count $30 \times 10^9 /\text{L}$.

Extended complement analyzes revealed impaired alternative complement pathway, but normal factor H, factor I and von Willebrand factor-cleaving protease. Regular weekly plasma infusions (Octaplas) were started. Serum creatinine stabilized around 60 $\mu\text{mol/L}$, hemoglobin and platelet count normalized slowly over several months.

6 months after the initial episode there was a relapse which was treated with 8 PE sessions with prompt effect.

Weekly plasma infusions continued. When trying to extend the treatment interval there was a drop in platelets and the weekly treatments were resumed.

Now at the age of 11 years, 6 years after diagnosis, the boy is doing perfectly well. There have been no more recurrences. Last GFR was 90 ml/min/1.73 m². He has mild proteinuria. Platelets and hemoglobin are normal. We have not found any factor H mutations, but further complement genetics have so far not been performed.

What is the prognosis? Further genetic studies? What are the treatment options in the long run? Should the present regime be continued? Can treatment intervals be extended? Is this boy a candidate for eculizumab or liver transplantation?

SUCCESSFUL TREATMENT OF A MPGN2/DDD PATIENT WHO DEVELOPED AUTOANTIBODIES TO COMPLEMENT COMPONENTS C3B AND FACTOR B

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Background Complement is a crucial component of innate immune system and several regulators control this spontaneously activated cascade system. Any disturbance in this self-mediated balance can result in tissue damage or in autoimmune disease. MPGN is a rare kidney disease associated with inappropriate complement regulation. This disease manifests with proteinuria, hematuria and acute nephrotic syndrome. MPGN patients have a relative poor prognosis and most of the patients progress to end-stage renal failure.

Aim Here we report on a special 12 years old MPGN2/DDD patient, who was treated upon autoantibody titers affecting complement pathway in plasma. Aim of our work is to find a suitable therapy of this autoimmune disease and to define the mechanisms how do the autoantibodies lead to disease pathology.

Methods Multiplex ligation-dependent probe amplification (MLPA) was used for screening copy number variations in genes encoding Factor H and Factor H related proteins. The patient was treated with plasmapheresis upon diagnosis of autoantibodies, she received immuno-suppressive therapy, including Rituximab. Autoantibody titers, together with measurement of C3 consumption and Factor B activation were followed closely over the course of therapy. Afterwards, a kidney transplantation was performed. Purified autoantibodies from this patient were used to test activation of complement alternative pathway.

Results This patient was diagnosed with heterozygous deficiency of complement factor H related genes *CFHR1* and *CFHR3*, and with autoantibodies to the central complement component C3b and to Factor B prior to therapy. After treatment, autoantibody titers to C3b were reduced from 0.8 to 0.3, as well as autoantibody titers to Factor B from 1.2 to 0.5 arbitrary units. When autoantibody titers to both C3b and Factor B were reduced to below 0.5 arbitrary units, C3 and Factor B levels went back to normal, kidney transplantation was performed. Kidney function is normal for a period of 18 months, under the regimen of plasmapheresis. Purified autoantibodies from this patient cause C3 convertase activation as determined by increased Factor B turnover B_a in fluid phase, and enhanced C3a release.

Conclusion Eighteen months post transplantation, the transplant is functional and the patient is without recurrence of disease. This demonstrates that autoantibodies to C3b and Factor B are associated with MPGN, and that reduction of autoantibody titers provides an option for successful therapy.

KIDNEY TRANSPLANTATION OF A GIRL WITH DENSE DEPOSIT DISEASE WITHOUT IDENTIFIED MUTATION

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A previously healthy girl was diagnosed with membranoproliferative glomerulonephritis, dense deposit disease (DDD), at the age of 7 years. At diagnosis she had macroscopic hematuria, proteinuria and increased serum creatinine of 270 $\mu\text{mol/L}$. She was hypertensive. She was treated with methylprednisolone pulses followed by oral prednisone and intravenous cyclophosphamide. S-creatinine normalized and proteinuria was reduced. Hypertension persisted and was treated with enalapril and candesartan. Besides alternate day prednisone she was given weekly infusions of plasma (Octaplas). The result of complement analysis showed normal C3, C3d, C5, factor H and factor I but positive C3 nephritic factor (C3NeF). Plasma treatment was then cancelled. Within 2½ weeks she had a relapse which was treated with plasma exchanges and rituximab. She improved but had lost renal function. C3NeF was repeatedly positive, but C3 and C3d were mostly normal. Rebiopsy after 1 year showed 75% glomerular global sclerosis and interstitial scarring. The renal function deteriorated and after another year peritoneal dialysis was started.

Genetics did not reveal any of the known mutations related to DDD (factor H, I, B, MCP). The following polymorphisms were found: Exon4 417A>G, Exon2 94C>T, Exon3 450A>G and Exon6 804G>A.

The girl was not doing very well. She was attending school but had daily nausea and vomiting, poor appetite and severe hypertension that was difficult to control. In this situation we are planning for renal transplantation after 3 weeks of intensive plasma exchange. We aim at returning to weekly infusions of Octaplas. With this regime we hope to avoid recurrence of disease. Is eculizumab an alternative?

ECULIZUMAB EFFECT AND PHARMACOKINETICS IN A NEWBORN WITH ATYPICAL HUS: AN OPTION IN NO-CANDIDATES TO PLASMAEXCHANGE

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Ecuzumab is effective in aHUS due C³ dysregulation, because it inhibits C5a generation & C5b-C9 complex formation. Few data are available in small infants who are not candidates to plasma exchange (PEX). *Case report:* 28 old day infant of 3.6 Kg admitted due severe aHUS (Hgb 9.2 g/dL, Hct 25.2%, platelets 32 x 10⁹/L, schistocytes 6%, LDH 1429 U/L, haptoglobin <0.07/dL) and ongoing renal failure, despite repeated plasma infusions. C³ levels were normal after plasma but meaningless. Homocysteine, methylmalonic acid, & ADAMTS13 levels were normal too. Patient rapidly deteriorated, PEX was attempted but not tolerated, and CVVHD initiated. After 5 days without plasma, low C3 (36 mg/dL), increased hemolytic and CFH activity but normal CFH levels were demonstrated. IV Ecuzumab (300 mg) was initiated and achieved disease remission. However aHUS relapsed afterwards, and higher Ecuzumab dose than expected was required to block C5b-C9. Table 1 shows pharmacokinetic data. Nowadays the disease is in remission, patient has normal renal function but proteinuria, and Ecuzumab is being very well tolerated. *Comment:* Ecuzumab pharmacokinetic studies are needed in infants and children.

Date of Draw	Time of Draw	Time Point	Platelets (x 10 ⁹ /L)	Date of Most Recent Dose	Amount of Most Recent Dose (mg)	PK (ug/mL)	PD (% Hemolysis)	SC5b-9 (ng/mL)
25-ago-10	20:00	0h	62	no dose	0	BLQ	6	55 [*]
29-ago-10	17:00	1h	3	29-ago-10	300	772,6	1	60,9
29-ago-10	19:00	3h	3	29-ago-10	300	696,6	1	59,1
30-ago-10	10:00	18h	6	29-ago-10	300	471,2	0	47,0
01-sep-10	12:00	68h	45	29-ago-10	300	212,1	8	39,6
21-sep-10	22:00	0h	50	10-sep-10	180	19,1	NA	181,4
23-sep-10	09:00	35h	33	22-sep-10	300	509,2	NA	60,2
28-sep-10	10:00	0h	120	28-sep-10	300	169,6	0	31,3
01-oct-10	12:00	74h	162	28-sep-10	300	694,7	2	46,8
15-oct-10	13:00	411h	276	28-sep-10	300	100,5	0	32,2

PLASMA INFUSION RESISTANT AHUS TREATED WITH ECULIZUMAB, AN ATYPICAL CLINICAL COURSE

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Ecuzumab has shown promise in treating atypical HUS (aHUS) in children, a disease with historically staggering end-stage renal failure and mortality rates. We report the case of a 4 year old girl who presented in April 2010 with diarrhea-negative severe abdominal pain, hematuria, proteinuria, hypertension (HTN), hemolytic anemia (HA) [6.3g/dL], and thrombocytopenia [87,000]. She had negative stool cultures, a low C3 [78 mg/dL], and was diagnosed with (aHUS). To date no mutations have been found in Factors H, I, or MCP. She was discharged after 18 plasmaphereses on 6 antihypertensives. Two months later she experienced severe clinical thrombotic microangiopathic (TMA) manifestations of aHUS with severe abdominal pain, HTN, and congestive heart failure. Despite 31 plasmaphereses she had persistent HA, severe HTN (BPs up to 216/141), and respiratory failure. Ecuzumab induction therapy was initiated (600mg on wk 1, 300mg on wk 2) with resolution of the HA and improved BP, and she was weaned off mechanical ventilation. She was started on maintenance ecuzumab infusions (300 mg every 2 wks). She received a partial dose (187.5 mg) of ecuzumab 4 months into therapy, and 1 wk later she developed a HTN crisis, and recurrence of her HA. While her BP levels improved and her HA was successfully treated with an induction dose of ecuzumab, she has had recurrent episodes of vomiting, abdominal pain and HTN crises that resolve with resolution of vomiting. In February 2011 while on maintenance therapy, pharmacokinetics showed full terminal complement inhibition during one of these episodes. Whereas the patient has never developed renal failure, she has persistent hematuria and proteinuria. Patient has been admitted for six urinary tract infections since presentation, and in February 2011 she was found to have a moderate pericardial effusion that resolved after discontinuation of amlodipine. At present, she is on 7 antihypertensives and on ecuzumab 600mg every 2 wks, and her average BP is 115/66 (95th 107/69). Thus, it can be concluded that ecuzumab therapy has proven effective in the treatment of hemolysis [Hb 11.3 g/dL], thrombocytopenia [282,000] and hypocomplementemia [C3 140 mg/dL] in our aHUS patient, and that it plays a role in the control of her HTN. At the same time, numerous re-admissions and non-life-threatening events have occurred during therapy without severe clinical TMA manifestations. The etiology and relationship of these events to ecuzumab therapy remain unclear.

**PRODUCTION AND GLYCO-ENGINEERING OF THERAPEUTICAL HUMAN
COMPLEMENT REGULATORS IN MOSS BIOREACTORS**

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At least three diseases, atypical hemolytic uremic syndrome (aHUS), dense-deposit disease (DDD) and age-related macular degeneration (AMD) are linked to mutations or polymorphisms causing changes in complement regulatory processes. In aHUS these mutations can effect uncontrolled activation of the complement system leading to a thrombotic microangiopathy with end stage renal disease occurring in more than half of the patients within a year and a risk of recurrence after renal transplantation between 20 to 80% dependent to the underlying genetic defect. Mutations in factor H are found in 40 to 45% of the patients with the familial form of aHUS. Therapeutical plasma infusion is performed to substitute deficient factor H because factor H is currently not available as a drug. However, its enormous potential was recently acknowledged by the assignment of the "orphan drug" status by the European Commission.

Recently, the group of Eva Decker was able to synthesize human factor H recombinantly in the moss *Physcomitrella patens*. The moss *Physcomitrella patens* perfectly suits the requirements for the production of complex biopharmaceuticals as this eukaryotic system not only offers an outstanding genetical accessibility, but moreover, proteins can be produced safely in scalable photobioreactors without the need for animal-derived medium compounds. The bioactivity of the produced recombinant factor H was shown by a complement cofactor assay.

This offers the possibility to develop recombinant factor H as a pharmaceutical. In an ongoing project we plan to perform the next essential step in that direction: As factor H and the members of the factor H family are highly glycosylated, a correctly glycosylated product would be mandatory for therapeutic applications. To reach this goal we will create glyco-optimized moss lines producing factor H with a glycosylation pattern adapted to that of humans. The glyco-optimized factor H will be analyzed by activity assays and especially in vitro-complementation with serum of affected patients testing its ability to serve as a future pharmaceutical. For this reason serum of aHUS / DDD patients is needed to perform this study (supported by the "Baden-Württemberg" foundation).

Please contact Karsten Häffner: karsten.haeffner@uniklinik-freiburg.de to include patients / patients serum in this study.

ABOUT INNSBRUCK



Dear Speakers, dear Participants,

We are very grateful that you have planned to visit Innsbruck for the 3rd international meeting on HUS-MPGN-related diseases and we hope we can offer you an interesting and inspiring conference.

Beside work and science we hope you'll find some time to discover the marvellous city of Innsbruck. Innsbruck offers a great variety of possibilities, there is certainly something for everyone.

In the 15th century under the Emperor of Maximilian I. Innsbruck became a centre of European politics and culture. Several buildings in the Old Town - which is near the hotel - date back to his reign. Walking through the small streets in the Old Town and admiring those old buildings is a nice past time. Furthermore shops, boutiques and bars/coffee shops are located there and in the nearby Museumsstrasse and Maria Theresienstrasse.

Innsbruck also offers a lot of possibilities for people interested in sports. Nearby you find a beautiful park and a nice route next to the river for those who like to go running. You will see that Innsbruck is located in a valley which is surrounded by big mountains. For a hiking trip you can start directly at the hotel or go with the Hungerburgbahn, a funicular, to almost the top of the mountains at 2256m above sea level.

A theatre, several cinemas and music festivals/concerts contribute to the cultural life in Innsbruck. The theatre, Tiroler Landestheater, is very close. Further information on the schedule is available under www.landestheater.at. Several museums show objects of arts starting from the Stone Age up to now (Ferdinandeum), give you an insight in the monarchy of the Habsburg dynasty (Hofburg) or show you the traditional way of living in Tyrol (Tiroler Volkskundemuseum).

This is just a glimpse of what you can expect in Innsbruck. If you have any questions don't hesitate to ask one of the organizers.

Looking forward to seeing you soon in Innsbruck

Local organizing team



MAP OF INNSBRUCK:

Goldenes Dachl



Hungerburgbahn



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