Thesis for the degree of doctor of philosophy (PhD)

PREDICTION OF DISEASE PROGRESSION WITH DIFFERENT SEROLOGIC MARKERS AMONG PATIENTS WITH CHRONIC LIVER DISEASE

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UNIVERSITY OF DEBRECEN

Doctoral School of Dentistry

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INTRODUCTION

Chronic liver diseases are characterized by progressive inflammation, tissue necrosis and regeneration of the liver. As they persist for years, fibrosis and later cirrhosis will develop, which is the end-stage of the diseases. According to a publication in 2014, the mortality due to liver cirrhosis was more than one million in 2010 worldwide, which is 1.95% of the overall mortality.

In Hungary, according to the latest (2016) data, liver disease related mortaliy is decreasing, but it is still the 6th most common cause of deaths.

The etiology of chronic liver diseases can be: extreme amount of alcohol consumption, chronic viral hepatitis (B, D, C), different metabolic diseases (Wilson's disease, haemochromatosis), circulatory disorders (portal vein thrombosis, Budd-Chiary-syndrome, right heart failure), non-alcoholic fatty liver disease (NAFLD) and – steatohepatitis (NASH), autoimmun liver diseases (primary biliary cholangitis [PBC], primary sclerosing cholangitis [PSC] and certain drugs. In Hungary and worlwide, the alcohol consumption is the main cause, but the pathogenetic role of obesity and thus NAFLD/NASH is growing.

Duo to the harmful factors, the hepatocytes progressively die, but even in the early cirrhotic stadium, patients are often asymptomatic, thanks to the large reserve capacity of the liver. This condition is called stable or compensated cirrhotic stadium (ST). For the prediction of disease severity Child-Pugh and MELD (Model for End-stage Liver Disease) scoring system is used. Complications can occur in any stadium of the disease progression: upper gastrointestinal tract bleeding (mostly from oesophageal varix rupture), presence of ascites, hepatic encephalopathy, bacterial infections and combination of the above mentioned conditions. This so called „acute decompensation" (AD) of the cirrhosis is a severe, even life threataning condition, these patients often need hospitalization, even in the Intensive Care Unit. In some of the AD patients acute-on-chronic liver failure (ACLF) could develop, which means the impairment of one or more extrahepatic organ and significantly worsen the patients status and short-term mortality. The most common cause of AD are bacterial infections, provoking a four-fold increase in mortality.

Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease characterized by persistent, progressive biliary inflammation, fibrosis and eventually end-stage liver disease. Clinical manifestations and progression of the disease are heterogeneous, the risk of biliary carcinoma is high. The only causative treatment is liver transplantation. No

reliable biomarkers have been identified to this point that able to predict the pace of progression. Consequently, patients with PSC cannot be stratified properly.

The etiology of PSC is poorly understood. Importance of gut-liver interaction in the pathogenesis of the disease however, has been certified by a large body of clinical evidence. On the one hand, there is a close relation of the disease with inflammatory bowel diseases (IBD), up to 80% of the patients have both conditions. On the other hand, in PSC patients with concomitant IBD, high peritransplant IBD activity was associated with higher, while colectomy before liver transplantation with considerably lower recurrent rate of PSC. Experimental findings also supported the role of inflamed permeable gut in the subsequent inflammation of the biliary tract. Patients with PSC display an exaggerated immune response to intestinal endotoxins with a lack of tolerance to repeated endotoxin exposure. Prolonged and active intestinal inflammation interrupts the gut mucosal barrier function with a subsequent endotoxin exposure to cholangiocytes. Disruption of cholangiocytes' tight junctions expose them various substances, such as bile acids, that could promote injury and inflammation. Disruption of cholangiocytes' tight junctions is an important step in the development of PSC in animal models.

Recently, reliable biomarkers of gut barrier function have been identified. Concerning enterocyte integrity, intestinal fatty acid-binding protein (I-FABP), a cytoplasmic protein of enterocytes, was reported as marker of enterocyte damage that could be considered as the "troponin of the gut". Anti-F-actin IgA antibodies (AAA-IgA) directed against intracellular cytoskeletal actin filaments and anti-gliadin IgA antibodies (AGA-IgA) serve as the markers of the structural intestinal mucosal damage. Presence of AAA-IgA strongly correlated with the histological findings of total or subtotal small intestinal atrophy in patients with celiac disease. Presence of AGA-IgA was associated with increased intestinal permeability in patients with cirrhosis and significant portal hypertension.

The possible role of these antibodies in PSC is yet unknown.

The liver plays a central role in the hemostasis by producing procoagulant and anticoagulant proteins. Despite the elongated coagulation parameters (prothrombin time [PT], international standardized ratio [INR], activated partial thromboplastin time [aPTT]), severe bleeding tendency can not be seen, as the decreased synthetic capacity affects both the pro- and also the anticoagulant factors. This altered, rebalanced, but unstable condition can easily change, bleeding- or prothrombotic tendency could develop due to endothel dysfunction, portal hypertension, infection and renal failure.

Von Willebrand factor (VWF) is a large, multimeric protein, playing a central role in platelet adhesion and primary haemostasis. It is produced in endothelial cells and megacariocytes as monomeric form. During multimerisation, it forms low- (LMWM), intermediate- (IMWM), high- (HMWM) and ultralarge molecular weight multimers (ULMWM). HMWM is the hemostatically most active multimer.

The level of VWF and factor VIII. are increased in patients with cirrhosis and correlate with the severity of the disease. There are studies where also a close correlation of VWF level and portal hypertension as well as mortality has been described. According to certain publications, platelet abnormalities (thrombocytopenia, hrombocytopathia), which are common in cirrhosis, can be compensated by elevated VWF.

The activity of VWF is strictly regulated by the VWF cleaving protease a disintegrin-like and metalloproteinase with thrombospondin type-1 motifs 13 (ADAMTS13), as the reactivity of VWF towards platelets is proportional to its multimeric size. In patients with either congenital or acquired deficiency of ADAMTS13, ultra-large VWF multimers are present leading to thrombotic micro-angiopathy, for example, thrombotic thrombocytopenic purpura (TTP). ADAMTS13 is synthesized by hepatic stellate cells, that are generally activated in liver cirrhosis. Previously variable activity and antigen levels of ADAMTS13 have been reported in patients with liver cirrhosis. *Mannucci et al.* and *Feys et al.* discovered that ADAMTS13 activity is significantly reduced in advanced liver disease.

Some researchers raised the possibility of pro-thrombotic tendency in advanced cirrhotic patients. Endothelial dysfunction together with systemic inflammation and bacterial infections can play a major role in the worsening of cirrhosis. There are no solid data about the characteristics of VWF parameters, like antigen level, functional activity, multimeric structure as well as ADAMTS13 in cirrhotic patients with AD as compared to stable (ST) cirrhosis. Since bacterial infections are common in our cirrhotic patients, and VWF is an acute phase protein, the correlation of the systemic inflammation with the alterations of this multimeric protein seem to be important.

AIMS

The aims of this work were to assess the prevalence of a panel of serologic markers that reflect gut barrier dysfunction in a mixed cohort of pediatric and adult PSC patients. We proposed to study the associations between these markers and both the clinical and laboratory characteristics of the disease. Besides, we proposed to exemine, whether serologic markers of gut failure is associated with the longterm disease course in PSC and if gut failure markers has an association with the markers of lipopolysaccharide exposure and mucosal immune reactivity.

We also aimed to analyse the quantitative parameters of VWF and the structure of the ultra large molecule as well as ADAMTS13 antigen and activity levels in patients with cirrhosis either in a stable outpatient cohort or hospitalized due to AD. Furthermore, we aimed to perform platelet adhesion and thrombin generation assay. We planned to clarify the effect of AD and systemic inflammation in patients with cirrhosis on VWF parameters as well as on ADAMTS13 activity and mortality. We also studied, whether the presence of a prothrombotic state can be confirmed in these patients, which also can contribute to the disease progression. Finally, we studied how these hemostatic parameters and C-reactive protein (CRP) effect on mortality.

PATIENTS

PSC patient group

We performed an observational cohort study among adult and pediatric PSC patients recruited in Hungarian referral hepatology centers (Hungarian Autoimmune Liver Disease Study Group). In total 67, well characterized PSC patients with a complete clinical follow-up [adult: 56 (male/female: 40/16), median age at presentation: 29 years (IQR: 19-37), median disease duration: 6 years (3-12) and children: 11 (male/female: 8/3), median age at presentation: 10 years (IQR: 6-12), disease duration: 5 (1-7)] were included between January, 2006 and December, 2007.

Diagnosis of PSC was based on clinical, biochemical, serological and cholangiographic (magnetic resonance or endoscopic imaging) features or, when indicated, on histological findings. Patients with any concomitant malignant disease were excluded. Blood samples and detailed description of clinical phenotypes were obtained at inclusion. Clinical data were determined by thorough review of patients' medical records, which had been collected in a uniform format. Medical records that documented disease phenotype (age at onset, duration, type of PSC - large duct or small duct), presence and type of concomitant IBD, presence of overlap syndrome, presence of cirrhosis and portal hypertension related complications (*e.g.*, ascites, encephalopathy, oesophageal varices or variceal bleeding), prior orthotopic liver transplantation (OLTx), co-morbidities and medication (*e.g.*, ursodeoxycholic acid, steroid, immunosuppressive and/or biological therapy) at inclusion were retrospectively analyzed for the period prior to the prospective follow-up. At enrolment, revised Mayo risk score was calculated and biochemical analyses were performed using standard routine laboratory protocols.

Fifty-five out of 67 PSC patients were available to be enrolled into a prospective follow-up study, where the treating physicians registered laboratory data, imaging and endoscopic findings, medical treatment, date and type of complications (cirrhosis, colorectal cancer, biliary tract cancer: cholangiocarcinoma gallbladder cancer or cholangitis) during regular and additional outpatient follow-up visits and inpatient stays. In Hungary, a follow-up visit is usually scheduled for every 6 mo at a specialized hepatology center (the actual interval varies between 3 mo-6 mo). Collected data were transferred and stored in a database for analysis. On December 1, 2015, all patients' charts and data were reviewed and updated for the data points mentioned above. Adverse outcome was defined as need for OLTx and/or liver-related death (composite end-point). Follow-up for a particular patient was terminated if

there was no further record available or adverse outcome occurred. Cases with non-liver related death were censored at the time of event. Median follow-up from inclusion was 2646 (IQR: 401-3130) days.

One hundred fifty-three healthy controls and 172 patients with ulcerative colitis (UC) as disease control group were included [male/female: 79/93, age: 34 (23-44) years].

Cirrhotic patient group

One hundred fifty-three well-characterized patients with cirrhosis of various causes were included consecutively from our in- and outpatient gastroenterology department into this study. The female/male ratio was 75/78, and the median age was 56 (range: 24–80) years.

The diagnosis was based on clinical, biochemical and imaging investigations aswell as on liver biopsy when available. The aetiology of cirrhosis was alcoholic liver disease (n=106), chronic viral hepatitis C or B (n=34) and others, like primary biliary cholangitis (previously cirrhosis), autoimmune hepatitis or cryptogenic cirrhosis (n=13). This cohort comprises 99 outpatients with ST cirrhosis and 54 hospitalized subjects due to an AD episode. There was no patient included into both groups due to disease progression. The presence of acute-on-chronic liver failure (ACLF) was evaluated retrospectively within the AD group (i.e. additional presence of organ failure(s)), after its definition was published in the CANONIC study. Patients with hepatocellular carcinoma were excluded, as it can influence the levels of VWF:Ag and ADAMTS13. Patients, who use aspirin or any non-steroidal anti-inflammatory drugs or received fresh frozen plasma, blood transfusion or platelet concentrate for 2 weeks prior to the time of inclusion, were excluded.

Ninetytwo healthy age-matched volunteers served as controls [male/female: 45/52, median age at inclusion: 50,5 (IQR: 31,75-64) years].

METHODS

Laboratory methods

Blood samples obtained by venipuncture from the antecubital vein into 3.2% sodium citrate (9:1, v/v) were centrifuged twice (2,000g for 15 minutes), snap frozen in aliquots and stored at –70°C until use.

Commercially available ELISAs were used according to the manufacturer's protocol to determine serologic markers of gut barrier function, lipopolysaccharide (LPS) exposure and anti-microbial antibodies. Positive cut-off levels for individual markers were defined either by the manufacturer's recommendation or by the 95th percentile of healthy controls and used to dichotomize absolute values.

Presence of IgA or IgG type antibodies against F-actin (AAA) and gliadin (AGA) (QUANTA Lite®; INOVA Diagnostics, San Diego, CA) and level of intestinal fatty acid-binding protein (I-FABP) (Hycult Biotechnology, Uden, Netherlands) were determined as the serologic markers of gut barrier function. The cut-off for positivity was 35 U and 25 U for both types of AAA and AGA, respectively.

Level of acute phase protein, lipopolysaccharide (LPS) - binding protein (LBP) and endotoxin core IgA antibody (EndoCAb IgA) (Hycult Biotechnology, Uden, Netherlands) were determined as markers of LPS exposure. The cut-off for positivity was 195 U/mL for EndoCAb IgA.

Of the classic anti-microbial serologic antibodies, anti-OMP Plus antibody (QUANTA Lite®, Inova Diagnostics, San Diego, CA, United States) was detected. This assay detects IgA antibody against multiple bacterial proteins derived from two species of intestinal bacteria (one Gram-positive and one Gram-negative). Neither bacteria are from the phylum Proteobacteria, of which Escherichia coli is a member. The cutoff for positivity was 25 U as recommended by the manufacturer.

Atypical P-ANCA and anti-endomysial (EMA) antibodies were determined by commercially available indirect immunofluorescence test (IIF) as a part of the differential diagnostic procedure of autoimmune liver diseases and celiac disease. Atypical P-ANCA (IgA and IgG) and EMA (IgA and IgG) antibody positivities were considered in case of specific fluorescencent patterns in IIFT (Euroimmun Medizinische Labordiagnostika AG, Lübeck, Germany) at a dilution $\geq$ 1:32 or 1:10, respectively as recommended by the manufacturer.

C-reactive protein (CRP) was determined using the Integra 700 automated analyzer system (Roche, Basel, Switzerland), where 10mg/L cut-off value—proved to be themost

accurate for prediction of infection-free survival,15 and therefore we used this level in this trial.

VWF:Ag was determined using polyclonal antibody against VWF for coating and its horseradish peroxidase-labelled product for detection in an enzyme-linked immunosorbent assay (ELISA). Both antibodies were purchased from Dako (Glostrup, Denmark).

VWF:CB was determined as was described in our previous publication.26 In short, human type III collagen (Sigma-Aldrich, St. Louis, Missouri, United States), 20 μg/mL in sodium phosphate-buffered saline (PBS), pH 7.4 was used for coating, followed bywashing with PBS containing 0.1% Tween-20 (PBS-T). Each well was then incubated with 100μL ofplasmadilutedinPBS-Tat roomtemperature. VWF:Ag and VWF:CB measurements were performed in parallel using the same plasma dilutions. The amount of VWF bound to collagen was detected using the same horseradish peroxidase-labelled polyclonal antibody against VWF (Dako). The World Health Organisation (WHO) 2nd International Standard von Willebrand Factor, Concentrate, National Institute for Biological Standards and Control (NIBSC) code: 09/182 was used for calibration of these methods (NIBSC, UK).

VWF:RCo was determined by using Ristocetin reagent that contained lyophilized platelets (Helena BioSciences, Sunderland, UK) in a Chrono-Log 810-CA lumi-aggregometer (Chronolog, Havertown, Pennsylvania,United States).

VWFpp level was measured by ELISA kit using mouse antibody pair clones CLB-Pro 35 and CLB-Pro 14.3 (Cell Sciences, Canton, Massachusetts, United States), according to the manufacturer's instruction.

ADAMTS13 activity was determined by fluorescein resonance energy transfer analysis,27 using the TECHNOZYME ADAMTS-13 kit (Technoclone, Vienna, Austria), according to the instruction of themanufacturer. ADAMTS13 antigen levels were measured by ELISA as previously published.

ADAMTS13 antigen level in pooled normal plasma was set at 100%.

Multimeric analysis of VWF was determined as previously described. Briefly, VWF multimers were separated by sodium dodecyl sulphate (SDS) 0.8% agarose gel electrophoresis, followed by Western blotting. Bands were visualized by horseradish peroxidase-labelled polyclonal antibody against VWF (Dako) and 3,3'-diaminobenzidine substrate (Sigma-Aldrich). The first 5 bands were considered as low, bands 6 to 10 as intermediate and larger than 10, including the area where VWF did not resolve into bands, as high molecular weight multimers (LMWM, IMWM, HMWM), respectively, as was previously described. Digital images of the bands were obtained by a GS-800 calibrated

densitometer and processed by its QuantityOne software (Bio-Rad Laboratories, Richmond, California, United States). The proportions of the three groups of bands were calculated from the area under the curve (AUC), constructed for all bands. Additionally, all samples were separated on 0.65% gels too and visually analysed for the presence of ultralarge molecular weight multimers (ULMWM) within the HMWM. ULMWM were defined as the high molecular weight bands not seen in controls. The quantity of VWF within each group of multimers was calculated by multiplying the relative ratios of the low, intermediate or high MWMs with the total VWF:Ag level.

The ability of plasma from 10 ST and 10 AD patients with cirrhosis aswell as 10 controls to support platelet adhesion was studied under flow conditions using reconstituted blood. Cellular elements of the reconstituted blood were isolated from healthy, O blood group donors. Adhesion study was completed within 2 hours after venipuncture. Platelet adhesion onto an uncoated polystyrene surfacewas carried out using an Impact-R cone and plate analyser (CPA; DiaMed, Switzerland). Platelet adhesion was quantified using acid phosphatase assay. Method details are the following. Citrated blood was centrifuged to obtain platelet-rich plasma (PRP, 150 g, 10 minutes, 25°C). Both the separated PRP and the remaining fraction of the blood were further centrifuged for platelet-poor plasma (PPP, 2,000 g, 10 minutes, 25°C). After removing the PPP, the cellular partswere suspended in HEPES buffered saline, containing 0.0129 M sodium citrate, 0.005 MD-glucose and 40 g/L bovine serum albumin (suspension buffer) to the original blood volume. Aliquots were pipetted into Eppendorf tubes for each adhesion experiment to obtain a platelet count of 200 g/L and a haematocrit of 0.37 in a final volume of 0.14 mL reconstituted blood. After a washing step (centrifugation at 2,000g, 10 minutes, 25°C), the supernatant was removed and the cells were suspended with the different cirrhotic or control plasmas to a final volume of 0.14 mL. Reagents were purchased from Sigma-Aldrich, Germany. For the adhesion, 0.13 mL of this reconstituted bloodwas pipetted into the wells and submitted at a shear rate of 1,800/s for 2.5 minutes. Each well of the CPA was gently washed five times with PBS to remove unbound platelets and all compartments of the blood. Bound platelets were then lysed with 0.2 mL of lysis buffer containing acid phosphatase substrate: 0.1 M citrate-phosphate buffer (pH 5.4), 0.1% Triton X-100, 0.01 Mp-nitrophenol phosphate. Wells were incubated for 1 hour at 37°C in a humid box incubator and rotated continuously. Out of this mixture, 0.15 mL was added to 0.05 mL 2 N NaOH into a 96-well plate to stop the enzyme reaction. The resultant colour was read at 405/545 nm and the number of the adhered platelets was calculated by the aid of the

standard curve, which was constructed in parallel using the original platelet suspension. Intraassay coefficient of variation for the adhesion results was 12%.

For the thrombin generation assay (TGA) citrated blood samples were centrifuged at 20°C for 15minutes at 3,200g within 1 hour of venipuncture. Plasma was separated and centrifuged again (3,200g, 10 minutes), and aliquots of the supernatant were stored at –70°C. Analysis was performed within 3 weeks. Individual aliquots were thawed in a 37°C water bath by tilting for 15 minutes, vortex mixed for 5 seconds and sampled immediately into black 96-well plates (Greiner GmbH, Germany). TGA were carried out using the Technothrombin TGA kit with fluorescent substrate and reagent C (50 pM tissue factor [TF] and low concentration of phospholipid) according to the manufacturer's instructions. The reader, the plate with plasma and the reagent/substrate mixture were pre-heated to 37°C, and then 0.06 mL reagent/ substrate mixture was added to 0.04 mL of plasma. The enzyme reaction was initiated by TF at a final concentration of 5 pM and detected with a BIOTEK Flx800 reader. Relative fluorescence units measured by the fluorimeter were converted into peak thrombin concentration (nM). A reference curve was constructed using a thrombin standard calibrated against the thrombin reference preparation of the WHO. The TGA kits, the Reader and the software were from Technoclone GmbH.

Statistical analysis

Variables were tested for normality using Shapiro Wilk's W test. Continuous variables were summarized as medians (interquartile range [IQR], 25–75th percentiles). Categorical variables were compared with Fisher's exact test or χ^2 test with Yates correction, as appropriate. Continuous variables were compared with Mann–Whitney U-test or Kruskal–Wallis H test with Dunn'smultiple comparison post hoc analysis. The Spearman's non-parametric rank correlation test was used to determine correlations. To discriminate between survivors and non-survivors, the receiver operating characteristic (ROC) curve was used. AUC and corresponding 95% confidence intervals (CIs) were calculated. Youden index was chosen to estimate the best discriminate threshold. We used Kaplan-Meier analysis to estimate the cumulative probability of 6- month survival of cirrhotic patients and to determine association between serological antibodies and adverse disease outcome (OLTx and/or liver-related mortality) in PSC patients. Differences in observed survival were assessed by the log-rank test. The association of categorical clinical variables or serological antibodies with

adverse disease outcome during the follow-up was evaluated by univariate Cox-regression analysis. Multivariate analyses were performed to adjust for the Mayo risk score or presence of cirrhosis with forced entry method. Associations are given as hazard ratio [HR] with 95% CI. A two-sided probability value of < 0.05 was considered to be statistically significant. For statistical analysis and graphical presentation, the SPSS Statistics (V.22.0/24.0, IBM, Armonk, NY, USA) and Prism (V.6, GraphPad Software Inc., La Jolla, CA, USA) programs were used.

ETHICAL PERMISSION

The study protocol was approved by the Regional and Institutional Research Ethics Committee of University of Debrecen and the National Scientific and Research Ethics Committee (DEOEC-RKEB/IKEB 5306-9/2011, 3515-2011, 3885/2012/EKU [60/PI/2012], 3880/2012/EKU [59/PI/2012]). Each patient was informed of the nature of the study and gave informed consent in writing.

RESULTS

Biomarkers of gut barrier function in PSC and their association with clinical or laboratory characteristics of PSC

A total of 40.3% (27/67) and 22.4% (15/67) of PSC patients were positive for IgA or IgG isotype AAA and AGA, respectively. Antibody prevalence was significantly higher compared to either that in patients with UC [14.7% (25/170), $P < 0.001$ for AAA and 11.6% (20/172), $P = 0.042$ for AGA] or in healthy controls [6.2% (7/113), $P < 0.001$ for AAA and 7.2% (11/153), $P = 0.003$ for AGA]. AGA positivity was mainly IgG isotype (9% *vs* 20.9%), while AAA was of both IgA and IgG isotypes (28.4% *vs* 25.4%) and there was no significant overlap between IgA and IgG subtypes of the given antibody. Likewise, no significant overlap was observed between AAA and AGA. Only 10.4% (7/67) PSC patients positive for either type of these antibodies had both AAA (IgA or IgG) and AGA (IgA or IgG) antibodies.

We analyzed clinical and laboratory characteristics of PSC patients according to their antibody status. AAA antibody status according to its immunoglobulin subtypes was not associated with gender, younger age at diagnosis, presence of cirrhosis, or concomitant IBD. However, values of several biochemical laboratory parameters and the Mayo risk score that indicate more severe disease were significantly higher in the presence of IgA isotype of AAA. In case of IgG isotype of AAA, only a single association was reported, namely the median level of ALP was significantly higher in antibody positive cases compared to antibody negative ones (715 *vs* 493 U/L, $P = 0.048$). Different AGA isotypes, however, were not associated with either the clinical or the laboratory characteristics of more severe PSC phenotype.

The presence of AAA IgG was higher in patients with overlapping autoimmune hepatitis [55.6% (5/9) *vs* 20.7% (12/58), $P = 0.040$] compared to patients without. In contrast, the frequency of AAA IgA was not different between the two groups [33.3% (3/9) *vs* 27.6% (16/58), $P = 0.706$].

Significance of gut failure markers in the risk of the progressive disease course in PSC

Development of colon cancer occurred in two, while biliary tract cancer in one patient during the followup period. Nine patients had at least one episode of cholangitis. Seven patients underwent OLTx. Five patients died due to liver-related complications. Composite end-point (OLTx and/or liver-related death) occurred in a total of 9 patients. One patient died due to acute myocardial infarction, this case was censored at time of death.

We analyzed the association of clinical variables and the different isotypes of AAA and AGA.

In Kaplan-Meier analysis, the median time to OLTx and/or liver-related death was 578 d (IQR: 212-1112). Presence of cirrhosis and increased Mayo risk score (*p*LogRank = 0.040 and < 0.001, respectively), but not gender (*P* = 0.547), age at onset (*P* = 0.845), disease location (*P* = 0.548) or concomitant IBD (*P* =0.762) were significantly associated with faster disease progression.

Positivity for IgA isotype AAA and AGA (*p*LogRank= 0.019 and 0.005, respectively), but not for IgG isotypes of these antibodies (*p*LogRank = 0.665 and 0.130, respectively) predicted OLTx and/or liverrelated death, see Figure 1. Accordingly, univariate Cox regression analysis revealed AAA IgA and AGA IgA-positivity as predictors of poor disease outcome [HR = 4.54 (1.14-18.18), *P* = 0.032 and 5.83 (1.45-23.41), *P* = 0.013]. Thereafter, we used Cox regression analysis to adjust for important clinical variables that influence disease progression. Both IgA isotype AAA and AGA remained significant predictors of disease progression after adjusting for the presence of cirrhosis [HR = 5.15 (1.27-20.86), *P* = 0.022 and 5.07 (1.25-20.54), *P* = 0.023, respectively]. Similar results, but with weakened statistical significance was observed in the case of AAA IgA-positivity [HR = 4.24 (0.99-18.21), *P* = 0.052] after adjusting for the Mayo risk score, however the significant association was lost in case of AGA IgA-positivity [3.67 (0.88-15.30), *P* =0.074].

Role of bacterial translocation and enterocyte damage markers in the risk of progressive disease course in PSC

As a next step, we analyzed various serological markers of bacterial translocation and enterocyte damage. Patients with AAA IgA-positivity had significantly higher EndoCab IgA titers [median (IQR): 123 (93-215) U *vs* 58 (40-92) U, *P* < 0.001] and higher frequency of anti-OMP Plus IgA antibody (36.8% *vs* 10.6%, *P* = 0.012) and also significantly higher level of the enterocyte damage marker, I-FABP [median (IQR): 365 (203-1079) pg/mL *vs* 166 (90-365) pg/mL, *P* = 0.011). However, LBP was not different between AAA IgA positive and negative patients. No other statistically significant association was found in case of AAA IgG or any isotypes of AGA.

Baseline characteristics of cirrhotic patients

Of 99 ST cirrhosis patients included, 51 had Child-Pugh A stage, 43 had Child-Pugh B and 5 had Child-Pugh C cirrhosis. At time of enrolment, 10 patients had stable chronic renal

impairment, with creatinine level between 135 and 329 μmol/L. AD events were defined as either bacterial infection, or HE, or sudden worsening of ascites or uppe rGI bleeding. The majority of the 54 AD patients had advanced cirrhosis, Child-Pugh B/C stage, but 6 patients had early Child-Pugh A cirrhosis. Thirty patients out of 54 had bacterial infection, with tense ascites in 17 patients, HE in 5 patients and upper GI bleeding in 5 patients. Out of the 24 patients without bacterial infection, 12 had upper GI bleeding (among them the 6 patients with Child-Pugh A stage), 8 had tense ascites and 6 had HE as the leading cause of AD. Out of 54 AD patients, 28 hadmore than one event simultaneously. ACLF was detected only in 17 AD patients, grade 1 in 10, grade 2 in 5 and grade 3 in 2 patients, respectively.

VWF:Ag and its functional activity levels

In cirrhosis, VWF:Ag, VWF:RCo level and VWF:CB activity was higher compared to healthy controls. Patients with AD had even higher values than patients with ST (VWF:Ag % AD vs. ST: 371 [275–513] vs. 269 [228–323], p=0.003; VWF:RCo % AD vs. ST: 180 [148–294] vs. 148 [128–192], p=0.035 and VWF:CB AD vs. ST: 335 [245–405] vs. 201 [156–269], p=0.001). Although both types of functional activities of VWF were elevated in each group of patients, they did not reach the level of VWF:Ag. The VWF:RCo/VWF:Ag ratio in the control group was 0.84 (0.72–0.98), whereas it was lower in both cirrhotic groups. In ST patients 0.55 (0.48–0.70) and in AD patients 0.53 (0.41–0.72) was found. The VWF:CB/VWF:Ag ratio was also reduced in both patient groups with cirrhosis. In ST patients 0.79 (0.65–0.93) and in AD patients 0.83 (0.73–0.93), whereas in controls 0.92 (0.85–1.00) could be obtained. A moderate correlation was found between VWF:Ag and VWF:RCo (r=0.521, p < 0.001), and strong correlation between VWF:Ag and VWF:CB (r=0.835, p < 0.001) as well as VWF:CB and VWF:RCo (r=0.619, p 0.001) in cirrhosis. In both cirrhotic groups, the level of VWFpp was higher as compared to the controls. In the ST group, 222 (169–260)%, in AD patients 257 (196–323)%, while in the controls 108 (99–121)% levels were found. VWF:Ag levels within the AD group significantly differed between patients with (475 [355–649]%) and without (350 [266–446]%) ACLF (p=0.013).

Analysis of VWF multimers

According to the visual analysis, the presence of ULMWM could be identified in 39 (25.5%) patients with cirrhosis. The majority of them was in the AD group (25 out of 54 patients, 46.3%), while 14/99 (14%) were found in the ST group. Computerized analysis of the densitometric curves was next performed, and from the total AUC, the relative ratios of

the LMWM, IMWM and HMWM were determined, respectively. We further analysed the samples according to the presence of ULMWM. The ratios of the LMWMs were 0.28 (0.25–0.31) in controls and the ratios were higher in both ST and AD patients with or without ULMWM. In the samples without ULMWM, we measured 0.38 (0.35–0.42) and 0.39 (0.32–0.46) LMWMs, for ST and AD patients, respectively. In patients with ULMWM, the proportion of LMWM decreased to 0.32 (0.26–0.36) in ST and 0.32 (0.29–0.36) in AD patients but both ratios remained higher than the controls ($p=0.018$ and $p<0.001$, respectively). The proportions of the IMWM did not show any difference between the ST group and the controls. However, in patients with AD, both with and without ULMWM, the ratios were significantly lower as compared to either controls or ST. Analysing the multimer proportions within the HMWM fraction, we observed 0.4 (0.37–0.44) in controls, whereas we detected only 0.30 (0.25–0.34) and 0.29 (0.24–0.39) in patients without ULMWM in ST and AD patients, respectively. Remarkably, in patients with ST as well as AD in the presence of ULMWM the ratios of HMWM were higher and became similar to controls, 0.37 (0.31–0.43) and 0.40 (0.32–0.44), respectively.

Within the HMWM group in the presence of ULMWM a significantly higher quantity of VWF:Ag was detected in both ST and AD patients.

ADAMTS13 antigen and activity levels

In patients with AD, ADAMTS13 activity level was decreased compared to ST and healthy controls (57[29–94] vs. 98 [67-132] and 106 [67–117]%, $p < 0.001$ for both). Similarly, low ADAMTS13 antigen levels were detected in patients with AD compared to ST and controls (66 [43–101] vs. 120 [89–155] and 101 [91–115]%, respectively, $p < 0.001$ for both). We further analysed the ADAMTS13 activity and antigen levels, according to the presence of ULMWM within both the cirrhotic groups. In patients without ULMWM, ADAMTS13 activity levels were not different from controls, in ST 104 (71–137)% and in AD 91 (60–110)% were found. In patients with ULMWM, the values were significantly lower as compared to controls and thosewithout ULMWM. In ST 65 (51–99)% and in AD 33 (24–49)% were measured. Similar alterations were observed for the ADAMTS13 antigen levels, except, that in ST patients without ULMWM, the level was higher than in the controls. In both patient groups with ULMWM, the antigen levels were significantly lower, than in patients without ULMWM. In the AD group, ADAMTS13 activity level in the presence or absence of ACLF was 49 (29–68)% and 70 (44–107)%, respectively ($p=0.171$). We also analysed the correlation between the quantity of VWF:Ag calculated within the HMWM

fraction and ADAMTS13 activity in ST and AD cirrhotic patients as well as in controls. A negative correlation (Spearman's r=–0.43, p=0.01) was found between these parameters in AD patients, that is, the lower the ADAMTS13 activity was, the higher the VWF:Ag level was found in the HMWM fraction. However, in ST patients (Spearman's r=–0.18, p=0.073), as well as in the controls there was no significant correlation (Spearman's r=–0.043, p=0.722).

We then investigated the association between ADAMTS13 activity and systemic inflammation, represented by CRP, in all AD patients. Patients with ADAMTS13 activity less than 50% (calculated by ROC analysis and Youden index), had higher CRP levels compared to those with ADAMTS13 activity above 50% (29.8 [6.6–97.4] vs. 10.9 [7.1–34.5] mg/L; p=0.048). In line with this, in AD patients with ULMWM the CRP level was higher as compared to the oneswithout ULMWM (22.96 [7.1–83.6] vs. 10.9 [4.8–36.8] mg/L; p=0.078).

Platelet adhesion assay

Adhesion of normal platelets showed a stepwise increase in the presence of cirrhotic plasmas, reaching the highest level in AD patients with ULMWM. VWF:Ag level was 106 (86–120)% in controls, 309 (279–327)% in ST and 356 (265–384)% in AD patients. VWF:Ag levels in the absence (n=14) or presence (n=6) of ULMWMin cirrhotic patients, were 305 (261–348)% and 340 (298–384)%, respectively. The VWF:Ag levels were not significantly different.

Thrombin generation assay

Peak thrombin concentration was increased in both cirrhotic groups as compared to controls. We measured 192 (158–238) nM in controls (n=31), 271 (223–293) nM in ST (n=62) and 362 (242–440) nM in AD (n=27) patients.

Association of VWF:CB, ADAMTS13 activity and CRP with survival

Within the ST group 4 out of 99 patients, whereas in the AD group 17 out of 54 patients died. The causes of death were liver-related in all patients, namely, GI bleeding in 3 patients, infection in 11 patients and hepatorenal syndrome in 7 patients. The areas under the ROC curves to predict 6-month survival were 0.708 (95% CI, 0.629–0.778) for VWF:Ag level, 0.717 (95% CI, 0.639–0.787) for VWF:CB level and 0.612 (95% CI, 0.530–0.6 90) for ADAMTS13 activity. The optimum cut-offs for predicting poor 6-month survival according to the Youden index were VWF:CB activity > 245% with 80.95% sensitivity and 58.33%

specificity and ADAMTS13 activity < 50% with 47.62% sensitivity and 82.44% specificity. Patients with either > 245% VWF:CB activity, or < 50% ADAMTS13 activity or > 10 mg/L CRP level had significantly poorer 6-month survival. The 6-month survivals were 74.6% versus 94.4% in patients according to the VWF:CB activity, 69.2% versus 89.5% according to the ADAMTS13 activity and 68.9% versus 92.3% according to the CRP level. Within the AD group, 6-month survivals were 60.1% versus 80.8% for VWF:CB activity, 57.2% versus 72.0% according to the ADAMTS13 activity and 55.7% versus 78.6% for CRP levels. Within the AD group, these survivals showed high numerical difference, but due to the low number of patients, these data did not reach statistical significance.

DISCUSSION

We tested different laboratory parameters, serologic markers for the prediction of disease progression in patiens with chronic liver disease.

The importance of gut-liver interaction in the pathogenesis of PSC has been certified by a variety of clinical and experimental evidence as discussed previously. In the present study, therefore we investigated the clinical importance of different serological biomarkers related to gut barrier function in the prediction of progressive disease course in a cohort of PSC patients. To our knowledge, this is the first study examining disease outcomes in PSC according to these serological markers.

The cytoskeleton protein, F-actin was identified as a specific target of smooth muscle cell antibodies (SMA) in autoimmune hepatitis and for over a decade IgG isotype AAA testing - either by immunofluorescence technique or ELISA - has been incorporated into the diagnostic procedure of the disease. Increased occurrence of this antibody was reported in other autoimmune diseases (*e.g.*, celiac disease or connective tissue disease)[22]. To our knowledge no previous study assessed, however, the prevalence and isotype characteristics of AAAs in PSC.

In the present study, we demonstrated - for the first time - that enhanced AAA formation is a feature of PSC regardless of overlapping AIH. A quarter of our PSC patients showed positivity for AAA that was significantly higher compared to either patients with UC or healthy controls. Contrary to routine laboratory practice, AAA was identified by anti-IgA secondary antibody in addition to anti-IgG one. This approach revealed isotype dependent association of AAA with clinical characteristics of the disease. The presence of IgA, but not IgG type AAA indicated more severe disease at baseline based on Mayo risk score and different biochemical parameters. Concordantly, previous studies in celiac disease reported that the presence of IgA isotype AAA were strongly associated with the degree of active tissue damage of the intestinal mucosa. At the same time, AAA IgA-positivity disappeared parallel to mucosal healing after gluten free diet was introduced. Of note, cases with persistent intestinal mucosa damage despite gluten-free diet, remained positive for AAA IgA antibody.

As a further novel finding of our study, the presence of AAA IgA-positivity predicted faster disease progression during follow-up, even after adjusting for the presence of cirrhosis or the Mayo risk score.

The mechanism how the breakdown of tolerance towards F-actin is associated with the development of enhanced fibrosis and thus disease progression in the liver remaines to be

elucidated. Interestingly, in our study patients with positivity for AAA IgA had an enhanced mucosal immune response to microbial antigens, like endotoxin (EndoCab IgA) or bacterial proteins (anti-OMP Plus IgA). This immune response seems to be restricted to the intestinal mucosal compartment without leading to systemic reaction since serum LBP concentration, the serologic hallmark of systemic LPS exposure were similar in AAA IgA positive and negative cases. This result is in agreement with the findings of previous studies. Namely, portal venous bacteraemia is not frequent in PSC. However, exposure of endotoxins to biliary epithelial cells leads to disruption of enterocytes' and cholangiocytes' tight junctions through TLR4 mediated signaling that is an important step in the pathogenesis in animal models of PSC. Our observation that serum level of I-FABP was significantly higher in the group of patients with AAA IgA-positivity compared to those with AAA IgA-negativity corresponds to these abovementioned literature findings. I-FABP is considered an accurate marker of enterocyte damage, since it is specifically produced by enterocytes and is released to systemic circulation in case of cellular injury during inflammatory processes.

Autoantibodies are generally not pathogenic in PSC, therefore it may be unlikely that the severity of biliary injury is driven by humoral factors. Production of AAA may reflect, however an enhanced (immune) reactivity of lymphocytes towards surrounding tissue and/or cellular debris. Of note, AAA is not organspecific since they may be present in a wide range of immune-mediated diseases other than PSC, as mentioned previously. IgG isotype AAA was associated with disease activity and poor survival in autoimmune hepatitis. In the study of Czaja *et al*. patients seropositive for AAA were more commonly HLADR3 positive, while seronegative patients had higher frequency of HLA-DR4 positivity than healthy subjects. In the same study AAA were also associated with HLA-B8 positivity. Interestingly, in PSC Wiencke *et al.* also demonstrated that HLA-DR3 and B8 are associated with progressive disease course. Conversely, patients with HLA-DR4 do not experience an accelerated disease progression, they also have a decreased risk for disease recurrence after liver transplantation. Based on these previous findings we speculate that the formation of AAA IgA in PSC might reflect a phenotype with distinct immunological function and genetic susceptibility for a more severe inflammatory process, similarly as in autoimmune hepatitis. A recent report on the association between autoantibodies, like atypical P-ANCA and HLA status in PSC might further support this hypothesis. Hypothetical sero-genotype linkage between AAA IgA-positivity and more aggressive HLA genotype warrants further exploration.

In the present study, occurrence of AGA was significantly higher in patients with PSC compared to either that in patients with UC or healthy subjects. Frequency of AGA IgG/IgA

corresponds to the findings of Sjöberg *et al.* (22.4% *vs* 24%). Distinctly only IgG but not IgA isotype were more frequent in our study. Higher frequency of AGA is observed in various disorders, such as celiac disease, neurodegenerative diseases, systemic lupus erythematosus and autoimmune liver diseases. Investigators have attributed the formation of these antibodies to an increased uptake of peptides from the gut lumen to the intestinal mucosa and presence of AGA is considered as a marker of a non-specific immune reaction towards a dietary peptide. Furthermore, Reiberger *et al.* reported a surprisingly high frequency (about 60%) of AGA in patients with liver cirrhosis, mainly of alcoholic origin. Patients with AGA had higher portal venous pressure and increased intestinal permeability assessed by the sucrose-lactulose-mannitol test. Nevertheless, a Swedish study including 22 patients with PSC failed to detect an altered intestinal permeability in PSC. Intestinal permeability in PSC might rather be considered as an immunological barrier dysfunction. We found an association between IgA type AGA and accelerated disease progression in our cohort, however due to the low number of positive cases, this finding should be interpreted with caution. The increased chance of a false positive finding as a result of the small number of positive cases is a limitation to be acknowledged. We speculate that the finding of an earlier study from a French research group might serve as a possible explanation. They showed that AGA IgA facilitated an increased transport of gliadin peptides from the intestinal lumen to the gut mucosa *via* CD71. In the subepithelial space these undigested toxic peptides are able to perpetuate intestinal inflammation. Of note, the design of our study was mainly explorative and did not include experimental methods that could give an exact explanation for the increased risk of disease progression in the presence of specific antibodies.

We performed detailed analyses of both VWF and ADAMTS13 in one of the largest group of patients with cirrhosis, published so far. We have tried to clarify hitherto unanswered questions in our study. Namely, what is the effect of AD and systemic inflammation in patients with cirrhosis on VWF parameters including multimer distribution as well as ADAMTS13 activity as compared to patients with stable cirrhosis; and can the presence of a prothrombotic state be confirmed in these patients?

It is known that VWF:Ag levels are highly elevated in patients with cirrhosis. Several previously published data provide strong evidence that the level of VWF:Ag is related to the severity of the disease, reaching the highest levels in Child C patients. The platelet glycoprotein Ib binding functional capacity of VWF, a feature that is measured by VWF:RCo, is also substantially elevated, albeit not to the same extent as the VWF:Ag level.

We could demonstrate that VWF:CB activity, which measures the binding capacity of VWF to collagen, was even higher than the VWF:RCo as we show here for the first time. Our finding is in contrast to the previous publications of Lisman's group. As they observed, VWF:CB was lower in any type of liver diseases than in controls, despite that this activity is more sensitive to the presence of high multimers than the VWF:RCo. There was, however, a major difference between their and the methodology that we and others are using. Our VWF:CB measurements were done in parallel to the VWF:Ag measurements from the same sample dilutions. Lisman et al, however, added the same amount of VWF:Ag into the wells (5% and 2.5% of the amount of VWF present in pooled normal plasma) for the determination of the VWF:CB, hence their data better reflect the VWF:CB/VWF:Ag ratio, which also in our hands was reduced. Moreover, they demonstrated that plasma from patients either with cirrhosis or acute liver failure induced an increased platelet adhesion to collagen surface, which definitively suggests a well functioningVWF:CB activity. These adhesion results under flow conditions could further confirm our findings.

The relative decrease of VWF:RCo and VWF:CB suggests a significant qualitative alteration of the multimeric structure of VWF. Indeed, we could demonstrate two major types of changes in the multimeric structure of VWF. In the majority of cirrhotic patients, where ULMWM was not present, the relative ratio of the LMWM fraction was increased, while the ratio of the haemostatically most active HMWM was reduced, that is, a shift from HMWM to LMWM could be observed. Remarkably, the absolute quantity of VWF within LMWM showed a threefold and in the HMWM only a twofold increase as compared to controls. Another major change could be seen in almost 50% of the AD patients where the presence of ULMWM of VWF could be detected. In these patients, the relative ratio as well as the absolute quantity of VWF:Ag within the HMWM fraction was much higher than in AD patients without ULMWM. Furthermore, we measured both lower activity and antigen levels of the VWF-cleaving protease ADAMTS13. In contrast, in patients without ULMWM, ADAMTS13 levels were similar to controls. Hence, in the group of patients with advanced liver cirrhosis, we suggest that probably the primary step is a decrease in ADAMTS13, which might be the consequence of the AD event combined with the systemic inflammation, represented by the higher CRP. Within the AD patient group ACLF represents an even more severe disease, in this minor group we found the highest VWF:Ag level and ADAMTS13 was also low. These findings are in line with previous results.

We put forward for consideration on the one hand, an ADAMTS13 independent alteration of VWF in those mostly stable cirrhotic patients in whom ADAMTS13 level is

normal and ULMWM are not present. In these patients, degradation of VWF multimers may be due to other proteolytic enzymes, like plasmin or other serine proteases. On the other hand, in the most advanced cirrhotic patients ULMWM can be detected due to the low level of ADAMTS13. The significantly increased VWF activity in cirrhotic plasmas containing ULMWM was confirmed by our platelet adhesion experiment. Our results suggest that indeed in the most severe cirrhotic patients a pro-thrombotic state can develop, to some extent reminiscent to what happens in TTP. Infections as well as systemic inflammation are supposed to induce a pro-thrombotic tendency in cirrhotic patients. This was proved by several lines of evidence, such as elevation of coagulation factor VIII or VWF levels and platelet activation due to gut-derived endotoxins; or demonstration of increased thrombin generation. Our observations are in line with these previous studies, since we could demonstrate increased platelet adhesion together with increased thrombin generation in half of our AD patients.

ADAMTS13 is secreted by hepatic stellate cells, which are known to be activated in cirrhotic patients. We speculate that in AD the synthesis of ADAMTS13 is reduced, since inflammatory cytokines are known to inhibit ADAMTS13 synthesis by the stellate cells. Furthermore, as the half-life of ADAMTS13 is about 2 to 3 days, the ULMWM can appear within a couple of days. In patients with AD, the very low ADAMTS13 activity together with the presence of ULMWM might represent an already significantly advanced disease stage. From these data, we can propose that multimeric analysis of VWF and ADAMTS13 activity measurement, albeit that they are not routine tests, could help to identify patients with a pro-thrombotic tendency. As standardized ADAMTS13 activity measurement, a WHO international standard and quality controls are available, we propose to measure ADAMTS13 activity especially in those centres where care is taken of the most advanced patients.

The severity of these events is clearly demonstrated by the poor 6-month survivals of these patients. Higher VWF:CB activity (> 245%), higher CRP (> 10 mg/L) as well as low ADAMTS13 activity (< 50%) either in the total group of patients or within the patients with AD resulted in higher mortality. Systemic inflammation together with the activation of the haemostatic system can play a role in the disease progression and this raises the need for thromboprophylaxis in these patients. *Villa et al* could demonstrate that low molecular weight heparin did prevent portal vein thrombosis and delayed hepatic decompensation. No data are available at present for antiplatelet prophylaxis.

There are some controversies onthemultimeric structure of VWF as well as ADAMTS13 levels in cirrhotic patients categorized by the Child-Pugh system. *Lisman et al*

suggested that there are less HMWM compared to controls and very variable ADAMTS13 levels. *Uemura et al* were the only ones who categorized the patients according to three different multimeric patterns, and LMWM dominated in the first group. In the second group, normal multimeric structure was demonstrated, whereas in the third one, the presence of ULMWM was detected. They showed a negative correlation between ADAMTS13 levels and the severity of cirrhosis and suggested an increased intra-hepatic formation of micro-thrombi. However, more than 50% of the Japanese patients (57/109) suffered also from hepatocellular carcinoma which significantly increased the heterogeneity of their patients. In another study, Reuken et al investigated the effect of systemic inflammation, mostly due to bacterial infections, on VWF. Both VWF levels as well as ADAMTS13 levels were very similar to our findings, but they could not demonstrate any change in the multimeric structure of VWF. They used, however, throughout their study, 1.6% SDS-agarose gel electrophoresis. As a consequence, the migration of the large multimers from the stacking gel into the separating gel was most probably less complete than into the 0.8 or 0.65% agarose gels we used, which therefore seems to be more sensitive to identify the changes of the multimeric structure of VWF, especially the ULMWM.

In summary, we reported that antibodies against F-actin are frequent in patients with PSC. Presence of IgA isotype of this antibody was associated with an enhanced mucosal immune response to various microbial antigens and also with the presence of enterocyte damage. Furthermore, AAA IgA identified a subgroup of patients with an increased risk of accelerated disease progression. These results serve as additional proof for the importance of the gut liver interaction in PSC. If they confirmed in large scale cohorts, IgA isotype of AAA could be a candidate biomarker for serological risk stratification of patients with PSC.

We here for the first time demonstrate, that VWF:CB activity measurement has similar value as VWF:RCo determination in cirrhosis. Also, we could quantitatively demonstrate that the ratio of the LMWM was significantly increased in stable cirrhotic patients. Furthermore, in the most severe cirrhotic patients with AD significantly reduced ADAMTS13 activity could be found, along with the presence of ULMWM, which are markers of thrombotic micro-angiopathy and possible contributors to the disease progression.

NEW FINDINGS

- IgA type anti-F-actin antibody was identified as a novel serologic marker of PSC patients with progressive disease course. Our findings highlighted new aspects of the gut liver interaction in PSC.
- Collagen-binding activity was similarly elevated as ristocetin co-factor activity and was strongly correlated with mortality of cirrhotic patients.
- The ratio of low molecular weight VWF multimers increased and that of the high multimers decreased in stable cirrhosis.
- Ultra-large VWF multimers and the lowest ADAMTS13 activity together with increased thrombin generation were evidenced in patients with acute decompensation resulting in increased pro-thrombotic activity.

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PUBLICATIONS OF THE AUTHOR

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List of publications related to the dissertation

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