**Programma najaarsvergadering 6 en 7 oktober 2005**

**NEDERLANDSE EN VLAAMSE VERENIGINGEN VOOR GASTROENTEROLOGIE**

Sectie Gastrointestinale Endoscopie

Netherlands Society for Parenteral and Enteral Nutrition

Sectie Neurogastroenterologie en Motiliteit

Sectie Experimentele Gastroenterologie

Sectie Kindergastroenterologie

Sectie Endoscopie Verpleegkundigen en Assistenten

Vereniging Maag Darm Lever Verpleegkundigen

★

**NEDERLANDSE VERENIGING VOOR HEPATOLOGIE**

★

**NEDERLANDSE VERENIGING VOOR GASTROINTESTINALE CHIRURGIE**

★

**NEDERLANDS GENOOTSCHAP VAN MAAG-DARM-LEVERARTSEN**

★

**NH KONINGSHOF**

**VELDHOVEN**

**INHOUDSOPGAVE** pag.

**INHOUDSOPGAVE** pag.



***Tijdstippen van de diverse ledenvergaderingen tijdens de najaarsvergadering:***

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|---|---|
| Assistentenvereniging Touché (mdl-artsen i.o.) | 6 oktober, 12.15 uur - Zaal 82/83 |
| Nederlandse Vereniging voor Hepatologie | 6 oktober, 15.00 uur - Parkzaal |
| Nederlandse Vereniging voor Gastroenterologie: | 7 oktober, 08.00 uur - Brabantzaal |
| Nederlands Genootschap van Maag-Darm-Leverartsen | 7 oktober, 12.00 uur - Zaal 81/83 |
| Sectie Endoscopie Verpleegkundigen en Assistenten | 7 oktober, 11.30 uur - Diezezaal |
| Sectie Experimentele Gastroenterologie | 7 oktober, 12.00 uur - Parkzaal |

## VOORWOORD

Hierbij treft u het volledige programma aan van de najaarsvergadering te Veldhoven.

Het programma zal van start gaan om 13.00 uur. De Nederlandse Vereniging voor Hepatologie start in een gezamenlijke sessie met de Vlaamse Vereniging voor Gastroenterologie iets eerder om 10.30 uur met vrije voordrachten. Op donderdagmiddag zijn er vrije voordrachten van de Nederlandse Vereniging voor Gastrointestinale Chirurgie, de Nederlandse Vereniging van Gastroenterologie en de Nederlandse Vereniging voor Hepatologie.
Daarnaast worden er drie symposia georganiseerd over Immunomodulation in IBD, Gastrointestinale stoornissen bij diabetes en tot slot een symposium georganiseerd door de Nederlandse Vereniging voor Hepatologie, getiteld: 'Liver fibrosis, a reversible condition?'

De Altana Lecture zal plaatsvinden om 17.00 uur en wordt ditmaal verzorgd door Prof. H.J. Hodgson. Deze voordracht is getiteld 'Clinical Perspectives of Genetherapy for IBD'.

De plenaire donderdagmiddagsessie zal van start gaan met de Presidential Selection. Om 18.30 uur zal de uitreiking van de AstraZeneca Gastrointestinale Researchprijs 2005 plaatsvinden. Alle aanwezigen worden van harte uitgenodigd bij deze sessie aanwezig te zijn!

Op vrijdag is er een symposium ter gelegenheid van het 25-jarig bestaan van de Sectie Gastrointestinale Endoscopie en voorts een oncologisch symposium: 'Gastrointestinale oncologie: een snel evoluerend gebied in de Hepato-Gastroenterologie'
Voorts zijn er sessies met vrije voordrachten van Nederlandse Vereniging van Gastroenterologie en de Nederlandse Vereniging voor Hepatologie en de Sectie Experimentele Gastroenterologie.
In de Diezezaal en het Auditorium worden tenslotte eigen programma's verzorgd door Sectie Endoscopie Assistenten en Verpleegkundigen en de Vereniging van Maag Darm Lever Verpleegkundigen.

**Tenslotte nog een aandachtspunt voor de sprekers:** u dient zich strikt te houden aan de beschikbare spreektijd! U vindt dit aangegeven bij de betreffende sessie. Indien u een eigen laptop gebruikt, dan dient u deze aan te sluiten tijdens de discussietijd van de spreker voor u. In **zaal 25** kunt u uw Power Point presentatie tevoren controleren.

Graag tot ziens in Veldhoven!

Dr. E.C. Klinkenberg-Knol, secretaris
Nederlandse Vereniging voor Gastroenterologie

***N.B. De met een asterisk gemerkte abstracts in het programma zijn ingezonden door leden van de Sectie Kindergastroenterologie.***

## Programma donderdag 6 oktober 2005

| DONDERDAG | BRABANTZAAL | BARONIEZAAL | PARKZAAL | AUDITORIUM | DIEZEZAAL |
|---|---|---|---|---|---|
| 10.30 | Geen ochtendprogramma in deze zaal. | Geen ochtendprogramma in deze zaal. | Voordrachten Nederlandse Vereniging voor Hepatologie en Vlaamse Vereniging voor Gastroenterologie (basaal) p. 10 | Tot 12.00 uur cursorisch onderwijs in MDL-ziekten in deze zaal. | Op donderdag 6 oktober geen programma in deze zaal. |
| 12.00 | | | Lunchbuffet Genderhal | | |
| 13.00 | Vrije voordrachten Nederlandse Vereniging voor Gastrointestinale Chirurgie p. 11 | 13.30 Aanvang **Symposium: 'Gastrointestinale motor disorders in diabetes mellitus'** p. 14 | Vervolg vrije voordrachten Nederlandse Vereniging voor Hepatologie (basaal) p. 17 | | |
| 15.00 | Theepauze | Theepauze | Thee/ledenvergadering | | |
| 15.30 | **IBD-symposium: 'Immunomodulation in IBD'** p. 12 | Vrije voordrachten Nederlandse Vereniging voor Gastroenterologie en MLDS-voordrachten p. 15 | Vervolg vrije voordrachten gevolgd door NVH-Symposium 'Liver fibrosis, a reversible condition?' p. 18 | | |
| 17.00 | **Altana Lecture door Prof. H.J. Hodgson, UK. 'Clinical Perspectives of Genetherapy for IBD'** p. 13 | Vervolg vrije voordrachten Nederlandse Vereniging voor Gastroenterologie en MLDS-voordrachten p. 16 | | | |
| 17.30 | **Presidential Selection NVGE / VVGE** p. 13 | | | | |
| 18.30 | **Uitreiking AstraZeneca GI Researchprijs 2005** p. 14 | | | | |
| 18.50 | Congresborrel expositiehal | Congresborrel expositiehal | Congresborrel expositiehal | | |
| 20.00 | Diner in Genderzaal | Diner in Genderzaal | Diner in Genderzaal | | |

## Programma vrijdag 7 oktober 2005

| VRIJDAG | BRABANTZAAL | BARONIEZAAL | PARKZAAL | AUDITORIUM | DIEZEZAAL |
|---|---|---|---|---|---|
| 08.00 | Ledenvergadering NVGE<br><br>p. 19 | | 8.10 Aanvang vrije voordrachten Nederlandse Vereniging voor Hepatologie (klinisch)<br>p. 23 | | |
| 08.30 | **Lustrumsymposium t.g.v. het 25-jarig bestaan van de Sectie Gastro-intestinale Endoscopie**<br>p. 19 | Vrije voordrachten Nederlandse Vereniging voor Gastroenterologie<br><br>p. 20 | Vervolg vrije voordrachten Nederlandse Vereniging voor Hepatologie (klinisch)<br><br>p. 23 | | |
| 10.00 | Koffiepauze | Koffiepauze | Koffiepauze | **Symposium VMDLV: 'Patiënten met dikke darm kanker'**<br>p. 30 | |
| 10.30 | **Vervolg lustrum-symposium t.g.v. het 25-jarig bestaan van de Sectie Gastrointestinale Endoscopie**<br>p. 19 | **Oncologisch symposium: 'Gastrointestinale Oncologie: een snel evolurend gebied in de Hepato-Gastroenterologie**<br>p. 22 | Vrije voordrachten Sectie Experimentele Gastroenterologie<br><br>p. 24 | **Vervolg symposium Vereniging Maag Darm Lever Verpleegkundigen: 'Patiënten met dikke darm kanker'** (11.00 koffiepauze)<br>p. 30 | Aanvang programma Sectie Endoscopie Verpleeg-kundigen en Assistenten en om 11.30 gevolgd door ledenvergadering SEVA<br>p. 31 |
| 12.00 | Lunch in expositiehal | Lunch in expositiehal | Lunch en ledenvergadering | Lunch in expositiehal | 12.30 Lunch in expositiehal |
| 13.30 | **Vervolg lustrum-symposium t.g.v. het 25-jarig bestaan van de Sectie Gastrointestinale Endoscopie** (tot 15.30 uur)<br><br>p. 19 | Vrije voordrachten Nederlandse Vereniging voor Gastroenterologie<br><br>p. 28 | Vervolg vrije voordrachten Sectie Experimentele Gastroenterologie<br><br>p. 26 | **Vervolg symposium Vereniging Maag Darm Lever Verpleegkundigen: 'Patiënten met dikke darm kanker'**<br><br>p. 30 | Vervolg programma Sectie Endoscopie Verpleeg-kundigen en Assistenten, met om 14.30 uur borrel ter gelegenheid van het 20-jarig bestaan SEVA<br>p. 31 |
| 15.00 | Thee / einde programma | Thee / einde programma | Thee / einde programma | Thee / einde programma | Einde programma |

| | |
|---|---|
| *Cursuscommissie:* | Prof. Dr. C.J.J. Mulder (voorzitter) (MDL-arts, VUmc)<br>Dr. H.M. van Dullemen (MDL-arts, UMCG)<br>Dr. C.M.F. Kneepkens (kinderarts, VUmc)<br>Dr. C.J.H.M. van Laarhoven (chirurg, Elisabeth Tilburg)<br>Dr. M.E. van Leerdam (MDL-arts i.o., Erasmus MC)<br>R. Quispel (MDL-arts i.o., UMCU) |

**Woensdag 5 oktober 2005**

| | |
|---|---|
| 20.30 - 21.00 uur | Dyspepsie in de huisartsenpraktijk<br>*Dr. H.E. van der Horst, HAG, Vumc, Amsterdam* |
| 21.00 - 21.30 uur | Gastropathie en dyspepsie; diagnostiek en therapie<br>*Prof. dr. M. Samsom, MDL, UMC Utrecht* |
| 21.30 - 22.00 uur | Epidemiologie en medicamenteuze therapie bij bloedingen<br>*Dr. M.E. van Leerdam, MDL, Erasmus MC, Rotterdam* |
| 22.00 - 22.30 uur | Nut van classificatie, endoscopische therapie en second look endoscopie bij ulcusbloedingen<br>*Dr. H.R. van Buuren, MDL, Erasmus MC, Rotterdam* |

**Donderdag 6 oktober 2005**

| | |
|---|---|
| 08.00 - 08.30 uur | Bovenbuikspijn bij kinderen<br>*Dr. R. Scheenstra, Kindergeneeskunde, UMC Groningen* |
| 08.30 - 09.00 uur | Refluxtherapie bij kinderen<br>*Prof. dr. Y. Vandenplas, Kindergeneeskunde, Brussel* |
| 09.00 - 09.30 uur | Motoriek van de slokdarm<br>*D. Sifrim, MDL, Universiteit Leuven* |
| 09.30 - 10.00 uur | Medicamenteuze therapie GORD<br>*Dr. A.A.M. Masclee, MDL, LUMC, Leiden* |
| 10.00 - 10.30 uur | Koffiepauze |
| 10.30 - 11.00 uur | Anti-reflux chirurgie (n=800)<br>*B. Dallemagne, Heelkunde, St. Joseph Kliniek, Luik* |
| 11.00 - 11.30 uur | Anti-reflux endoscopic therapy<br>*Prof. P. Swain, Chirurgische Oncologie, St. Mary's, Londen* |
| 11.30 - 11.45 uur | Refluxtherapie; endoscopie versus laparoscopie; een helicopter view<br>*Prof. dr. H.G. Gooszen, Heelkunde, UMC Utrecht* |
| 11.45 - 12.10 uur | Discussie reflux interventie<br>Lunch |

**Nederlandse Vereniging voor Hepatologie en Vlaamse Vereniging voor Gastroenterologie (basale voordrachten)** **Parkzaal**

10.00 Ontvangst, inschrijving, koffie

**Voorzitters:** H. Reynaert / L. Klomp

Voordrachten in het Engels, spreektijd 10 minuten, discussietijd 5 minuten.

10.30 Liver-X-receptor agonist T0901317 suppresses endotoxin-induced expression of inflammatory genes in Kupffer cells in vitro (p. 33)
*J. Mulder[1], J.A.A.M. Kamps[2], S.J. Karpen[3], F. Kuipers[1], E. Sturm[1]. Dept of Pediatrics[1], Center for Liver, Digestive and Metabolic Diseases, and Dept of Pathology & Laboratory Medicine/Medical Biology[2], University Medical Center Groningen, The Netherlands, and Dept of Pediatrics[3], Baylor College of Medicine, Houston, TX, USA*

10.45 Simultaneous targeting of HCV replication and viral entry by a single gene therapy vector containing multiple RNA interference cassettes: prevention of mutational escape (p. 34)
*S.D. Henry[1], P.G. van der Wegen[2], H.J. Metselaar[3], R. Bartenschlager[4], H.W. Tilanus[1], B.J. Scholte[2], L.J.W. van der Laan[1]. Depts of Surgery[1], Cell Biology[2] and Gastroenterology and Hepatology[3], Erasmus Medical Center, Rotterdam, The Netherlands, and Dept of Molecular Virology[4], University of Heidelberg, Germany*

11.00 The inhibition by regulatory T cells of the immune response against HBV is partially HBV-specific but not mediated through IL10 and TGF-β (p. 35)
*J.N. Stoop[1], J.G. Kusters[1], E.J. Kuipers[1], C.C. Baan[2], H.L.A. Janssen[1], R.G. van der Molen[1]. Depts of Gastroenterology and Hepatology[1], and Internal Medicine[2], Erasmus Medical Center, Rotterdam, The Netherlands*

11.15 Reduced expansion of HBV specific $CD8^+$ T cells by myeloid dendritic cells isolated from chronic HBV patients (p. 36)
*R.G. van der Molen[1], D. Sprengers[1], P.J. Biesta[1], P.P.C. Boor[1], M.K. Maini[2], J.G. Kusters[1], H.L.A. Janssen[1]. Dept of Gastroenterology and Hepatology[1], Erasmus Medical Center, Rotterdam, The Netherlands, and Division of Infection and Immunity[2], University College London, United Kingdom*

11.30 The expression of E-selectin, P-selectin and ICAM-1 in the peritoneal microvessels of portal hypertensive and cirrhotic rats treated with or without peginterferon-alpha2a (p. 37)
*A.M. Geerts, K.Y. Cheung, E. Vanheule, H. Van Vlierberghe, M. De Vos, I. Colle. Dept of Hepatology & Gastroenterology, Ghent University Hospital, Belgium*

11.45 Effect of MARS and PROMETHEUS on systemic hemodynamics and vasoactive agents in patients with acute on-chronic alcoholic liver failure: the clash of the titans (p. 38)
*W. Laleman[1], A. Wilmer[2], P. Evenepoel[3], C. Verslype[1], J. Fevery[1], F. Nevens[1]. Dept of Hepatology[1], Medical Intensive Care[2] and Nephrology[3], University Clinic Gasthuisberg, KU Leuven, Belgium*

12.00 Lunch

**Nederlandse Vereniging voor Gastrointestinale Chirurgie** **Brabantzaal**

12.30 Inschrijving, koffie

**Voorzitters:** O.R.C. Busch / C.J.H.M. van Laarhoven

Voordrachten in het Nederlands, spreektijd 7 minuten, discussietijd 3 minuten.

13.00 Robot-assisted thoracoscopic esophago-lymphadenectomy for esophageal cancer (p. 39)
*J. Boone[1], W.A. Draaisma[1], I.A.M.J. Broeders[1], M.J.M.M. Giezeman[2], I.H.M. Borel Rinkes[1], R. van Hillegersberg[1]. Dept of Surgery[1] and Anesthesiology[2], University Medical Center Utrecht, The Netherlands*

13.10 Long-term results of laparoscopic fundoplication (p. 40)
*P.W.J. Maljaars[1], A.D. Roopram[1], J. Ringers[2], A.A.M. Masclee[1]. Depts of Gastroenterology-Hepatology[1] and Surgery[2], Leiden University Medical Center, Leiden, The Netherlands*

13.20 Re-operation rate after laparoscopic adjustable gastric banding (LAGB) (p. 41)
*R. Schouten, F.M.H. van Dielen, J.W.M. Greve. University Hospital Maastricht, The Netherlands*

13.30 Fast track major liver surgery; initial experience with an enhanced recovery programme (p. 42). *R.M. van Dam[1], M.M.E. Coolsen[1], M.C.G. van de Poll[1], M.F. von Meyenfeldt[1], M.H.A. Bemelmans[1], J.W.M. Greve[1], K. Lassen[2], A. Revhaug[2], C.H.C. Dejong[1], on behalf of the Enhanced Recovery After Surgery (ERAS) group. Dept of Surgery[1], University Hospital Maastricht, The Netherlands, and Dept of Surgery[2], University Hospital Tromso, Norway*

13.40 Bile Salt Toxicity Aggravates Cold Ischemic Injury of Bile Ducts after Liver Transplantation in Mdr2+/- Mice (p. 43)
*H. Hoekstra[1,2], R.J. Porte[2], Y. Tian[1], W. Jochum[3], B. Stieger[4], R. Graf[1], W. Moritz[1], P.A. Clavien[1]. Dept of Visceral and Transplant Surgery[1], University Hospital Zurich, Section of Hepatobiliary Surgery and Liver Transplantation, Dept of Surgery[2], University Medical Center Groningen, The Netherlands, Dept of Pathology[3], University Hospital Zurich, Division of Clinical Pharmacology and Toxicology, Dept of Internal Medicine[4], University Hospital Zurich, Switzerland*

13.50 Magnetic Resonance Cholangiopancreatography (MRCP) as Initial Examination in Biliary Pancreatitis (p. 44). *E. De Waele[1], B. Op de Beeck[2], H. Devriendt[1], G. Delvaux[3]. Dept of Surgery[1], AZ Maria Middelares - Sint-Jozef, Gentbrugge, and Dept of Radiology[2], Surgery[3], VUB University Hospital, Brussels, Belgium*

14.00 RT-PCR and immunohistochemical evaluation of sentinel lymph nodes after in vivo mapping with Patent Blue V in colon cancer patients (p. 45)
*W. Kelder[1,2], A. van den Berg[3], A.T.M.G. Tiebosch[1], P.C. Baas[1], J.T.M. Plukker. Dept of Surgery[1], Martini Hospital Groningen, Depts of Surgery[2], and Pathology[3], University Medical Center Groningen, The Netherlands*

14.10 Rectal Cancer staging for distant metastases: Ultra Small Paramagnetic Iron Oxide (USPIO) enhanced Whole body MRI versus PET-CT (p. 46)
*R.M. van Dam[1], M. Lahaye[4], J.G. Bloemen[1], G. Lammering[3], A. Arens[2], G.J.J. Teule[2], G.L. Beets[1], C.H.C. Dejong[1], R.G.H. Beets-Tan[4]. Depts of Surgery[1], Nuclear Medicine[2], Radiation Oncology[3] and Radiology[4], University Hospital Maastricht, The Netherlands*

14.20 Efficacy and cost-efficiency of follow-up after curative surgery for colorectal carcinoma (p. 47).
*M.C.E. van Reedt Dortland[1], H.M. Teijgeler[1], A.B. Bijnen[1,2]. Dept of surgery[1], Medical Center Alkmaar, and Dept of Medical Education[2], Free University Medical Center, Amsterdam, The Netherlands*

14.30 The influence of delivery on Long-term pouch-function: an underestimated risk-factor (p. 48).
*S.W. Polle[1], M.S. Vlug[1], J.F.M. Slors[1], A.H. Zwinderman[2], A. Gerritsen van de Hoop[3], M.A. Cuesta[4], R. Bakx.[1], D.J. Gouma[1] and W.A. Bemelman[1]. Depts of Surgery[1] and Medical Statistics[2], Academic Medical Center, Amsterdam, Dept of Surgery[3], Leiden University Medical Center, Leiden, Dept of Surgery[4], Free University Medical Center, Amsterdam, The Netherlands*

14.40 Elastic band ligation of hemorrhoids: flexible endoscope versus rigid proctoscope (p. 49)
*M. Cazemier[1], R.J.F. Felt-Bersma[1], M.A. Cuesta[2], C.J.J. Mulder[1]. Depts of Gastro-enterology and Hepatology[1] and Gastrointestinal Surgery[2], Free University Medical Center Amsterdam, The Netherlands*

14.50 Temperature controlled radiofrequency energy (Secca®) to the anal canal for the treatment of fecal incontince: pilot seems promising (p. 50)
*R.J.F. Felt-Bersma, C.J.J. Mulder. Free University Medical Center, Amsterdam, The Netherlands*

15.00 Theepauze

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**IBD-symposium** **Brabantzaal**

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**'Immunomodulation in IBD'**

**Voorzitters:** M. de Vos en D.J. de Jong

15.15 Laudatio van Prof. K. Geboes

15.30 Immunomodulation in severe ulcerative colitis
*S. Travis (Oxford)*

15.50 Mucosal immunity in IBD as target for therapy
*D.W. Hommes (AMC, Amsterdam)*

16.10 Step-up or top-down strategy in Crohn's disease
*G. D'Haens (Leuven)*

16.30 Panel discussion
When to start with immunomodulators and biologicals in IBD
*Moderator: M. De Vos*

17.00 Einde programma

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**Nederlandse Vereniging voor Gastroenterologie** **Brabantzaal**

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**Voorzitter:** J.B.M.J. Jansen

17.00 **Altana Lecture: 'Clinical Perspectives of Genetherapy for IBD'**
*verzorgd door Prof. H.J. Hodgson*
*Royal Free and University College Medical School, Londen, UK*

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**Presidential Selection NVGE – VVGE - plenaire sessie** **Brabantzaal**

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**Voorzitters:** J.B.M.J. Jansen en M. Peeters

Voordrachten in het Nederlands, spreektijd 10 minuten, discussietijd 5 minuten.

17.30 Enhanced recovery after surgery ("fast track") programs in colonic surgery: a systematic review (p. 51)
*J. Wind[1], S.W. Polle[1], P.H.P. Fung Kon Jin[1], C.H.C. Dejong[2], W.A. Bemelman[1]. Depts of Surgery, Academic Medical Center Amsterdam[1] and University Hospital Maastricht[2], The Netherlands*

17.45 Polymorphisms in the T-cell regulatory gene CTLA-4 are associated with susceptibility for acute rejection after liver transplantation (p. 52)
*Ö. Tapirdamaz[1], V. Pravica[4], H.J. Metselaar[1], B. Hansen[1,2], L.M.G. Moons[1], A. Yousefi Arash[4], J. van Meurs[3], I.V. Hutchinson[4], J. Shaw[5], K. Agarwal[6], C.P. Day[6], D.H. Adams[5], J. Kwekkeboom[1]. Dept of Gastroenterology and Hepatology[1], Epidemiology and Biostatistics[2], and Internal Medicine[3], Erasmus Medical Center Rotterdam, The Netherlands, Immunology Research Group[4], University of Manchester, Liver Research Laboratories[5], MRC Center for Immune Regulation, the University of Birmingham Institute of Clinical Science, and Centre for Liver Research[6], University of Newcastle-upon-Tyne, United Kingdom*

18.00 Asymmetric Dimethyl-L-Arginine (ADMA): a possible cause of endothelial dysfunction in the bile-duct excised cirrhotic rat (p. 53)
*W. Laleman, I. Vander Elst, M. Zeegers, J. Fevery and F. Nevens. Dept of Hepatology, University Hospital Gasthuisberg, KU Leuven, Belgium*

18.15 Role of the nuclear orphan receptor Rev-erbα; in the regulation of bile acid synthesis (p. 54)
*T. Claudel[1], H. Duez[2], J. van der Veen[1], C. Fontaine[2], R. Havinga[1],C. Duhem[2], V.W. Bloks[1], B. Vennström[3], J.C. Fruchart[2], B. Staels[2], F. Kuipers[1]. Center for Liver[1], Laboratory of Pediatrics, University Medical Center Groningen, The Netherlands, UR.545 INSERM, Dépt d'Athérosclérose[2], Institut Pasteur Lille, France, Laboratory of Developmental Biology[3], Dept of Cell and Molecular Biology, Karolinska Institute, Stockholm, Sweden*

18.30 **Uitreiking AstraZeneca Gastrointestinale Researchprijs 2005**
*door de voorzitter van de jury Dr. R.J. De Knegt, gevolgd, door een erevoordracht door de prijswinnaar.*

18.50 Congresborrel in de expositiehallen

20.00 Diner in de Genderzaal

22.00 Borrel in de Brabantzaal

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**Symposium Motiliteit** **Baroniezaal**

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**Voorzitters:** G.E.E. Boeckxstaens en A.A.M. Masclee

"**Gastrointestinal motor disorders in diabetes mellitus"**

13.30 Epidemiology and pathophysiology
*Prof. dr. M. Samsom, UMCU , Utrecht.*

14.00 Gastroparesis : diagnosis and therapy
*Prof. dr. J. Tack, Leuven.*

14.30 Intestinal and colonic dysfunction
*Prof. dr. V.Stanghellini, Bologna*

15.00 Theepauze

**Nederlandse Vereniging voor Gastroenterologie** **Baroniezaal**

**Voorzitters:** E.C. Klinkenberg-Knol en W. Hameeteman

Voordrachten in het Nederlands, spreektijd 7 minuten, discussietijd 3 minuten.

15.30 Lipopolysaccharide detoxification and clearance by enterocytes. (p. 55)
(Final report Maag Lever Darm Stichting project no. WS 00-73)
*A.C.E. Vreugdenhil, M. Hadfoune, J.W.M. Greve, W.A. Buurman. Dept of General Surgery, Maastricht University, The Netherlands*

15.40 No association between referral indications for open access upper GI endoscopy and endoscopic findings (p. 56)
*S.J. van Rijswijck[1], L.A.S. van Kerkhoven[1], L.G.M. van Rossum[1], A.C.I.T.L. Tan[2], J.B.M.J. Jansen[1]. Dept of Gastroenterology & Hepatology[1], Radboud University Nijmegen Medical Center, and Dept of Internal Medicine[2], Canisius Wilhelmina Hospital, Nijmegen, The Netherlands*

15.50 Nurse-administered Propofol Sedation for ERCP (p. 57)
*G. Wolff-Geerders[1], J. Haringsma[1], G.J. Los[2], M. Klimek[2], E.J. Kuipers[1]. Depts of Gastroenterology & Hepatology[1] and Anesthesiology[2], Erasmus Medical Center, Rotterdam, The Netherlands*

16.00 Insulin-like growth factor I (IGF-I) replacement therapy increases albumin concentration in liver cirrhosis: Results of a pilot randomized controlled clinical trial. (p. 58)
(Final report Maag Lever Darm Stichting projectnr. WS 00-78 (WS 97/87))
*M. Conchillo[1], R.J. de Knegt[2], M. Payeras[1], J. Quiroga[1], B. Sangro[1], J.I. Herrero[1], I.Castilla-Cortazar[1], Jan Frystyk[4], Allan Flyvbjerg[4], C. Yoshizawa[3], P.L.M. Jansen[2], B. Scharschmidt[3], J. Prieto[1]. Department of Medicine and Liver Unit[1], Clinica Universitaria, Medical School and Center for Applied Medical Research (CIMA), University of Navarra, Pamplona, Spain, Department of Gastroenterology and Hepatology[2], University Hospital[3], Groningen, The Netherlands, Chiron Corp.Emeryville[3], CA, USA, The Medical Research Laboratories[4], Clinical Institute, Aarhus University Hospital, Aarhus C, Denmark*

16.10 The quality of endoscopic ultrasonographic staging of esophageal cancer during the learning period using electronic consultation (p. 59)
*M.J. van Campen[1], B. Oldenburg[1], P. Fockens[2], M. Samsom[1], M.P. Schwartz[1]. Dept of Gastroenterology[1], University Medical Center, Utrecht, and Dept of Gastroenterology[2], Academic Medical Center, Amsterdam, The Netherlands*

16.20 Prevalence of Barrett's esophagus in a population not primarily referred for gastro-esophageal reflux disease (p. 60)
*B.C. van Eijck, L.M.M. Wolters, H. van Dekken, E.J. Kuipers, P.D. Siersema. Depts of Gastroenterology & Hepatology and Pathology, Erasmus Medical Center Rotterdam, The Netherlands*

16.30 Acid reflux in patients with achalasia before and after pneumatic dilation (p. 61)
*I. Leeuwenburgh, J. Haringsma, E.J. Kuipers. Dept of Gastroenterology and Hepatology, Erasmus University Medical Center, Rotterdam, The Netherlands*

16.40 Colonoscopy Using High Resolution Endoscopy, Video Autofluorescence Imaging and Narrow Band Imaging: A Feasibility Study (p. 62)
*M.A. Kara[1], E. Dekker[1], C.C. Cohen[1], J.J. Bergman[1], J. Hardwick[1], J. Offerhaus[2], P. Fockens[1]. Departments of Gastroenterology and Hepatology[1] and Pathology[2], Academic Medical Center Amsterdam, The Netherlands*

16.50 CDX2 is an early marker of epithelial intestinal differentiation in columnar epithelium of the esophagus (p. 63, 64)
*M. Kerkhof[1], D.A. Bax[1], L.M.G. Moons[1], A.J. van Vuuren[1], H. van Dekken[2], E.J. Kuipers[1], J.G. Kusters[1], P.D. Siersema[1]. Depts of Gastroenterology and Hepatology[1], Pathology[2] and Surgery[3], Erasmus Medical Center, Rotterdam, The Netherlands*

17.00 Postoperative chemoradiotherapy in gastric cancer - results of two parallel phase I-II studies of a fixed radiotherapy regimen with escalating doses of cisplatin and capecitabine (p. 65)
*H. Boot[1], E.P.M. Jansen[2], A. Cats[1], R. Dubbelman[1], M.P. Saunders[3], V.S. Khoo[3], T.D.L. Crosby[4], H. Bartelink[2], M. Verheij[2]. Depts of Gastroenterology[1] and Radiotherapy[2], The Netherlands Cancer Institute-Antoni van Leeuwenhoekziekenhuis, Amsterdam, The Netherlands, and Christie Hospital NHS Trust[3], Manchester, United Kingdom, and Velindre Hospital, Cardiff, Wales*

17.10 Palliation of patients with malignant gastric outlet obstruction with the WallFlex enteral stent: a retrospective multicentre study (p. 66)
*J. van Hooft[1], M. Mutignani[2], A. Repici[3], H. Messmann[4], H. Neuhaus[5], P. Fockens[1]. Academic Medical Centre[1], Amsterdam, The Netherlands, Hospital A. Gemelli[2], Rome, Italy Molinette Hospital[3], Torino, Italy, Klinikum Augsburg[4], and Evangelisches Krankenhaus [5], Düsseldorf, Germany*

17.20 Colonic function in slow transit constipation: motor and sensory disorder? (p. 67)
(Final report Maag Lever Darm Stichting projectno. WS 01-40
*E.A. van Hoboken, A.A.M. Masclee. Leids Universitair Medisch Centrum, Leiden, The Netherlands*

17.30 Einde programma
Voor de plenaire sessie kunt u zich begeven naar de Brabantzaal.

**Nederlandse Vereniging voor Hepatologie (basale voordrachten) Parkzaal**

**Voorzitter:** P.L.M. Jansen / J. Kwekkeboom

Voordrachten in het Engels, spreektijd 10 minuten, discussietijd 5 minuten.

13.00 Conditional inactivation of the mouse glutamine synthetase gene in the liver (p. 68)
*Y. He, T. Hakvoort, M. van Roon, J. Vermeulen, W. Lamers. Amsterdam Liver Center, Amsterdam, The Netherlands*

13.15 The Role of Anion Exchanger 2 in Bile Secretion (p. 69)
*P. Mardones Hiche[1], J. Medina[2], J. Prieto[2], R. Oude Elferink[1], Amsterdam Liver Center[1], The Netherlands, Division of Gene Therapy and Hepatology[2], University of Navarra, Spain*

13.30 Metformin protects rat hepatocytes against bile acid and cytokine-induced apoptosis (p. 70)
*T.E. Vrenken, L. Conde de la Rosa, M. Buist-Homan, H. Moshage. Dept of Gastro-enterology and Hepatology, University Medical Center Groningen, The Netherlands*

13.45 Oxidative stress induced apoptosis is inhibited by metformin via an ERK and Src dependent pathway in rat hepatocytes (p. 71)
*L. Conde de la Rosa, T.E. Vrenken, M. Buist-Homan, H. Moshage. Dept Gastro-enterology and Hepatology, University Medical Center Groningen, The Netherlands*

14.00 Different inducers of oxidative stress have divergent effects on survival and proliferation of activated rat hepatic stellate cells (p. 72)
*S. Dunning, R.A. Hannivoort, M. Buist-Homan, H. Moshage. Dept Gastroenterology and Hepatology, Center for Liver, Digestive and Metabolic diseases, University Medical Center Groningen, The Netherlands*

14.15 Ischemic-Type Biliary Lesions after Human Liver Transplantation are preceded by abnormal Bile Composition (p. 73)
*C.I. Buis[1,2], E. Geuken[1], D.S. Visser[1], H.G.D. Leuvenink[1], M.J.H. Slooff[2], R.J. Porte[2]. Surgical Research Laboratory[1], Hepatobiliairy Surgery and Liver Transplantation[2], University Medical Center Groningen, The Netherlands*

14.30 Several COMMD proteins interact with ATP7B; possible candidate genes for hepatic copper overload disorders with unknown etiology (p. 74)
*P. de Bie[1,2], E. Burstein[3], B. van de Sluis[1,2], R. Berger[1], C. Wijmenga[2], C.S. Duckett[3,4], L.W.J. Klomp[1]. Laboratory of Metabolic and Endocrine Diseases[1], University Medical Center Utrecht, Complex Genetics Section[2], DBG-Dept of Medical Genetics, University Medical Center Utrecht, The Netherlands, Depts of Internal Medicine[3] and Pathology[4], University of Michigan Medical School, Ann Arbor, MI, USA*

14.45 A proteomics analysis of the cholestatic rat hepatocyte (p. 75)
*J. Woudenberg, A. Gerbens, M. Buist-Homan, R. Havinga, H. Moshage, K.N. Faber. Center for Liver, Digestive and Metabolic Diseases, University Medical Center Groningen, The Netherlands*

*Donderdag 6 oktober 2005*

15.00 Theepauze en ledenvergadering

15.30 Atp8b1 renders the canalicular membrane resistant to hydrophobic bile salts (p. 76)
*C. Paulusma[1], A. Groen[1], C. Kunne[1], K. Mok[1], H. Vreeling[2], K. Houben[2], W. Frederiks[2], J. van Marle[2], L. Bull[3], A. Knisely[4], R.P.J. Oude Elferink[1]. Amsterdam Liver Center[1], Dept Cell Biology and Histology[2], Academic Medical Center Amsterdam, The Netherlands, UCSF Liver Center[3], San Francisco General Hospital, USA, and Institute of Liver Studies[4], King's College Hospital, London, United Kingdom*

15.45 Excess phospholipid excretion in Fic1 mutant mice is Mdr2 independent (p. 77)
*A. Groen[1], C. Kunne[1], C. Paulusma[1], L. Bull[2], R. Oude Elferink[1]. Amsterdam Liver Center[1], Amsterdam, The Netherlands, and UCSF Liver Center[2], San Francisco General Hospital, USA*

**Symposium Nederlandse Vereniging voor Hepatologie** **Parkzaal**

***Chairman:*** *P.L.M. Jansen*

**"Liver fibrosis, a reversible condition?"**

16.00 "Life and death in the injured liver"
*Prof. dr. Han Moshage, Universitair Medisch Centrum Groningen*

16.30 "Is the liver biopsy obsolete?"
*Prof. dr. Tanja Roskams, Afdeling Pathologie, KU Leuven*

17.00 "The role of hepatic stellate cells in the pathogenesis of liver fibrosis".
*Prof. dr. Albert Geerts, Laboratorium voor Cel Biologie, Vrije Universiteit Brussel*

17.30 Voor de plenaire sessie kunt u zich begeven naar de Brabantzaal.

| **Nederlandse Vereniging voor Gastroenterologie** | **Brabantzaal** |
|---|---|

08.00 - 08.30 Ledenvergadering

| **Sectie Gastrointestinale Endoscopie** | **Brabantzaal** |
|---|---|

**Symposium ter gelegenheid van het 25-jarig bestaan van de Sectie Gastrointestinale Endoscopie**

**Morning Program**

08.30 - 08.50 Session I: "The early days of endoscopy in the Netherlands".
*Joep Bartelsman*

08.50 – 09.40 Session II: Imaging and treatment of early neoplasia of the GI tract
*Jelle Haringsma: New developments in GI imaging*
*Jacques Bergman: Future of endoscopic treatment of early neoplasia*

09.40 - 10.10 Session III: ERCP
*Martin Freeman: Is ERCP dead*?

10.10 - 10.30 Coffee

10.30 - 11.00 Session IV: EUS
*Annette Fritscher Ravens: EUS: What is accomplished en where do we go?*

11.00 - 11.30 Session V: Small bowel imaging and therapy
*Chris Mulder: VCE or double balloon enteroscopy?*

11.30 - 12.00 Session VI: Videoforum
*Cases, preferably, with videofragments and pictures*

12.00 - 13.30 Lunch

**Afternoon Program**

13.30 - 15.30 Session VII: The future of gastro-intestinal endoscopy.

A technician's view on the future of gastro-intestinal endoscopy
*Paul Breedveld, University Delft*

A teacher's view on the future of gastro-intestinal endoscopy: training, certification, expertise and complications
*Peter Cotton*

A clinician's view on the future of gastro-intestinal endoscopy
*Guido Tytgat*

15.25- 16.30 Drinks

**Nederlandse Vereniging voor Gastroenterologie** **Baroniezaal**

**Voorzitters:** M. Hiele en P.D. Siersema

Voordrachten in het Nederlands, spreektijd 7 minuten, discussietijd 3 minuten.

08.30 Adherence to proton pump inhibitor therapy is low in daily clinical practice (p. 78)
*E.M. van Soest[1,2], P.D. Siersema[1], M.C.J.M. Sturkenboom[2,3], J.P. Dieleman[2], E.J. Kuipers[1]. Dept of Gastroenterology and Hepatology[1], Medical Informatics[2], and Epidemiology and Biostatistics[3], Erasmus University Medical Center, Rotterdam, The Netherlands*

08.40 Proton pump inhibitor use is associated with the development of sporadic fundic gland polyps (p. 79)
*M. Jalving[1,2], J.J. Koornstra[1], S. de Jong[2], J. Wesseling[3], J.H. Kleibeuker[1]. Dept of Gastro-enterology and Hepatology[1], Medical Oncology[2] and Pathology[3], University Medical Center Groningen, The Netherlands*

08.50 Inappropriate prescription of proton pump inhibitors in a hospital setting (p. 80)
*E.P.M. van Vliet[1], H.J.A.M. Otten[2], H.C. Hoogsteden[2], P.D. Knoester[3], A. Rudolphus[4], E.J. Kuipers[1], P.D. Siersema[1]. Depts of Gastroenterology and Hepatology[1], Pulmonary Medicine[2] and Clinical Pharmacy[3], Erasmus Medical Center Rotterdam, and Dept of Pulmonary Medicine[4], Sint Franciscus Gasthuis Rotterdam, The Netherlands*

09.00 E266K CARD4/NOD1 gene polymorphism increases the risk for peptic ulceration in Helicobacter pylori infected patients (p. 81)
*M.G.H. van Oijen[1], T. van Nguyen[2], M.J.R. Janssen[1], S. Bergevoet[1], D.J. de Jong[1], J.B.M.J. Jansen[1], J.P.H. Drenth[1]. Dept Gastroenterology & Hepatology[1], Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands, and Dept Gastroenterology[2], Can Tho University of Medicine and Pharmacy, Can Tho, Vietnam*

09.10 Evaluation of endoscopy outcomes a decade after nationwide introduction of protonpump inhibitors and guidelines for *Helicobacter pylori* eradication (p. 82)
*S.J. van Rijswijck[1], L.A.S. van Kerkhoven[1], L.G.M. van Rossum[1], A.C.I.T.L. Tan[2], J.B.M.J. Jansen[1]. Dept of Gastroenterology & Hepatology[1], Radboud University Nijmegen Medical Center, and Dept of Internal Medicine[2], Canisius Wilhelmina Hospital, Nijmegen, The Netherlands*

09.20 Colonic manometry as predictor of clinical success with cecostomy in children with defecation disorders* (p. 83)
*M.M. van den Berg[1], M. Hogan[2], D.A. Caniano[3], C. Di Lorenzo[4],M.A. Benninga[1], H.M. Mousa[4]. Dept of Pediatric Gastroenterology and Nutrition, Academic Medical Center Amsterdam, The Netherlands[1], Depts of Radiology[2], Pediatric Surgery[3], Pediatric Gastroenterology[4], Children's Hospital, Columbus, Ohio, USA*

09.30 Prevalence and clinical presentation of constipation in a representative cohort of children with severe generalized cerebral palsy* (p. 84)
*R. Veugelers[1], C. Penning[1], E.A.C. Calis[1], M.A. Benninga[2], D. Tibboel[3], H.M. Evenhuis[1]. Dept of Intellectual Disability Medicine/General Practice[1], Erasmus Medical Center Rotterdam, Dept of Pediatric Gastroenterology and Nutrition[2], Academic Medical Center Amsterdam, Dept of Pediatric Surgery/Intensive Care[3], Erasmus Medical Center - Sophia Children's Hospital, Rotterdam, The Netherlands*

09.40 Flow cytometry patterns specifying celiac disease in lymphocytic enteritis (MARSH I enteropathy): a pilot study (p. 85)
*A.A. Vrij[1], A. Martens[2]. Depts of Gastroenterology[1] and Clinical Chemistry[2],Twenteborg Hospital, Almelo, The Netherlands*

09.50 Diagnostic value of measuring disaccharidase activities in duodenal biopsies of children* (p. 86)
*Y.B. de Rijke[1], G.P. Koelewijn[1], J. Bouquet[2]. Depts of Clinical Chemistry[1] and Gastro-enterology[2], Erasmus Medical Center - Sophia Children's Hospital, Rotterdam, The Netherlands*

10.00 Koffiepauze

**Oncologisch symposium** **Baroniezaal**

**Voorzitters:** A. Cats en M. Peeters

‘**Gastrointestinale Oncologie:**
**een snel evoluerend gebied in de Hepato-Gastroenterologie’**

***De adjuverende behandeling bij het maagcarcinoom***

10.00 Chemotherapie?
*H. Boot, maag-darm-leverarts, Antoni van Leeuwenhoekhuis, Amsterdam*

10.20 Radiotherapie?
*T. Boterberg, radiotherapeut-oncoloog, Universitair Ziekenhuis Gent*

10.40 De adjuverende behandeling bij het coloncarcinoom
*A. Cats, maag-darm-leverarts, Antoni van Leeuwenhoekhuis, Amsterdam*

11.00 De behandeling van het gemetastaseerd coloncarcinoom
*M. Peeters, maag-darm-leverarts, Universitair Ziekenhuis Gent*

***Optimalisering van de behandeling van het rectumcarcinoom***

11.20 De Nederlandse ervaring
*H. Rutten, chirurg, Catharina Ziekenhuis Eindhoven*

11.40 De Belgische ervaring
*F. Penninckx, diensthoofd abdominale heelkunde, Universitair Ziekenhuis Leuven*

12.00 Lunch

**Nederlandse Vereniging voor Hepatologie (klinisch)** **Parkzaal**

**Voorzitter:** W. van Steenbergen / R.J. de Knegt

Voordrachten in het Nederlands, spreektijd 7 minuten, discussietijd 3 minuten.

08.10 Prediction of response based on viral decline during PEG-interferon alpha-2b therapy in HBeAg-positive chronic hepatitis B (p. 87, 88)
*M.J. ter Borg[1], B.E. Hansen[1,2], R.A. de Man[1], S.W. Schalm[1], H.L.A. Janssen[1], for the HBV 99-01 Study Group. Dept of Gastroenterology & Hepatology[1] and Epidemiology & Biostatistics[2], Erasmus Medical Center, Rotterdam, The Netherlands*

08.20 Relapse in HBeAg-positive chronic hepatitis B after Peg-Interferon alpha-2b therapy alone or in combination with lamivudine (p. 89)
*H.J. Flink[1], B.E. Hansen[1,2], R.A. de Man[1], S.W. Schalm[1], H.L.A. Jansen[1]. Depts of Gastroenterology and Hepatology[1], Epidemiology and Biostatistics[2], Erasmus Medical Center, Rotterdam, The Netherlands*

08.30 Adefovir treatment of chronic hepatitis B patients recovers circulating numbers and function of myeloid dendritic cells (p. 90)
*R.G. van der Molen[1], D. Sprengers[1], P.J. Biesta[1], R.A. de Man[1], H.G.M. Niesters[2], J.G. Kusters[1], H.L.A. Janssen[1]. Depts of Gastroenterology and Hepatology[1], and Virology[2], Erasmus Medical Center, Rotterdam, The Netherlands*

08.40 Switching lamivudine resistant chronic Hepatitis B patients from tenofovir to adefovir results in less potent HBV DNA suppression (p. 91)
*W.F. Leemans[1], H.L.A. Janssen[1], H.G.M. Niesters[2], S.W. Schalm[1], R.A. de Man[1]. Depts of Gastroenterology & Hepatology[1] and Virology[2], Erasmus Medical Center, Rotterdam, The Netherlands*

08.50 Additional value of genetic fingerprinting in source and contact tracing of hepatitis B virus infection in the community (p. 92)
*M.C. Mostert[1], R.A. de Man[2], H. Götz[1], G.J.J. van Doornum[3], H.G.M. Niesters[3], J.H. Richardus[1,4]. Dept of Infectious Diseasess[1], Municipal Health Service, Rotterdam, and Depts of Gastroenterology and Hepatology[2], Virology[3], Public Health[4], Erasmus Medical Center, Rotterdam, The Netherlands*

09.00 Non-invasive assessment of liver fibrosis in patients with hepatitis B and C. The first Dutch experience (p. 93)
*C. Verveer[1], H.R. van Buuren[1], P. Zondervan[2], R.J. de Knegt[1]. Depts Gastroenterology & Hepatology[1] and Pathology[2], Erasmus Medical Center, Rotterdam, The Netherlands*

09.10 Decline in serum neopterin concentration correlates with HCV RNA decline during administration of VX-950, a Hepatitis C Virus Protease Inhibitor (p. 94)
*H.C. Gelderblom[1], S. Zeuzem[2], C.J. Weegink[1], N. Forestier[2], L. McNair[3], S. Purdy[3], P.L.M. Jansen[1], H.W. Reesink[1]. Dept of Gastroenterology and Hepatology[1], Liver Center Amsterdam, The Netherlands, Saarland University Hospital[2], Homburg/Saar, Germany, Vertex Pharmaceuticals[3], Cambridge, Massachusetts, USA*

09.20 Viral kinetics in patients with hepatitis C genotype 1, treated with high-dose daily interferon, high-dose pegylated interferon and pegylated interferon combined with daily interferon (p. 95)
*R.J. de Knegt[1], J.M. Vrolijk[1], B. Niesters[2], S.W. Schalm[1]. Depts Gastroenterology & Hepatology[1] and Virology[2], Erasmus Medical Center, Rotterdam, The Netherlands*

09.30 Variant mannose-binding lectin gene alleles in donor livers constitute a major risk for severe infections after orthotopic transplantation (p. 96)
*L.H. Bouwman[1], H.W. Verspaget[2], B. van Hoek[2], A. Roos[3], O.T. Terpstra[1], P. de Knijff[4], S.P. Berger[3], M.R. Daha[3], M. Frölich[5], A.R. van der Slik[6], I.I. Doxiadis[6], B.O. Roep[6], A.F.M. Schaapherder[1]. Depts of Surgery[1], Gastroenterology and Hepatology[2], Nephrology[3], Human and Clinical Genetics[4], Clinical Chemistry[5], and Immunohematology and Blood Transfusion[6], Leiden University Medical Center, The Netherlands*

09.40 Anastomotic biliary strictures after liver transplantation: prevalence, presentation, management and outcome (p. 97)
*R.C. Verdonk[1], C.I. Buis[2], R.J. Porte[2], E.J. van der Jagt[3], A.J. Limburg[1], A.P. van den Berg[1], M.J.H. Slooff[2], J.H. Kleibeuker[1], E.B. Haagsma[1]. University Medical Center Groningen, The Netherlands*

09.50 Actual non-estimated ten-year survival in adults after liver transplantation (LT) (p. 98)
*C.S. van der Hilst[1], A.J.C. IJtsma[2], T.J. Boelstra[2], E.B. Haagsma[3], A.P. van den Berg[3], E.M. TenVergert[1], M.J.H. Slooff[2]. Office for Medical Technology Assessment[1], Dept of Surgery, Division of Hepatobiliary Surgery and Liver Transplantation[2], Dept of Gastro-enterology and Hepatology[3], Groningen University Hospital, The Netherlands*

10.00 Koffiepauze

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**Sectie Experimentele Gastroenterologie** **Parkzaal**

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**Voorzitter:** M.A.C. Meijssen en E.A.F. van Tol

Voordrachten in het Nederlands, spreektijd 7 minuten, discussietijd 3 minuten.

10.30 Early hepatocellular injury during liver surgery leads to systemic inflammation (p. 99)
*M.C.G. van de Poll[1], J.P.M. Derikx[1], E. Heineman[1], W.A. Buurman[1], A. van Bijnen[1], M.H.A. Bemelmans[1], C.H.C. Dejong[1]. Dept of Surgery[1], University Hospital Maastricht, The Netherlands*

10.40 Characterization of mice with gut specific expression of IL-12 family genes (p.100)
*L. Quaglietta[1], I. Pronk[2], A. Staiano[1], R. Troncone[1], M.S. Rodriguez Pena[2], F.J. ten Kate[3], D.W. Hommes[2], A.A. te Velde[2]. Dept of Pediatrics and European Laboratory for the Investigation of Food-Induced Diseases[1], University Federico II, Naples, Italy, Depts of Experimental Internal Medicine[2], and Pathology[3], Academic Medical Center, Amsterdam, The Netherlands*

10.50 Probing the role of peroxisome proliferator-activated receptor alpha (PPARα) in the small intestine (p.101)
*M. Bünger, H. van den Bosch, J. van der Meijde, S.Kersten, G. Hooiveld, M. Müller. Nutrition, Metabolism & Genomics Group, Division of Human Nutrition, Wageningen University, The Netherlands*

11.00 Intestinal expression of cholesterol transporter genes and chylomicron formation are not affected by dietary phytosterols and stanols in rodents* (p.102)
*J.K. Kruit[1], Y. Lin[2], T. Plösch[1], R. Havinga[1], W.J. Kloots[2], V.W. Bloks[1], R. Boverhof[1], G.S.M.J.E. Duchateau[2], A.K. Groen[3], F. Kuipers[1]. Dept of Pediatrics[1], University Medical Center Groningen, Unilever Health Institute[2], Unilever R&D, Vlaardingen, Dept of Experimental Hepatology[3], Academic Medical Center, Amsterdam, The Netherlands*

11.10 Cytoprotective role of Multidrug Resistance associated Protein (MRP1) in severe intestinal inflammation: involvement of the leukotriene pathway (p.103)
*A. van Steenpaal[1], H. Blokzijl[1], S. Vander Borght[2], L.I.H. Bok[1], M. Geuken[1], G. Dijkstra11[1], T.A.D. Roskams[2], P.L.M. Jansen[3], K.N. Faber[1]. Dept of Gastroenterology and Hepatology[1], University Medical Center Groningen, Dept of Gastroenterology and Hepatology[3], Academic Medical Center Amsterdam, The Netherlands, Dept of Liver Pathology[2], University of Leuven, Belgium*

11.20 Chemokine production by buccal epithelium as a distinctive feature of pediatric Crohn's disease* (p.104)
*G. Damen[1], J. Hol[1], L. de Ruiter[1], J. Bouquet[1], M. Sinaasappel[1], J. van der Woude[2], J. Laman[3], W. Hop[4], H. Escher[1], E. Nieuwenhui [1]. Dept of Pediatric Gastroenterology and Laboratory of Pediatrics[1], Erasmus Medical Center – Sophia Children's Hospital, Rotterdam, Depts of Gastro-enterology[2], Immunology[3], Epidemiology and Biostatistics[4], Erasmus Medical Center, Rotterdam, The Netherlands*

11.30 Regulation of murine *Muc5ac* mucin gene expression by GATA-6 and HNF-4α transcription factors during stomach development* (p.105)
*I.B. Renes[1], N. Jonckheere[2], A.M. Korteland-van Male[3], M.P. Ducourouble[2], A.W.C. Einerhand[3], I. van Seuningen[2]. Dept of Pediatrics, Divisions of Neonatology[1] and Pediatric Gastroenterology & Nutrition[3], Erasmus Medical Center - Sophia Children's Hospital, Rotterdam, The Netherlands, and Unité INSERM N°560[2], Lille Cedex, France*

11.40 The Neutrophil Activating Protein (HP-NAP) of Helicobacter pylori plays a role in adherence to gastric epithelial cells (p.106)
*M.M. Gerrits[1], F. Namavar[2], M. Sparrius [2], A.H.M. van Vliet[1], E.J. Kuipers[1], C.M.J.E. Vandenbroucke-Grauls[2], J.G. Kusters[1]. Dept of Gastroenterology and Hepatology[1], Erasmus Medical Center, Rotterdam, and Dept of Medical Microbiology and Infection Control[2], Free University Medical Center, Amsterdam, The Netherlands*

11.50 Evaluation of small bowel bacterial overgrowth in human duodenal biopsies by FISH (p.107). *D.H.H. Huijbregts, L.A. van der Waaij, H.J.M. Harmsen, B.J. Boersma, A.E. Smith, G.W. Welling. Dept of Gastroenterology and Medical Microbiology, University Medical Center Groningen, The Netherlands*

12.00 Lunchbuffet

*Vrijdag 7 oktober 2005*

13.00 Ledenvergadering Sectie Experimentele Gastroenterologie

**Sectie Experimentele Gastroenterologie** **Parkzaal**

**Voorzitters:** E.H.H.M. Rings en H.W. Verspaget

Voordrachten in het Nederlands, spreektijd 7 minuten, discussietijd 3 minuten.

13.30 TRAIL and Fas are not responsible for apoptosis of crypt epithelial cells in intestinal transplant rejection (p.108)
*G. Dijkstra[1], R. Ymker[1], L. Bok[1], J. Koornstra[1], S. Radio[2], R. Ploeg[3], A. Langnas[4], D. Sudans[4]. Dept of Gastroenterology and Hepatology[1], University Medical Center Groningen, The Netherlands, Depts of Pathology[2] and Surgery[4], Division of Transplantation, University of Nebraska Medical Center, Omaha, USA, and Dept of Surgery[3], Division of Transplantation and Organ Donation, University Medical Center Groningen, The Netherlands*

13.40 5-Aminosalicylic acid inhibits colorectal cancer cell growth by inducing mitotic arrest and apoptosis (p.109)
*P.J. Koelink, M.A.C. Mieremet-Ooms, G. Griffioen, H.W. Verspaget. Dept of Gastro-enterology-Hepatology, Leiden University Medical Center, Leiden, The Netherlands*

13.50 Important role for Mucin 2 in colonic protection against Dextran Sulfate Sodium* (p. 110)
*M. van der Sluis[1], I. van Seuningen[2], A. Velcich[3], J.B. van Goudoever[1], H.A. Büller[4], J. Dekker[4], A.W.C. Einerhand[4], I.B. Renes[1]. Division of Neonatology[1], Laboratory of Pediatrics, Erasmus Medical Center - Sophia Children's Hospital, Rotterdam, The Netherlands, Unité INSERM No560[2], 59045 Lille Cedex, France, Dept Oncology[3], Albert Einstein Cancer Center/ Montefiore Medical Centre, New York USA Pediatric, Division of Gastroenterology and Nutrition[4], Laboratory of Pediatrics, Erasmus Medical Center - Sophia Children's Hospital, Rotterdam, The Netherlands*

14.00 Protection against experimental colitis by Filamentous Hemagglutinine A of Bordetella Pertussis is not exclusively mediated by IL-10 (p. 111)
*I.L. Huibregtse[1], H. Braat[1], P. McGuirk[2] , K. Mills[2], S.J.H. van Deventer[1]. Laboratory of Experimental Internal Medicine[1], Academic Medical Center Amsterdam, The Netherlands, Dept of Biochemistry[2], Immune Regulation Research Group, Trinity College, Dublin, Ireland*

14.10 Methotrexate-induced mucositis in mucin 2 deficient mice* (p. 112)
*B.A.E. de Koning[1*], M. van der Sluis[2*], D.J. Lindenbergh-Kortleve[1], A. Velcich[3], R. Pieters[4], H.A. Büller[1], A.W.C. Einerhand[1], I.B. Renes[2], *Both authors participated equally in this study. Laboratory of Pediatrics, Division of Pediatric Gastro-enterology[1], Division of Neonatology[2], and Pediatric Oncology[4], Erasmus Medical Center - Sophia Children's Hospital, Rotterdam, The Netherlands, Dept Oncology[3], Albert Einstein Cancer Center/Montefiore Medical Centre, New York, USA*

14.20 Colonic application of tacrolimus inhibits local mucosal immune responses in mice (p. 113)
*J.M. van Dieren[1], C.J. van der Woude[1], E.J. Kuipers[1], J.N. Samsom[2], E.E.S. Nieuwenhuis[2]. Dept of Gastroenterology and Hepatology[1], Erasmus Medical Center, Rotterdam, Laboratory of Pediatrics[2], Division of Gastroenterology, Erasmus Medical Center - Sophia Children's Hospital, Rotterdam, The Netherlands*

14.30 Mucosal delivery of Ovalbumin by the genetically modified L. lactis suppresses inflammatory T-cell responses in DO11.10 mice (p. 114)
*I.L. Huibregtse[1], H. Braat[1], K. van Laer[2], A.A. te Velde[1], P. Rottiers[2], S.J.H. van Deventer[1]. Laboratory of Experimental Internal Medicine[1], Academic Medical Center, Amsterdam, The Netherlands, and Dept of Molecular Biomedical Research[2], VIB, Ghent, Belgium*

14.40 Can probiotics inhibit the growth of antibiotic resistant micro-organisms? In-vitro study (p. 115)
*L. van Bindsbergen[1,2], B.U. Ridwan[1], M.G.H. Besselink[2], H.M. Timmerman[2], J. Verhoef[1], H.G. Gooszen[2], L.M.A. Akkermans[2]. Depts of Medical Microbiology[1] and Surgery[2], University Medical Center, Utrecht, The Netherlands*

14.50 Activation induced unresponsiveness to peptidoglycan of intestinal epithelial cells upon short term exposure to Lactobacillus casei* (p. 116)
*J. Hol[1], H.C. Raatgeep[1], E. Kerkhof[1], L. de Ruiter[1], J.N. Samsom[1], E.E.S. Nieuwenhuis[1]. Laboratory of Pediatrics[1], Erasmus Medical Center - Sophia Children's Hospital, Rotterdam, The Netherlands*

15.00 Theepauze, einde programma

**Nederlandse Vereniging voor Gastroenterologie** **Baroniezaal**

**Voorzitter:** J.B. van Goudoever en C.M.F. Kneepkens

Voordrachten in het Nederlands, spreektijd 7 minuten, discussietijd 3 minuten.

13.30 Host-microbe interactions in vivo in the human small intestine; the effects of lactobacillus plantarumWCFS1 using functional transcriptomics and proteomics (p. 117)
*F.J. Troost[1], R.J.M. Brummer[12]. Division of Gastroenterology, Depts of Internal medicine[1] and Clinical Dietetics[2], The Netherlands*

13.40 Increased portal blood flow maintains hepatic ammonia clearance capacity following major liver resection in man (p. 118)
*M.C.G. van de Poll[1], S.J. Wigmore[2], P.A.M. van Leeuwen[1], N.E.P. Deutz[1], C.H.C. Dejong[1]. Depts of Surgery, University Hospital Maastricht[1], The Netherlands, Royal Infirmary Edinburgh[2], Scotland, Free University Medical Center[3], Amsterdam, The Netherlands*

13.50 Albumin synthesis in preterm ventilated infants on the first day of life, studied with [1-$^{13}$C]leucine* (p. 119)
*J.E.H. Bunt[1], T. Rietveld[2], H. Schierbeek[2], D. Wattimena[2], L.J.I. Zimmermann[3], J.B. van Goudoever[1]. Divisions Neonatology[1,3], Dept Pediatrics, Erasmus Medical Center - Sophia Children's Hospital, Rotterdam, and Internal Medicine[2], Erasmus Medical Center, Rotterdam, The Netherlands*

14.00 Reduced circulating TNF receptor p75/p80 after enriching enteral nutrition with glutamine and antioxidants following major upper GI surgery (p. 120)
*P.G. Boelens, G.C. Melis, J. Diks, P.A.M. van Leeuwen. Dept of Surgery, Free University Medical Center, Amsterdam, The Netherlands*

14.10 Cachectic tumor (MAC16) induces poor post-operative response with arginine deficiency (p. 121)
*Y.L.J. Vissers, M.F. von Meyenfeldt, Y.C. Luiking, C.H.C. Dejong, N.E.P. Deutz. Dept of Surgery, Maastricht University Hospital, Maastricht, The Netherlands*

14.20 Exposure to bacterial DNA before hemorrhagic shock leads to massive liver damage and apoptosis via an IFN-gamma dependent route (p. 122)
*J.P.M. Derikx, M.D.P. Luyer, J.W. Greve, E. Heineman, W.A. Buurman. Dept of Surgery, University Hospital Maastricht, The Netherlands*

14.30 Natural history of early gallbladder stones: a follow-up study in patients with colorectal carcinoma (p. 123)
*W.P. Metsaars WP[1], A.B. Bijnen[1,2]. Depts of Surgery[1], Medical Center Alkmaar, and Medical Education[1], Free University Medical Center, The Netherlands*

14.40 Laser capture microdissection is compatible with 16S rDNA-based genomic technoogies in bowel biopsies from Crohn's disease patients (p. 124)
*G. De Hertogh[1], J. Aerssens[2], R. de Hoogt[2], P. Peeters[2], P. Verhasselt[2], P. Van Eyken[1], P. Rutgeerts[3], B. Coulie[2], K. Geboes[1]. Depts of Morphology & Molecular Pathology[1] and Gastroenterology[3], University Hospitals, KU Leuven, Johnson & Johnson Pharmaceutical Research & Development[2], Beerse, Belgium*

14.50 Theepauze en einde programma

**Symposium: 'Patiënten met dikke darm kanker'**

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|---|---|
| 09.30 | Ontvangst met koffie/thee |
| 10.00 | Welkomstwoord en inleiding<br>*door de voorzitter van de VMDLV: dhr. H. Welling* |
| 10.05 | Anatomie, fysiologie en pathologie bij coloncarcinoom<br>*Dr. F.M. Nagengast, Universitair Medisch Centrum Nijmegen St. Radboud* |
| 11.00 | Koffie/theepauze |
| 11.15 | ERAS (Enhanced Recovery After Surgery)<br>Fast track leverchirurgie<br>*Dr. C.H.C. Dejong, chirurg, en Dr. R.M. van Dam, chirurg,*<br>*Kimm Luyten, verpleegkundige gastro-intestinale chirurgie*<br>*Academisch Ziekenhuis Maastricht* |
| 12.15 | Lunch |
| 13.15 | Voedingsaspecten bij het ERAS project<br>*José Maessen, research medewerker afd. diëtetiek, Academisch Ziekenhuis Maastricht* |
| 14.15 | Verpleegkundig spreekuur polikliniek darmkankerpatiënten<br>*Hanneke Balk en Paulien Staal: coloncare verpleegkundigen Gelderse Vallei* |
| 14.35 | Belang van een vereniging, Maag Lever Darm Stichting<br>*Coördinator voorlichter van MLDS: J. Dierx* |
| 14.55 | Sluiting van de dag door dhr. H. Welling |

**Ochtendprogramma SEVA**

10.30 Tonometrie
*Mevr. A. Goossens, verpleegkundige, Medisch Spectrum Twente, Enschede*

10.50 Endo Mucosale Resectie
*Mevr. H. Foekema, endoscopie verpleegkundige, AMC Amsterdam*

11.10 Werken zonder wachtlijst
*Mevr. T. van der Meulen, endoscopie verpleegkundige, VUMC Amsterdam*

11.30 Ledenvergadering

12.30 Lunchbuffet in de expositiehal

**Middagprogramma SEVA**

13.30 Werken op de endoscopieafdeling in België
*Dhr. W. Claes, voorzitter Vlaamse Vereniging voor Endoscopie*

14.00 Voorstelling "Op stap naar de toekomst"
Pleegtheater, Amsterdam

14.30 Borrel ter gelegenheid van het 20-jarig jubileum SEVA

**Liver-X-receptor agonist T0901317 suppresses endotoxin-induced expression of inflammatory genes in Kupffer cells in vitro**

J. Mulder[1], J.A.A.M. Kamps[2], S.J. Karpen[3], F. Kuipers[1], E. Sturm[1]. Dept of Pediatrics[1], Center for Liver, Digestive and Metabolic Diseases, and Dept of Pathology & Laboratory Medicine/Medical Biology[2], University Medical Center Groningen, The Netherlands, and Dept of Pediatrics[3], Baylor College of Medicine, Houston, TX, USA.

Kupffer cells, the resident liver macrophages, play a key role in inflammation-induced cholestasis and thus represent a potential target for pharmacological intervention. The liver-X-receptor (LXR), a ligand-activated transcription factor which heterodimerizes with the retinoid-X-receptor (RXR), has been shown to possess anti-inflammatory properties in peritoneal macrophages. Recent work indicates that the synthetic LXR-agonist T0901317 (T09) is able to attenuate endotoxin-induced effects on hepatic transporter expression *in vivo*. However, it remains unknown whether T09 exerts its *in vivo* effects in Kupffer cells or directly in hepatocytes. Hence, we sought to determine whether T09 has anti-inflammatory effects in endotoxin-stimulated Kupffer cells *in vitro*.

Primary rat Kupffer cells (PRKC) and a mouse peritoneal macrophage cell-line (IC-21) were pretreated with T09 (0.1-20μM) for 1-24hrs. Subsequently, cells were exposed to endotoxin (lipopolysaccharide (LPS), 1μg/ml) for 3hrs. Gene expression was assessed by quantitative real-time PCR.

LXRα and LXRβ gene expression was similar in IC-21 cells and PRKC. At 3hrs after LPS-treatment, IL-6 expression was induced 35-fold, TNFα 94-fold, IL-1β 115-fold and iNOS 25-fold in IC-21 cells. T09-pretreatment suppressed IL-6 expression in LPS-stimulated IC-21 cells in a dose-dependent manner and this effect was dependent on pretreatment time (at least 3-5hrs for maximal suppression).

In PRKC pretreated with T09 for 20hrs, LPS-induced expression of IL-6, TNFα, IL-1β and iNOS was dose-dependently suppressed. At 20μM T09, IL-6 expression was suppressed by 70%, TNFα by 39%, IL-1β by 50% and iNOS by 84% compared to LPS-treated control cells.

Finally, the efficacy of T09-pretreatment was increased by addition of the RXR-ligand LG1069. Co-pretreatment with LG1069 (0.1μM) enforced T09-suppression of IL-1β mRNA from 42% to 91% in LPS-stimulated IC-21 cells.

In conclusion, T09 is able to inhibit the inflammatory response in Kupffer cells *in vitro*. This effect is dose-dependent and comparable to that in IC-21 macrophages. This is the first evidence that Kupffer cells are susceptible to anti-inflammatory effects of an LXR-ligand. Targeting of LXR-ligands to Kupffer cells *in vivo*, particularly in combination with an RXR-ligand, may represent a novel strategy to treat inflammation-induced cholestasis.

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**Simultaneous targeting of HCV replication and viral entry by a single gene therapy vector containing multiple RNA interference cassettes: prevention of mutational escape**

S.D. Henry[1], P.G. van der Wegen[2], H.J. Metselaar[3], R. Bartenschlager[4], H.W. Tilanus[1], B.J. Scholte[2], L.J.W. van der Laan[1]. Depts of Surgery[1], Cell Biology[2] and Gastroenterology and Hepatology[3], Erasmus Medical Center, Rotterdam, The Netherlands, and Dept of Molecular Virology[4], University of Heidelberg, Germany

Chronic Hepatitis C Virus (HCV) is a major medical problem. Current treatments are often unsuccessful. Targeted gene therapy of the liver presents a promising new approach to tackle this problem. Aim of the current study is to construct and test state-of-the-art lentiviral vectors that utilize RNA interference (RNAi) to prevent HCV infection.
Vectors were constructed with single (Con, 321, 6367, CD81), double (321-6367, 321-CD81, 6367-CD81) and triple (321-6367-CD81) self expressing short hairpin RNA (shRNA) constructs targeting two regions of the HCV 1b genome and host cell viral entry receptor CD81. The anti-viral effect of the RNAi therapy was tested in Huh-7 hepatoma cell line, containing the HCV replicon with a luciferase reporter. Luciferase activity, mRNA levels, and helicase (NS3) expression were used to determine effectiveness of RNAi. IFN production was detected by BioAssay and ELISA. CD81 expression was determined by FACS staining and immunocytochemistry.
Vectors expressing different shRNA targeting the HCV 5' UTR(321) or NS5 (6367) were shown to be effective in inhibiting HCV replication in vitro by 82 and 98%, respectively. Vectors containing shRNA targeting CD81 reduced surface expression by 83% and showed no effect on the expression of unrelated surface protein (Ber-EP4) or HCV replication. Vectors expressing both anti-HCV shRNA cassettes or HCV and CD81 targets showed similar reduction in HCV levels (321-6367, 85%; 6367-CD81, 89%). Triple expressing vectors (321-6367-CD81) showed effective reduction of HCV replication (75% inhibition) and also reduced CD81 surface expression by 76%. It is important to note that none of the cells expressing shRNA produced a detectable interferon response.
Conclusion: This study has demonstrated that RNA interference is effective in reducing HCV replication in vitro. This effect was not dependant on an IFN response to the shRNA. The lentiviral vectors that deliver multiple shRNA payloads which target different viral and host cell sequences simultaneously were similar in effectiveness to individual constructs. In addition, cells expressing multiple shRNAs should prevent mutational escape of HCV. We are currently testing the effectiveness these vectors on viral entry and replication of primary virus.

**The inhibition by regulatory T cells of the immune response against HBV is partially HBV-specific but not mediated through IL10 and TGF-β**

J.N. Stoop[1], J.G. Kusters[1], E.J. Kuipers[1], C.C. Baan[2], H.L.A. Janssen[1], R.G. van der Molen[1]. Depts of Gastroenterology and Hepatology[1], and Internal Medicine[2], Erasmus Medical Center, Rotterdam, The Netherlands

Chronicity of hepatitis B virus (HBV) infection is characterized by a weak immune response to the virus. Previously, we have shown that the proportion of CD4+CD25+*FoxP3*+ regulatory T cells (Treg) in peripheral blood of patients with a chronic HBV infection was increased compared to both healthy controls and to those who have resolved their HBV infection. These Treg were capable of specifically inhibiting the T cell response against HBV core antigen (HBcAg) in a dose dependent manner. Since it is unclear how these Treg suppress the HBV specific immune response, the aim of this study was to determine the underlying mechanism.

Treg and Treg-depleted cell fractions were isolated from peripheral blood of 5 patients with a chronic HBV infection using MACS beads. The relative *FoxP3* mRNA expression of the isolated Treg was determined by RT-PCR and compared to the *FoxP3* mRNA expression in Treg-depleted cells. Treg-depleted cells and Treg-depleted cells reconstituted with 20% Treg were stimulated with HBcAg or with tetanus toxin. Neutralizing antibodies against IL10 and/or TGF-β or isotype matched control antibodies were added to the HBcAg stimulated cells. After 6 days the proliferation was determined by incorporation of $^{3}$[H]-thymidine.

The isolated CD4+CD25+ cells showed a 192-fold higher expression of the Treg-specific transcription factor *FoxP3* compared to the Treg-depleted cell fraction, indicating that the added CD4+CD25+ cells are indeed CD4+CD25+*FoxP3*+ Treg. These Treg were capable of inhibiting the response against both HBcAg and tetanus toxin, however the suppressive effect on the proliferation against HBcAg was stronger (52% inhibition for HBcAg vs. 33% inhibition for tetanus toxin). Neutralizing antibodies to IL-10 or TGF-β alone or combined did not abrogate the suppression caused by the Treg, compared to control antibodies.

In conclusion, CD4+CD25+FoxP3+ Treg isolated from chronic HBV patients display a much stronger inhibiting effect on the proliferation against HBcAg compared to the proliferation against tetanus toxin. This suggests that chronic HBV patients have more HBV-specific Treg in their peripheral blood. The inhibition by Treg is not mediated through an IL10 and TGF-β dependent mechanism.

**Reduced expansion of HBV specific CD8$^+$ T cells by myeloid dendritic cells isolated from chronic HBV patients**

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Dendritic cells (DC) play an important role in the induction of T cell responses. We hypothesize that the defective anti-viral T cell response in chronic hepatitis B patients is a result of impaired DC function. Previously, it was shown that mDC of chronic HBV patients are indeed impaired in their maturation and allostimulatory capacity. In this study we analyzed whether these mDC are also hampered in the expansion of HBV specific CD8$^+$ T cells. MDC were isolated from peripheral blood mononuclear cell (PBMC) of HLA-A2$^+$ chronic HBV patients and healthy controls, using magnetic cell sorting techniques. MDC were pulsed for 2 hours with or without HBcore18-27 peptide, washed and matured overnight with IL-1$\beta$ and TNF$\alpha$. Next, PBMC or purified CD8$^+$ T cells from an HLA-A2$^+$ individual with memory to HBV were added to the mDC and co-cultured for 9 days in the presence of IL-2. Subsequently, HBV specific CD8$^+$ T cells of total CD8$^+$ T cells were detected by flowcytometry using a HBc18-27 specific pentamer. The expansion of HBV specific CD8$^+$ T cells was significantly reduced by mDC of chronic HBV patients compared to healthy controls, both in whole PBMC cultures (mean ± SEM; 4.08% ± 0.79 vs 8.56% ± 1.26, $p<0.01$; n=11), as in purified CD8$^+$ T cell cultures (2.22% ± 0.31 vs 5.02% ± 1.19; $p<0.05$; n=14). The outgrowth using mDC pulsed without peptide was always lower than 0.05%. Addition of a neutralizing antibody to IL-10 completely restored the reduced expansion of HBV specific CD8$^+$ T cells as compared to healthy control mDC with anti-IL-10 (4.58 ± 0.95 vs 4.67 ± 0.95; n=14). To investigate whether the impaired expansion of CD8$^+$ T cells was HBV specific, we pulsed the mDC with CMV pp65-derived peptide NLVPTMATV. Next, CD8$^+$ T cells from an individual who has CMV specific CD8$^+$ T cells were added and the outgrowth was determined using a tetramer specific for CMVpp65. The results showed that there was a trend towards reduction of the expansion of CMV specific CD8$^+$ T cells by mDC of chronic HBV patients as compared to healthy controls (39.6% ± 3.84 vs 48.2% ± 4.89; n=7).

In conclusion: These results indicate that mDC of chronic HBV patients display a preferential reduced capacity for the expansion of HBV specific CD8$^+$ T cells, which was IL-10 dependent. Correcting this defect may reverse HBV tolerance and could thus lead to novel therapeutic strategies to overcome chronic HBV infection.

**The expression of E-selectin, P-selectin and ICAM-1 in the peritoneal microvessels of portal hypertensive and cirrhotic rats treated with or without peginterferon-alpha2a**

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Patients with portal hypertension (PHT) and cirrhosis are more likely to develop bacterial infections. Previously, we showed significantly impaired leukocyte recruitment in peritoneal microvessels of rats with PHT and cirrhosis. A treatment with peginterferon (pegIFN) improved number of rolling leukocytes, probably through upregulation of selectins, but had no influence on adhesion and extravasation. E-selectin, P-selectin and ICAM-1 are important mediators to regulate respectively rolling and adhesion. Aim was to investigate E-, P-selectin and ICAM-1 expression in response to endotoxin in peritoneal microvessels of rats with PHT and cirrhosis with or without peginterferon-alpha2a treatment. Tissue samples from visceral peritoneum (after LPS stimulation) were taken in Sham (n=8), partial portal vein ligated (PPVL) (n=8) and common bile duct ligated rats (CBDL) (n=8), placebo or pegIFN treated in a dose of 18 µg/rat per week SC. These were immunohistochemically stained for E-, P-selectin and ICAM-1 and a score from 0 to 3 was given for the intensity of staining on the endothelial cells.
P-selectin expression was similar in Sham, PPVL and CBDL placebo rats. PegIFN increased P-selectin expression significantly in Sham and CBDL rats compared to placebo treated, while there was no effect in PPVL rats. E-selectin expression was significantly higher in Sham placebo rats compared to PPVL and CBDL placebo rats. PegIFN increased and normalized E-selectin expression in PPVL similar to the expression in Sham, while E-selectin expression in CBDL only slightly increased and remained significantly lower compared to PPVL group. ICAM-1 expression was significant higher in Sham placebo rats compared with PPVL and CBDL placebo rats. PegIFN did not increase ICAM-1 expression in the PPVL and CBDL rats.
In conclusion: This study provides for the first time evidence of an underlying defect in expression of E-selectin and ICAM-1 on endothelial cells in PPVL and CBDL rats. PegIFN treatment showed upregulation of P-selectin expression in Sham and CBDL rats. Also E-selectin expression was increased significantly in PPVL and CBDL rats with normalization in PPVL rats. There was no effect on ICAM-1 expression after pegIFN treatment. These results showed for the first time that pegIFN caused an increase in the number of rolling leukocytes through an upregulation of expression of P- and E-selectins. Despite improving rolling by pegIFN, adhesion and emigration remained impaired in vivo in PPVL and CBDL rats. We showed that a decreased ICAM-1 expression was responsible for these findings.

**Effect of MARS and PROMETHEUS on systemic hemodynamics and vasoactive agents in patients with acute on-chronic alcoholic liver failure: the clash of the titans**

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Following acute-on-chronic liver failure (AoCLF), patients with cirrhosis and portal hypertension show an aggravated hyperdynamic circulation, which is presumed to originate in excessive endogenous vasoactive substances and is deemed co-responsible for multiple organ dysfunction. Liver detoxification devices, such as the Molecular Adsorbent Recirculating System (MARS) and Fractionated Plasma Separation, Adsorption, and Dialysis (Prometheus [PROM]), have been proposed as treatment options for severe liver failure and its complications. We aimed to evaluate, in a controlled manner, potential changes on systemic haemodynamics induced by MARS or PROM in patients with AoCLF. 18 patients (51.2±2.3 yr, 10M/8F, MELD 25.6±1.4, Child-Pugh 12.5±0.2, Maddrey 63.1±5.0, HVPG 17.6±0.9mmHg [mean±SEM]) with biopsy-proven alcoholic cirrhosis and superimposed alcoholic hepatitis were treated with either standard medical therapy (SMT) combined with MARS (n=6) or PROM (n=6) or SMT alone (n=6) on 3 consecutive days (6 hours/session). Besides liver tests, systemic hemodynamics and vasoactive agents such as plasma renin activity (PRA), aldosteron (ALDO) and the catabolic products of nitric oxide (NOx) were determined before and after sessions. Baseline hemodynamics and levels of vasoactive substances were comparable. Both MARS and PROM decreased serum bilirubin (P<.005 vs SMT), PROM being more effective than MARS (removal rate 39.3±5.2% vs 14±2.9%, P=.002). In contrast, only MARS showed significant improvement in MAP (Δ change: +9±2.4 mmHg vs -0.3±2.4mmHg with PROM and -5.2±2.1mmHg with SMT, P<.05) and in SVRI (Δ change: +131.5±46.2dyne.s.cm-5.m-2 vs -92.8±85.2dyne.s.cm-5.m-2 with PROM and -30.7±32.5dyne.s.cm-5.m-2 with SMT, P<.05) while cardiac index and central filling remained constant. This circulatory improvement in the MARS group was paralleled by a significant decrease in PRA (12.6±3.9 to 5.5±2.3µg/L/h vs PROM 10.7±3.4 to 12.4±4.2 µg/L/h and SMT 9.8±3.0 to 10.8±3.4µg/L/h, P<.03), ALDO (461±123 to 138±30ng/L vs PROM 394±177 to 433±235ng/L and SMT 417±102 to 483±125ng/L, P<.02) and NOx levels (91.2±16.7 to 40.4±5.9µM vs PROM 73.6±14.4 to 84.4±17.6µM and SMT 68.6±9.9 to 71.7±13.1µM, P<.005). In conclusion, PROM shows a more effective reduction in bilirubin, but MARS greatly attenuates the hyperdynamic circulation typical of AoCLF probably by the demonstrated decrease in vasoactive agents. This emphases conspicuous conceptual differences amongst the albumin dialysis devices.

## Robot-assisted thoracoscopic esophago-lymphadenectomy for esophageal cancer

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Transthoracic esophagectomy offers the best chance for cure in patients with esophageal cancer. This operation carries a high morbidity rate mainly caused by pulmonary complications. Minimally invasive esophagectomy aims to reduce postoperative morbidity and mortality. The feasibility and safety of robot-assisted thoracoscopic esophago-lymphadenectomy was assessed prospectively. Twenty-one consecutive patients with esophageal cancer underwent robot-assisted thoracoscopic esophago-lymphadenectomy using the Da Vinci™ system. The first 16 patients underwent a thoracoscopic dissection followed by laparotomy to create the gastric conduit and a cervical anastomosis. In the last five cases, the entire abdominal phase was performed laparoscopically as well. Eighteen (86%) procedures were completed thoracoscopically. Conversion to thoracotomy occurred in 3 patients due to either extensive pulmonary adhesions, a bulky adhesive tumor in the upper esophagus, or bleeding from an aortic branch. The median blood loss was 400 (150-700) ml for the thoracoscopic phase and 950 (250-5300) ml for the complete procedure. Median operating time for the thoracoscopic phase was 180 (120-240) min and 450 (372-550) min for the complete procedure. A median of 20 (9-30) lymph nodes were retrieved. Median ICU stay was 4 (1-129) days and postoperative ventilation time was 2 (0-126) days. Pulmonary complications occurred in 10 (48%) patients, cardiac in 3 (14%), anastomic leakage in 3 (14%), chylous leakage in 3 (14%) and vocal cord paralysis in 3 (14%). Overall median hospital stay was 18 (11-182) days. One (4.7%) patient died from a tracheo-esophageal fistula. There was a steep learning curve with a reduced median operation time of the robot-assisted thoracoscopic part (2.5 hrs respectively 3.8 hrs) and reduced pulmonary complications (20% respectively 60%) in the last 10 patients compared to the first 10.
Conclusions: Combined robot-assisted thoracoscopic and standard laparoscopic eso-phago-lymphadenectomy for cancer of the esophagus has demonstrated to be feasible and safe. It allows an effective mediastinal lymphadenectomy with low blood loss.

## Long-term results of laparoscopic fundoplication

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Nowadays laparascopic (partial) fundoplication is the preferred surgical treatment for therapy resistant reflux disease. Previously we have reported a high success rate (symptom relief in 85% and normalization of 24 hr pH metry in 80%) after short-term follow up of 6-12 months. Little is known however on long-term results and symptom relief after laparoscopic antireflux surgery. Our aim was to evaluate the long-term results of 130 patients operated at our institution between 1991 and 2001 for therapy resistant reflux disease. From 1991 to 1995 complete fundoplication was performed, thereafter partial fundoplication. We evaluated symptoms (severity score 0-3, frequency score 0-3, combined score 0-9), use of anti-reflux medication, patient satisfaction and quality of life (SF-36) by questionnaires. Response rate was over 70%.
Results: Mean age of patients was 54 ± 1.4 yr (women 53%). The mean follow up time after surgery was 7.8 ± 0.5 yrs. Indication for surgery was therapy resistant reflux disease or unwillingness to maintenance therapy. Reflux was documented by endoscopy (esophagitis) or 24 hr ph metry. Eight patients underwent reoperation because of recurrent reflux or dysphagia. These data were also included. The heartburn scores decreased sign. ($p<0.0001$) from 6.6 ± 0.3 (pre-op) to 1.6 ± 0.3 (long term post op), retrosternal pain from 5.0 ± 0.4 to 1.8 ± 0.3 ($p<0.0001$) and dysphagia from 4.0 ± 0.4 to 2.2 ± 0.3 ($p<0.0001$). Concerning antireflux medication: 31% still needed acid reducing medication on a daily basis, 9% on a weekly basis and 60% had no need for medication. Satisfaction with surgical result: 25% was not satisfied with the procedure, 15% moderately satisfied and 60% fully satisfied. When compared to age matched controls, quality of life by SF-36 was significantly ($p<0.05$) reduced for all domains in the patients post fundoplication. In the patients with follow up 10-15 yrs and 5-10 yrs, QOL was significantly ($p<0.008$) higher versus patients with 0-5 yrs follow up. These results were not affected by the type of surgery (10-15 yrs complete fundoplication, other groups partial fundoplication) nor by age or gender.
In conclusion: Long term follow up of patients after laparoscopic surgery shows a less favorable outcome compared to short term follow up. One third of the patients still needs acid reducing drugs to control symptoms and 25% is not satisfied with the procedure.

## Re-operation rate after laparoscopic adjustable gastric banding (LAGB)

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Introduction: Morbid obesity is a rapidly growing problem in western society. Different operation techniques with a gastric restrictive or malabsorbtive effect are used to induce weight loss and reduce obesity related comorbidities. A type of gastric restrictive surgery that gained interest the last few years is laparoscopic adjustable gastric banding (LAGB). This is a safe technique with few direct postoperative complications. However, there are complications such as slippage of the band and pouch dilatation, for which re-operation is necessary. In this study laparoscopic re-operation after LAGB is evaluated.
Results: From February 1997 to March 2002 74 bands were placed in our clinic in 61 females and 13 males (mean age: 36.9 years). Initial body mass index (BMI) was 44 kg/m². During the first operation a 9.75 cm band was placed in all patients. There were no direct postoperative complications. Postoperative follow-up was 100%.Re-operation was necessary in 21 patients (28%); 19 females and 2 males. Initial band placement was perigastric in 17 patients and pars flaccida in 4 patients. At the time of re-operation mean BMI was decreased to 36 kg/m² and comorbidities were significantly reduced. Mean time between the first band placement and re-operation was 28.8 months (range: 7-61).The cause of band dysfunction was anterior slippage with pouch dilatation (n=17), band erosion (n=2), posterior slippage (n=2), band leakage (n=2) or a combination of slippage with band leakage (n=2).Symptoms leading to the diagnosis of band dysfunction were vomiting (n=11), pyrosis (n=8), nausea (n=7), retrosternal pain (n=5) and regurgitation (n=3). Laparoscopic revision was successful in 19 patients. In 2 patients the laparoscopic procedure was converted to open surgery because of adhesions. During re-operation the perigastric technique was converted to the pars flaccida technique in 9 patients. In 10 patients pouch reduction and refixation was performed. In 2 patients with band erosion the 9.75 cm band was replaced, after primary removal, by a wider band. Postoperatively, there were no direct complications except for wound infections (n=2).The median follow-up after laparoscopic re-operation was 32 months (range: 2-45). BMI and obesity related comorbidity both decreased further after revision. Except for 2 re-slippages (10%) after 27 en 48 months, there were no long-term complications.
Conclusion: Complications after Lap-Band, mainly anterior slippage and pouch dilatation, can be successfully managed with laparoscopic revision with further decrease of BMI and obesity related comorbidities.

**Fast track major liver surgery; initial experience with an enhanced recovery programme**

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Introduction: Recovery and outcome after surgery can be improved with multimodal perioperative care protocols. Pre-operative oral carbohydrate loading, epidural analgesia and limited use of opioids reduce nausea and allow removal of naso-gastric (NG) tubes and oral feeding early after surgery. This in combination with early mobilization and prescheduled care seem to contribute most to the improvement in postoperative recovery. However, it remains unclear whether such programmes can be applied to major resectional liver surgery. This study was conducted to evaluate whether an enhanced recovery programme can accelerate postoperative recovery in major resectional liver surgery.
Methods: An enhanced recovery feasibility study of a consecutive series of 11 patients undergoing major liver resection was conducted (Group I, n=11). The collaborative Enhanced Recovery After Surgery group (ERAS group) multimodal core protocol for colonic surgery was modified to cover all aspects of major liver surgery. Data on naso-gastric drainage, restart of oral intake after surgery, length of postoperative hospital stay (LOS), readmissions, morbidity and mortality were collected prospectively and compared with historical data from a prospectively collected data base of all major liver resections in our hospital from 2000 to 2004 (group II, n=49). The Mann-Whitney U test and the Chi-Square test were used for statistical analysis.
Results: In all patients the NG tube could be removed and oral intake restarted within 4 hours after surgery. Median LOS (including readmissions) was 6 days (range 4 – 42) in group I and 9 days (range 6 - 64) in group II (p=0,14), with 55% of patients discharged after 4 days in group I (1 readmission). The readmission rate was 18% in group I and 21% in group II (p=0,77). The complication rate was 45 % in group I and 23 % in group II (p=0,12). Complications in group I consisted of 3 bile leakages, 2 temporary liver failures, 1 fluid collection and 1 pleural effusion, all of these were managed non-operatively. Mortality for the subgroups was 0 % and 2 %, group I and II respectively (p=0,63).
Conclusion: An enhanced recovery programme after major liver resection is feasible and safe. Oral intake can be restarted within 4 hours after surgery. A LOS of 4 days is achievable in the majority of patients when complications are absent. Median length of postoperative hospital stay is reduced to 6 days without an increase in morbidity or mortality.

**Bile Salt Toxicity Aggravates Cold Ischemic Injury of Bile Ducts after Liver Transplantation in Mdr2+/- Mice**

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Intrahepatic bile duct strictures are a serious type of biliary complication after orthotopic liver transplantation (OLT). We aimed to define the role of endogenous bile salt toxicity in the pathogenesis of bile duct injury after OLT.
Methods: Livers from wild-type mice and mice heterozygous for disruption of the multidrug resistance protein 2 Mdr2 gene (Mdr2+/-) were transplanted into wild-type recipient mice. Mdr2+/- mice secrete only 50% of the normal amount of phospholipids into their bile, leading to an abnormally high bile salt/phospholipid ratio. In contrast to homozygous Mdr2 -/- mice, the Mdr2+/- mice, however, have normal liver histology and function under normal conditions. Two weeks after OLT, bile duct injury and cholestasis were assessed by light and electron microscopy, as well as through molecular and biochemical markers.
Results: There were no signs of bile duct injury or intrahepatic cholestasis in liver grafts from wild-type donors. Liver grafts from Mdr2+/- donors, however, had enlarged portal tracts with cellular damage, ductular proliferation, bilio-stasis, and a dense inflammatory infiltrate after OLT. Parallel to this observation, recipients of Mdr2+/- livers had significantly higher serum bilirubin, transaminases, alkaline phosphatase levels, and bile salts, compared to recipients of wild-type livers. In addition, molecular signs of intrahepatic cholestasis were found in Mdr2+/- grafts but not in wild-type grafts.
Conclusion: This data indicates that toxic bile composition, due to a high biliary bile salt/phospholipid ratio, acts synergistically to cold ischemia in the pathogenesis of bile duct injury after transplantation.

**Magnetic Resonance Cholangiopancreatography (MRCP) as Initial Examination in Biliary Pancreatitis**

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MRCP is a noninvasive imaging modality that can be used for the visualization of the common bile duct (CBD) before laparoscopic cholecystectomy (LC). However, by its cost and limited availability, it should only be performed when CBD stones are suspected. We evaluated MRCP in patients with biliary pancreatitis (BP) in whom, by definition, gallstones either are present in, or have passed through the CBD.
Methods: A series of 84 consecutive patients with BP was prospectively studied. MRCP findings were compared with subsequent endoscopic retrograde cholangiopancreatography (ERCP), intraoperative cholangiography (IOC) or clinical follow-up.
Results: In 19 out of 84 patients (22,6%) MRCP revealed evidence of CBD calculi. Eighteen patients underwent ERCP and in all cases CBD stones were extracted. One patient underwent LC without ERCP, but no stones were seen at IOC. MRCP performed within 48 hours after admission disclosed a 33% incidence of CBD stones, decreasing to only 9% after one week. Sixty-five patients had no CBD stones on MRCP. In 2 of them, however, CBD stones were preoperatively demonstrated by endosonography or ERCP. In the remaining 63 patients IOC was performed in 54 and was negative in 52. Two patients had a small stone in the distal CBD. In 9 patients no IOC could be realized for various reasons but no patient has represented afterwards with choledocholithiasis. MRCP also provided information with respect to other bilio-pancreatic pathology, such as insertion anomalies of the cystic duct, cholecystitis, pancreatic pseudocysts, etc.
Conclusion: MRCP proved to be of great value for patients with BP by identifying hepatobiliary and pancreatic pathology and by reducing significantly the need for preoperative ERCP. Small stones, however, could be missed and the presence of CBD stones depended largely on the time lapse between the onset of pancreatitis and the MRCP examination.

**RT-PCR and immunohistochemical evaluation of sentinel lymph nodes after in vivo mapping with Patent Blue V in colon cancer patients**

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The value and technique of detecting sentinel lymph node (SLN) by subserosal blue dye injection is still a matter of debate. We aimed to study the feasibility of Patent Blue V dye of in vivo SLN detection in a multicenter setting and to evaluate nodal microstaging and ultrastaging using cytokeratin immunohistochemistry and RT-PCR methods on these nodes. In 30 consecutive patients operated for colon cancer subserosal injection with Patent Blue dye was used in the SLN detection in 4 different hospitals under supervision of a regional coordinator. In searching for occult micrometastases each SLN was examined at three levels. In tumor-negative SLN's at routine hematoxylin-eosin (H&E) examination (pN0) we performed CK8/CK18 immunohistochemistry (IHC) and RT-PCR for CEA.The procedure was successful in 29 patients (97%). The sentinel node was negative in 18 patients. In 16 patients the non-sentinel nodes were also negative. This leads to a negative predictive value of 89% and an accuracy of 93%. There was upstaging in 9 patients (31%); 7 by IHC and 2 by RT-PCR. Aberrant drainage was seen in 3 patients (10%).
Conclusions: The sentinel node concept in colon carcinoma using Patent Blue V is feasible and accurate. It leads to an upstaging of nodal status in 31% of patients when IHC and PCR techniques are combined. This may have diagnostic and therapeutic consequences in stage II colon cancer patients in the future.

**Rectal Cancer staging for distant metastases: Ultra Small Paramagnetic Iron Oxide (USPIO) enhanced Whole body MRI versus PET-CT**

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Introduction: Metastases is the major problem influencing long-term survival in patients with rectal cancer. In order to determine the best treatment strategy, accurate and detailed anatomical information on distant extent of the disease is as important as information on the loco regional status. The aim of the present study is to compare Whole Body PET-CT (PET-CT) and USPIO enhanced Whole Body MRI (USPIO MRI) for staging distant metastases in patients with rectal cancer.
Methods: A consecutive series of 25 patients with rectal cancer was studied prospectively. All underwent PET-CT and USPIO-MRI. All lesions suspicious for distant metastases were identified on PET-CT by a nuclear medicine specialist in consensus with an abdominal radiologist, on USPIO-MRI by an experienced abdominal MR radiologist, independent and blinded for each other's results. Each patient's staging results with USPIO-MRI and PET-CT were then presented with a 2 week interval to a specialist rectal/liver surgeon who was asked to determine the treatment strategy. The need for further characterisation of visible lesions and the number of alterations of treatment were assessed.
Results: There were 17 T3 and 7 T4 tumours as staged on USPIO-MRI. Suspicious liver lesions were found in 10 patients (40%), 6 (24%) detected by PET-CT and 9 (36%) by USPIO-MRI, lung lesions were found in 4 patients (16%), 3 (12 %) detected by PET- CT and 2 (8 %) by USPIO-MRI, lesions in other body parts were found in 6 patients (24%), 6 (24%) detected by PET-CT and 3 (12%) by USPIO-MRI. Need for further investigation to characterise lesions was 6x for PET-CT and 8x for USPIO-MRI. Standard treatment strategy was altered 4x after PET-CT (16%) and 5x after USPIO-MRI (20%). (All $p > 0.05$, chi-square). TME was postponed in 2 to conduct a livermets resection and a primary lung tumour resection. TME was abandoned in 2 as result of extensive metastases. All 4 patients were identified with both techniques. 1 TME was extended to pelvic exenteration for bladder cancer identified by WB-MRI.
Conclusion: In patients with rectal cancer USPIO enhanced Whole Body MRI and Whole Body PET-CT seem to be comparable in the detection of distant metastases and alter the treatment strategy in 20% of patients. As USPIO enhanced Whole Body MRI is also accurate for prediction of local tumor and nodal status, it therefore serves as a potential single modality staging tool for rectal cancer. Larger series are needed to draw definitive conclusions.

## Efficacy and cost-efficiency of follow-up after curative surgery for colorectal carcinoma

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Routine follow-up after curative surgery for colorectal carcinoma (CRC) is under debate because of the low yield of curable recurrences that are detected. Available data however are mainly derived from series that include calculations based on assumptions or models. This study evaluates the efficacy and cost-efficiency in the practice of a standardised follow-up program. Between 1993 and 2001, 753 patients were operated for cure. Of these, Dukes' stage and location of the primary tumour, (time to) recurrence, location of recurrence, type of treatment, clinical outcome, follow-up frequency and loss to follow-up were entered into a database. Survival rates were calculated using the Kaplan Meier Method. Overall 2- and 5-year survival was 80,8% and 61,3% respectively. A recurrence was found in 183 patients. Of these, 31 patients were treated with curative intent. Five year survival after discovery of the recurrence was 33%. Of the 610 patients that participated in our follow-up program, 341 patients followed the complete program and 269 only in part. More asymptomatic recurrences were found in patients that participated in the complete program (p=0,001) and patients with an asymptomatic recurrence lived significantly longer than patients with a symptomatic recurrence (p=0,0008). However, when comparing the results of complete and partial follow-up, curability nor survival advantage reached statistical significance (p=0,238 and p=0,3531 respectively). With 2189 chest X-rays (costs: € 46) curable lung metastases were discovered in 6 patients, of which 4 had symptoms. The costs to discover curable lung metastases in one patient are €16.782. With 2326 liver ultrasounds (costs: € 73) curable liver metastases were discovered in 12 patients, of which 11 had no symptoms. The costs to discover curable liver metastases in one patient are € 14.150. A colonoscopy (costs: € 394) was performed 645 times. A curable symptomatic local recurrence was found in 13 cases. A curable asymptomatic metachronous carcinoma was discovered 4 times. The costs to discover one curable local recurrence or curable metachronous carcinoma are € 14.987.
Conclusion: Although our follow-up does increase the number of asymptomatic recurrences discovered, it does not significantly influence the survival-rate. Lead-time advantage is clearest in liver metastases and metachronous tumour. Overall costs to detect one patient with curable disease are around € 15.000.

**The influence of delivery on Long-term pouch-function: an underestimated risk-factor**

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In the general population damage to the anal sphincter is reported in one third of females after the first two vaginal deliveries. Therefore anal sphincter damage after delivery can also be expected in female patients who underwent restorative proctocolectomy. Occult obstetric injury after delivery that occurred before or after restorative proctocolectomy might be associated with decreased long-term pouch function. Aim of this study was to evaluate if vaginal delivery before or after restorative proctocolectomy had influence on long-term pouch function in female patients. All female patients who underwent restorative proctocolectomy in 3 academic centres in the Netherlands between 1985 and 2004 were sent the COREFO questionnaire to assess functional outcome. From these questions a score Fwas calculated to determine incontinence. Higher scores correlated with worse continence. Patients were asked about their pregnancies and potential obstetric risk-factors. Responding patients with a minimal post-operative follow-up of 1 year and a functioning pouch were analysed. Multiple linear regression analysis was done to study the effect of pre-defined potential risk-factors on incontinence. The response-rate was 82.6%. Median follow-up of the 172 analysed patients was 7.2 years (range 1.0-19.7) of which 100 gave birth to a child; by vaginal delivery in 83 (78 before restorative proctocolectomy) and by Caesarean section in 17 (13 after restorative proctocolectomy). Fifty-two patients (63%) had a delivery with potential obstetric injury. Ageing and longer follow-up had no influence on pouch-function in patients never pregnant and in those with an uncomplicated delivery. In patients with potential obstetric injury however, ageing and longer follow-up were statistically significant risk-factors for incontinence.
Conclusions: This study shows that previous vaginal delivery not necessarily deteriorates future pouch function. However, more than 60% of the patients had a delivery with potential obstetric injury which resulted in more incontinence with ageing and longer follow-up. Vaginal delivery therefore is a significant risk-factor for long-term pouch function.

## Elastic band ligation of hemorrhoids: flexible endoscope versus rigid proctoscope

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Introduction: First choice in treatment of persisting internal hemorrhoids is elastic band ligation by means of a rigid proctoscope. Some studies evaluated the use of a flexible endoscope equipped with a ligation cap, normally used for ligation of esophageal varices, in treating hemorrhoids. A flexible endoscope has more maneuverability, a wider view and can provide photographic documentation. A prospective randomized trial was performed to compare both techniques.
Aim: To evaluate the efficacy of two different hemorrhoidal ligation devices.
Materials and Methods: Patients between 18 and 80 years, with chronic complaints (blood loss/ pain/ itching/ prolapse) of internal hemorrhoids grade I – III, were randomized to elastic band ligation by rigid proctoscope or flexible endoscope (preloaded with 7 bands). Patients were re-treated every 6 weeks till cessation of complaints.
Results: Forty-one patients were included (median age 52.1, range 27-79 years, 20 men). Nineteen patients were treated with a rigid proctoscope and twenty two with a flexible endoscope. Twenty-nine, 9, and 3 patients had Grade I, II and III hemorrhoids respectively. Patients treated with a rigid proctoscope needed a mean of 1.32 treatments (maximum: 3) and patients treated with a flexible endoscope needed a mean of 1.23 treatments (maximum: 3, $p = 0.613$). A mean of 4.2 bands were used in the rigid proctoscope group and a mean of 6.0 bands were used in the flexible endoscope group ($p < 0.05$). In the group treated with a rigid proctoscope, 5 patients needed 1 re-treatment and 1 patient needed 2 re-treatments. In the group treated with a flexible endocope, 2 patients needed 1 re-treatment and 1 patient needed 2 re-treatments. Two patients were excluded from follow up: 1 patient had a rupture of an abdominal aortic aneurysm and 1 patient developed an anal fissure. Pain after ligation tended to be more frequent in patients treated with the flexible endoscope (first treatment: 3 vs 10 patients, $p < 0.05$).
Conclusion: Both techniques are easy to perform, well tolerated and have a good and fast effect.It is easier to perform more ligations with the flexible endoscope. Additional advantages of the flexible scope are the maneuverability and photographic documentation. However, treatment with the flexible endoscope seems to be more painful, is more expensive and a rectal enema is sometimes needed.

**Temperature controlled radiofrequency energy (Secca®) to the anal canal for the treatment of fecal incontince: pilot seems promising**

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Fecal incontinence is a devastating complaint. Even after conservative measures like diet adaptation, fibers and physiotherapy a majority of patients still has complaints. Few patients have a sphincter defect suitable for repair. Other emerging therapies like dynamic gracilis plasty or neuromodulation carry side effects and are not generally available due to financial restrictions. Temperature controlled radio frequency energy (RF), (Secca®) (equivalent to Stretta® of the esophagus) has shown promising results in the USA. The mode of action is not totally clear, local fibrosis plays a role.We treated 5 females, mean age 62 years (53-73) with the Secca® procedure. Patients with diarrhea (defecation more than 3 x day), sphincter defects and relative anal stenosis were excluded. The procedure was performed under conscious sedation and local anesthesia. Antibiotics were given 3 x around the procedure. In 4 quadrants on 4-5 levels RF was delivered with multiple needle electrodes. Laxative was prescribed in case of hard stools first days after treatment. Anal endosonography and anorectal function tests were performed before and at 3 months. AE was also performed after the procedure and after 6 weeks. Patients were evaluated at 3, 6 and 12 weeks. At 6 weeks, 3 of the 5 patients had a good improvement, one slight and one none. At 12 weeks, the good improvement persisted in the 3 patients, the patient who had improved slightly become incontinent and the patient without effect was improved. Side effects were local hematoma (2), bleeding 3 days (1), pain persisting 2-3 weeks (4) and diarrhea persisting 1-3 weeks (4). The Vaizey score improved from 19,5 to 12. There was a tendency of improved rectal in 3 of the 4 improved patients (few data for statistical analysis). Anal endosonography did not show any changes.
Conclusion: The Secca® procedure seems promising for patients with fecal incontinence. Mild side effects are dominating the first 3 weeks, effect should be judged after 6-12 weeks. Further studies and long term follow up are needed and financial reimbursing needs to be solved.

**Enhanced recovery after surgery ("fast track") programs in colonic surgery: a systematic review**

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A major development in colonic surgery are fast track (FT) programs optimizing perioperative care resulting in an enhanced recovery and shorter hospital stay. Optimization includes preoperative counseling of the patient, no bowel preparation, no premedication, no preoperative fasting but carbohydrate loaded liquids, thoracic epidural anaesthesia and short acting anaesthetics, fluid restriction, minimal or transverse incisions, non-opioid pain management, no routine use of drains and nasogastric tubes, early removal of bladder catheter, standard laxatives and early postoperative feeding and mobilization. FT reduces the stress response that leads to profound changes in endocrine, metabolic, neural and organ function seen after surgery. Aim of this systematic review is to assess the current evidence on FT rehabilitation after segmental colonic resections compared to traditional care.

Medline, EMBASE and the Cochrane Library were searched for randomized and clinical controlled trials. Search terms were colectomy, colorectal, colon, sigmoid, fast track, enhanced, recovery, convalescence, accelerated, early ambulation, perioperative care, clinical protocols, recovery of function, multimodal, care pathway and rehabilitation. The literature search, data extraction and quality assessment were performed by three independent observers.

Between 1998-2005, 39 manuscripts were retrieved, of which 32 were excluded due to duplicate publications, insufficient data description or the used protocol was not a FT program. Seven studies were included for analysis (2 RCT's and 5 CCT's with 984 patients). The nature of perioperative care varied widely both within the traditional care and the FT approach. The FT care programs contained an average of 7.6 (4-11) of 13 pre-determined FT modalities. The primary and overall hospital stay (mean) ranged from 2-5.2 and 3.4-6 days with FT compared with 5.8-12 and 7.1-11 days after traditional care. Readmissions were reported in up to 22% of cases in the FT group. There was no increase in morbidity and mortality. Post operative ileus, in terms of duration of nasogastric decompression, first defecation or tolerance of solid food intake was reduced.

Multimodal FT rehabilitation programs in colonic surgery are safe and shorten primary and overall hospital discharge. However, only 7 comparative studies were identified. Therefore a multi-center prospective randomized trial is advisable to confirm its broad applicability and favourable results.

**Polymorphisms in the T-cell regulatory gene CTLA-4 are associated with susceptibility for acute rejection after liver transplantation**

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The CTLA-4 gene encodes for a membrane-bound (mCTLA-4) and a soluble (sCTLA-4) isoform, which are both involved in regulation of T-cell function. mCTLA-4 is expressed on activated effector T-cells and CD4+CD25+ regulatory T-cells, and plays a critical role in suppression of effector T-cell functions. sCTLA-4 is secreted by resting T-cells and inhibits co-stimulation of T-cells via CD28. The CTLA-4 +49A/G single nucleotide polymorphism (SNP) influences the glycosylation and expression of mCTLA-4; the +6230A/G SNP affects the production of sCTLA-4. The aim of this study was to examine whether these functional SNP influence the risk of acute rejection after liver transplantation. Liver graft recipients (n=483) were genotyped for both SNP by allele-specific PCR and haplotypes were reconstructed. Association with rejection was tested by the log-rank test using the Kaplan-Meier method with the time to the first acute rejection episode as outcome. Adjustment of the association between SNP and rejection for patient demographic factors was performed by Cox regression analysis. Three haplotypes were observed in the cohort: +49A/+6230A, +49A/+6230G and +49G/+6230G. The +49A/+6230G haplotype was significantly and dose-dependently associated with acute rejection (p=0.01). Of the demographic factors tested, only the underlying liver disease was significantly associated with rejection. Adjusted for underlying liver disease, each additional +49A/+6230G haplotype allele resulted in a significantly higher risk of acute rejection (Risk ratio = 1.35; 95% CI: 1.05-1.74; p=0.02). Patients who lacked this haplotype had the lowest, carriers an intermediate, and homozygotes the highest risk of acute rejection.
Conclusion: The CTLA-4 +49A/+6230G haplotype of the recipient is a co-dominant risk allele for acute rejection after liver transplantation. This implies that CTLA-4 is critically involved in regulation of the human immune response to allogeneic liver grafts.

**Asymmetric Dimethyl-L-Arginine (ADMA): a possible cause of endothelial dysfunction in the bile-duct excised cirrhotic rat**

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Reduced production of nitric oxide in the cirrhotic liver results from a defect in hepatic endothelial nitric oxide synthase (eNOS) and contributes to the high intrahepatic resistance and portal hypertension (PHT) typical of cirrhosis. We aimed to study whether asymmetric dimethylarginine (ADMA), a putative endogenous NOS inhibitor which is metabolized intrahepatically, might be involved. Two cirrhotic rat models of PHT [$CCl_4$ and bile-duct excised (BDE) (n=10 each)] were used with sham-operated rats (n=10) as controls. We assessed hepatic NOS activity, plasma ADMA levels and endothelial dysfunction. The latter was evaluated by performing concentration-effect curves to acetylcholine in in-situ perfused normal and BDE-cirrhotic livers (n=10), preincubated either with vehicle or ADMA, and with additional measurement of ADMA levels in perfusate and bile. To further characterize potential NOS inhibiting features of ADMA, a comparison was made in healthy perfused rat liver with L-NAME, a well known NOS inhibitor, and with symmetric dimethylarginine (SDMA), the presumed vasoinactive stereoisomer of ADMA. Additionally, nitrate/nitrite (NOx) were measured in these perfusates. Both cirrhotic models showed decreased hepatic NOS activity (2.8±0.3 for CCl4 rats and 1.9±0.1 for BDE rats vs 4.1±0.3 pmol/min/mg protein for sham-controls, *P* <.05). The plasma concentration of ADMA was significantly increased in BDE cirrhotic rats (2.92±0.59µM) but not in sham (0.69±0.37µM) or $CCl_4$ cirrhotic rats (0.82±0.29µM) (*P* <.001). In normal perfused liver, ADMA caused impaired vasorelaxation at each dose of acetylcholine used ($10^{-7}$M: -0.6±1.6% vs -7.0±0.7% [ADMA vs vehicle], *P* =.006; $10^{-6}$M: -2.9±1.8% vs -11.7±1.3%, *P* =.016; $10^{-5}$ M: -2.5±2.4% vs -16.9±2.3%, *P* =.002), which was associated with decreased NOx production in perfusate (-29.6±3.1nM vs -0.4± 5nM [ADMA vs vehicle], P <.05). These effects, both perfusion and NOx levels, mimicked these of L-NAME. In BDE perfused rat liver, impaired endothelial relaxation was aggravated when ADMA was added. This was associated with a decreased removal rate of ADMA as compared to healthy livers (73.6±5.4% vs 34.3±6.0%, P <.001). No ADMA could be retrieved upon analysis of bile of BDE rats.
Conclusion: In biliary cirrhotic rats, ADMA, an endogenous NOS inhibitor, might cause endothelial dysfunction through intrahepatic accumulation and might therefore also be responsible for the increased intrahepatic resistance seen in bilary cirrhosis.

## Role of the nuclear orphan receptor Rev-erbα; in the regulation of bile acid synthesis

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Conversion into bile acids represents an important route to remove excess cholesterol from the body. Cholesterol 7$\alpha$-hydroxylase (CYP7A1) is the rate-controlling enzyme in the classical pathway of bile acid synthesis. Hepatic CYP7A1 expression is tightly controlled at the transcriptional level and displays circadian rhythmicity. Rev-erbα is a nuclear receptor that participates, as one of the clock genes, in the control of circadian rhythmicity. Here, we identify a role for Rev-erbα in the regulation of CYP7A1 expression and bile acid metabolism. CYP7A1 expression is decreased in livers of Rev-erb$\alpha$-deficient mice, whereas adenovirus-mediated Rev-erb$\alpha$ over-expression induces CYP7A1 expression in human hepatocytes. The induction of CYP7A1 by Rev-erbα is associated with repression of the orphan nuclear receptor SHP, a potent CYP7A1 repressor. Gel shift and transfection assays identified a response element in the murine and human SHP promoter *via* which Rev-erb$\alpha$ represses its transciption. Moreover, Rev-erb$\alpha$ modulates bile acid metabolism *in vivo* in mice, since Rev-erb$\alpha$-deficient mice display a lower synthesis rate and an impaired excretion of bile acids into the bile. Bile acids induce the expression of Rev-erb$\alpha$ *via* activation of the nuclear receptor FXR. Altogether these data identify Rev-erb$\alpha$ as a regulator of bile acid metabolism and suggest that Rev-erb participates in the circadian regulation of hepatic CYP7A1 expression and as such may coordinate diurnal bile acid homeostasis.

**Lipopolysaccharide detoxification and clearance by enterocytes.**
**(Final report Maag Lever Darm Stichting project no. WS 00-73)**

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Enteric bacteria and their toxins are involved in the pathogenesis of systemic and local intestinal inflammation. The aim of this study was to further explore the natural intestinal defense against bacterial toxins. In a murine endotoxemia model, chylomicrons have been shown to prevent endotoxin induced inflammation and to improve survival. Detoxification of LPS by other lipoproteins, i.e. VLDL, LDL and HDL, is catalyzed by LPS Binding Protein (LBP). We recently reported evidence for the synthesis of this acute phase protein by enterocytes. This triggered us to investigate whether LBP participates in the interaction of LPS with chylomicrons.

The binding of LBP and LPS to chylomicrons was studied in a solid phase ligand-binding assay. LBP was demonstrated to associate with chylomicrons and enhance LPS binding to chylomicrons in a dose dependent fashion. Subsequent, the effect of LBP and chylomicrons on the ability of LPS to induce cytokine release by peripheral blood mononuclear cells was studied. LBP induced binding of LPS to chylomicrons prevented endotoxin toxicity within 30 minutes. This function is not limited to gram-negative bacterial toxins since also fragments of gram-positive bacteria are neutralized by chylomicrons in the presence of LBP.

Others demonstrated that the intestinal epithelium takes up circulating endotoxin followed by secretion of endotoxin into the intestinal lumen. Next, we asked whether the binding of LPS to LBP-chylomicron complexes at the basolateral site of the intestinal epithelium influences the clearance of LPS by enterocytes. The transport of LPS from the basolateral towards the apical surface of Caco-2 cells was found to be markedly enhanced by basolateral presence of chylomicrons and further augmented by the presence of both chylomicrons and LBP.

The data of this study imply that the intestine contributes to detoxification and clearance of LPS by secretion of LBP and chylomicrons. We consider systemic clearance of bacterial toxins and inactivation of bacterial toxins by LBP-chylomicron complexes in the circulation of importance. In addition, we hypothesize that this is also part of a local defense mechanism of the intestine against bacterial toxins translocated across the mucosa of a compromised gut.

## No association between referral indications for open access upper GI endoscopy and endoscopic findings

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In accordance with current guidelines, general practitioners refer patients with gastro-intestinal symptoms for open access upper gastrointestinal endoscopy when empirical treatment fails to relief symptoms, when alarm symptoms are present or for reassurance that symptoms are not due to serious pathology. To our knowledge it was not thoroughly investigated whether these indications are associated with relevant organic findings at endoscopy. Therefore, the aim of this study was to assess the association between referral indications given by general practitioners and endoscopic outcomes.
Consecutive patients referred to a primary care hospital for open access endoscopy between January 2002 and December 2004 were included. Their general practitioners were asked to specify the reason for referral on a specially designed form. Information about endoscopic findings were obtained from medical files. Relevant organic disorders were defined as findings at endoscopy that might explain symptoms or influence treatment strategy, i.e. gastroesophageal carcinoma, peptic and duodenal ulcer, duodenitis, Barrett's oesophagus or reflux-oesophagitis.
A total of 1298 subjects was included: 49% was male and mean age was 53.7 (SD=15.0). Overall, a relevant organic abnormality was found in 35% of patients. Alarm symptoms had a positive predictive value (PPV) of 4% for carcinoma and 5% for Barrett's oesophagus, while prevalences were 2% and 5%, respectively. The PPVs of treatment failure for carcinoma and Barrett's oesophagus are 1% and 5%, respectively. The only indication with a slightly increased PPV was reflux-like symptoms; PPV was 33% for reflux-oesophagitis, while prevalence was 22%.
None of the positive predictive values of different referral indications varies substantially from prevalences of organic endoscopic findings. Only reflux-like symptoms have a slightly increased PPV for reflux-oesophagitis, which is, however, of little clinical relevance since this rarely alters therapeutic strategy. In particular treatment failure and the presence of alarm symptoms, which are referral indications for open access endoscopy as described in the Dutch guideline for general practitioners, are not associated with any relevant organic finding. In conclusion, referral indications do not distinguish organic from functional diseases.

## Nurse-administered Propofol Sedation for ERCP

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Conscious sedation for ERCP is generally obtained with a combination of midazolam and a morfin agonist. Propofol is a short acting hypnotic agent with a rapid onset of action and rapid recovery. It's major adverse effect is severe respiratory depression with apnea. According to CBO guidelines propofol can only be administered by anesthesiologists. However, according to the literature propofol can safely be applied by non-physicians under particularly careful monitoring. The aim of our study was to prospectively evaluate the efficacy and safety of propofol sedation in therapeutic ERCP.
In this prospective open study sedations were carried out by a trained nurse in the presence of an anesthesiologist, using a continuous intravenous infusion technique with a target controlled infusion (TCI) system (Base Primae, Fresenius/KB medical). All patients received intranasal oxygen. Heart rate, blood pressure and oxygen saturation were continuously monitored during the administration of propofol and for at least 30 minutes after regaining full consciousness.
Sixteen patients ASA class I and II, 31% female, mean age 63 years (36-88), undergoing therapeutic ERCP were included. Average duration of the procedure was 44 min (14-135). Adequate sedation was obtained in all patients, on average in 2.25 min. (0.5-5). Full recovery of consciousness occurred in 8 min. (3-20). Propofol was administered at a mean dosage of 450 mg (178-1200). No episodes of apnea occurred. The oxygen saturation fell below 90% in 1 patient (6%), necessitating brief mask ventilation.
Conclusions: The administration of propofol for sedation by nurses, trained in assisted ventilation and the pharmacological properties of this drug, is feasible. Propofol sedation for ERCP is safe, comfortable and effective. Careful selection of patients and adequate monitoring is mandatory.

**Insulin-like growth factor I (IGF-I) replacement therapy increases albumin concentration in liver cirrhosis: Results of a pilot randomized controlled clinical trial. (Final report Maag Lever Darm Stichting projectno. WS 00-78 (WS 97/87))**

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Background: Insulin-like growth factor I (IGF-I) is an anabolic hormone synthesized in the liver whose levels decrease sharply in liver cirrhosis.
Methods: We conducted a randomized double-blind placebo-controlled clinical trial to evaluate the effect of subcutaneous administration of IGF-I (20 mg/kg/day with dose escalation to 50–100 mg/kg/day) for 4 months in patients with alcoholic or primary biliary cirrhosis (PBC) and subnormal IGF-I levels. Eight alcoholics and one PBC entered the placebo group and seven alcoholics and two PBC the treatment group. Biochemistry, body composition, muscle mass and strength, and resting energy expenditure (REE) were evaluated.
Results: Total serum IGF-I and IGF-I/IGFBP-3 ratio (a surrogate marker of IGF-I biovailability) increased in the treatment group but IGF-I values still remained below normal limits in the treated patients. No differences were observed in body composition, muscle strength or muscle mass between groups. However, IGF-I therapy increased significantly serum albumin (P=0.038) and this improvement correlated positively with variation of IGF-I/IGFBP-3 ratio. IGF-I treatment also tended to increase REE (P=0.085); this difference was significant (P=0.049) in thesubgroup of alcoholic patients.
Conclusions: A short course of IGF-I increased albumin levels and tended to improve energy metabolism in liver cirrhosis. These findings warrant larger clinical trials to assess the clinical benefit of IGF-I in cirrhotic patients.

**The quality of endoscopic ultrasonographic staging of esophageal cancer during the learning period using electronic consultation**

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Endoscopic ultrasonography (EUS) is superior for preoperative T-staging of esophageal cancer. Training guidelines recommend a minimum of 75 examinations to achieve adequate accuracy. The aim of this study was to assess the accuracy of esophageal cancer staging by EUS using two endoscopists with on-demand electronic supervision.
Since July 2001 all our EUS examinations were performed by two experienced gastroenterologists (MPS and BO). They were both self-taught in EUS by attending multiple EUS-courses and observing about 50 EUS cases each in an expert center. During their learning period they interpreted EUS cases together using video recordings to reach consensus. In case of discordance, ultrasound images were electronically sent to an experienced investigator (PF) in another hospital. The EUS database containing the first 75 examinations of primary staging of esophageal cancer was reviewed. The EUS T-stage was compared to the pathologic T-stage, when available.
From July 2001 to December 2004, 75 patients with esophageal cancer underwent preoperative EUS. In all cases it was possible to perform adequate T-staging. Forty-one patients were referred for primary endoscopic or surgical treatment. Thirty-six tumors were resected. In 2 cases resection specimens were unsuitable for pathologic T-staging. The overall accuracy of endosonographic T-staging was 88% (n=34). Sensitivity was 67% for staging T1 (n=6), 93% for staging T3 (n=27), and 100% for staging T4 tumors (n=1). There were no T2 tumors in this group. Conclusion: This study shows that EUS staging of esophageal cancer was reliable even during the learning period when using a consensus model and electronic consultation. This method might be adopted more often as the need for training new endosonographers increases.

**Prevalence of Barrett's esophagus in a population not primarily referred for gastro-esophageal reflux disease**

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Background: Cancer of the esophagus, particularly esophageal adenocarcinoma (EAC), is the fastest growing malignancy worldwide. Barrett's esophagus (BE) is an important risk factor for EAC. As it is largely unknown what the prevalence of BE is in asymptomatic individuals, we aimed to study the prevalence of BE in a population which was not primarily referred for upper gastrointestinal (GI) complaints.
Methods: A total of 150 patients undergoing routine, non urgent colonoscopy were included in this study. All patients underwent an upper GI endoscopy prior to colonoscopy. All patients filled out the Gastrointestinal Symptom Rating Scale (GSRS). Biopsies were taken from the Z-line (n=4), and, if BE was suspected, additional 4-quadrant biopsies were taken. All biopsies were reviewed by an experienced GI pathologist.
Results: In 17 of 150 (11%) patients, endoscopical 15 SSBE (≤2 cm) and 2 LSBE (>2 cm) and histologically confirmed (intestinal metaplasia) BE was found. In these 17 patients, 12 had no dysplasia and 5 low-grade dysplasia. In 9 (6%) patients, an endoscopic diagnosis of BE (9 SSBE, 0 LSBE) was histologically not confirmed. In 6 (4%) patients, intestinal metaplasia of the cardia was found without endoscopical evidence of columnar epithelium in the distal esophagus. Two patients were diagnosed with early stage EAC and underwent a resection. BE patients (mean ± SD: 60.2 ± 10.6 yrs) were older than patients (51.6 ± 15.1 yrs) without BE (p=0.023), but gender was not different (M/F ratio: 1 vs. 1.1 resp.). In addition, no significant differences were found between smoking (37.5 (%) vs. 22.5 (%), resp.), alcohol intake (68.8 (%) vs. 60.0 (%), resp.), use of proton pomp inhibitors (17.6 (%) vs. 20.9 (%), resp.), use of NSAID/aspirine (31.3 (%) vs. 20.8 (%), resp.) and BMI>25 (62.5 (%) vs. 46.5 (%), resp.). According to the GSRS questionnaire, patients with BE had not more commonly reflux symptoms compared to those without BE (37.5% vs. 32.6%).
Conclusions: BE was detected in 11% and EAC in 1.3% of patients not primarily referred for evaluation of GERD symptoms. A major question that arises from this prospective study is whether endoscopic screening for BE should be applied to the general population in an effort to early identify BE patients, who are at an increased risk for the development of cancer.

## Acid reflux in patients with achalasia before and after pneumatic dilation

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Introduction: Achalasia is an esophageal motor disorder in which aperistalsis of the distal part of the esophagus and failed relaxation of the lower esophageal sphincter (LES) leads to a functional esophageal obstruction. Goal of treatment is lowering of LES pressure and improving passage of food. The two most applied treatment modalities are pneumatic dilation and laparoscopic myotomy. This therapy may enhance the risk of gastro-esophageal reflux disease (GERD), reported in 5-30% of the patients after myotomy and 0-20% after pneumatic dilation. We therefore treat all our patients with a proton-pump inhibitor for two weeks after pneumatic dilation. We studied the prevalence of acid reflux prior to, during and shortly after pneumatic dilation.
Methods: All patients newly diagnosed with achalasia between 15 November 2004 and May 2005 were treated with pneumatic dilation. A dilation treatment consisted of 3 dilations on three consecutive days with increasing diameter (30-35-40 mm) Rigiflex balloon dilator (Boston Microvasive) on an outpatient basis. Twenty-four hours before first treatment, a wireless pH-capsule (BRAVO, Medtronic) was inserted 5 cm above the LES. If the capsule was still in place after the first pneumatic dilation, the pH recording was continued for 72 hours. No acid lowering therapies were used during the pH recording.
Results: Seven patients with newly diagnosed achalasia were treated with pneumatic dilation according the protocol. In 1 patient the capsule migrated before the first dilation. One measurement failed due to technical failure. In 5 patients a pH-recording could be evaluated. The mean recording time was 60 hours (30 – 96). The mean number of reflux episodes before dilatation was 3.7 (0-8) and 1.8 (0-5) after treatment. The mean total reflux time before dilatation is 0.5% (0 – 1.7) and 0.1% (0 – 0.3) after treatment. There was no significant difference between these values on the first, second and third day after pneumatic dilation.
Conclusions: Newly diagnosed patients with achalasia have virtually no acid reflux episodes. Repeated pneumatic dilation does not induce pathological gastro-esophageal reflux. Therefore there is no need for standard acid lowering therapy after pneumatic dilatation.

## Colonoscopy Using High Resolution Endoscopy, Video Autofluorescence Imaging and Narrow Band Imaging: A Feasibility Study

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Background and Aim: Video autofluorescence imaging (AFI) and narrow band imaging (NBI) are novel techniques that may improve the detection rate and the accuracy of classifying colonic polyps. In a recent prototype, high resolution endoscopy (HRE) has been combined with AFI and NBI (AFI-NBI, Olympus Corp. Tokyo, Japan). This study aimed to evaluate the feasibility of using this device for the detection and classification of adenomas to improve the diagnostic accuracy of colonoscopy.
Methods: The prototype used in this study has a RGB sequential illumination light source and the system enables switching between HRE, AFI and NBI by using two knobs on the handle of the endoscope. During AFI, blue light is used for autofluorescence excitation and a narrow band of green light was used for reflectance. During NBI, narrowed non-overlapping red, green and blue light bands, and a higher relative intensity of blue light are used for excitation. This enables optimal visualization of the mucosal patterns since blue light does not penetrate deep in the tissue owing to its short wavelength. The colonoscope has two separate CCD's, one for HRE and NBI and one for AFI and it has no magnifying capabilities. We enrolled patients attending for surveillance colonoscopy. Segmental examination of the colon during withdrawal was performed using HRE and AFI in a randomized sequence. Narrow band imaging was used to classify the pit-patterns of polyps according to the Kudo classification. Lesions were photographed using all three modalities prior to biopsy or polypectomy.
Results: 31 patients were enrolled; indication for colonoscopy was ulcerative colitis (n=9) and prior adenoma, family history or genetic mutation (n=22). Adenomas were detected in 8 patients; 2 patients were first examined with AFI with which 4 adenomas were detected; subsequent examination with HRE did not lead to the detection of additional adenomas. Six patients were first examined with HRE with which 14 adenomas were detected which were also visible with AFI; 5 adenomas missed with HRE were detected with AFI in 3 patients. With NBI, adenomas were diagnosed with 85% sensitivity, 90% specificity, 74% positive predictive value and 81% negative predictive value.
Conclusions: The consecutive use of HRE, AFI and NBI incorporated in one system is feasible and may improve the diagnostic capability of colonoscopy by increasing the detection rate of adenomas (AFI) and improving the accuracy of polyp classification (NBI). The addition of a magnifying technique to this device may improve the accuracy of polyp classification with NBI.

**Intestinal marker expression in columnar epithelium in the remnant esophagus of patients who have undergone esophagectomy**

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Patients who have undergone esophagectomy with gastric pull-up reconstruction often have complaints of gastroesophageal reflux. A subset of these patients develop columnar epithelium in the remnant esophagus. This metaplastic epithelium can be of the gastric or intestinal type (Barrett's esophagus). The aim of this study was to determine whether gastric epithelium and intestinal metaplasia in the esophagus are different entities or consecutive stages of metaplastic progression. The medical records of 613 patients that had undergone esophagectomy with gastric pull-up reconstruction between 1994 and 2004 in the Erasmus MC were reviewed for the presence of columnar metaplasia in the remnant esophagus. The presence of intestinal metaplasia was determined histologically by haematoxylin and eosin, alcian blue, and periodic acid-Schiff staining in archival biopsy samples. Intestinal characteristics were identified by immunohistochemical staining for CDX2, which is a transcription factor for many intestinal proteins, MUC2 and cytokeratins 7 and 20 (CK7/20). Fifteen (2.5%) patients with histologically proven columnar epithelium in the remnant esophagus were included in this study. The mean interval between resection and detection of columnar epithelium was 65 (3-118) months. Histological analysis revealed gastric epithelium in all patients. Four patients with gastric epithelium also had foci of intestinal metaplasia. CDX2 and MUC2 expression was observed in the regions with intestinal metaplasia, and in one patient, CDX2 expression was observed in gastric epithelium adjacent to intestinal glands. Diffuse CK7 and superficial CK20 expression (Barrett-like pattern), was identified in columnar epithelium of patients with and without intestinal metaplasia. In conclusion, in the majority of patients, gastric epithelium did not express CDX2 and MUC2, but CDX2 has been observed in gastric epithelium adjacent to intestinal metaplasia. This indicates that a part of the gastric epithelium and the intestinal metaplasia may share a common pathway, eventually leading to the development of specialised intestinal epithelium. Furthermore, in this study CK7/20 staining did not discriminate between gastric epithelium and intestinal metaplasia.

## CDX2 is an early marker of epithelial intestinal differentation in columnar epithelium of the esophagus

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Barrett's esophagus (BE) is a premalignant condition defined by the presence of columnar epithelium of the esophagus with intestinal metaplasia (IM) of the esophagus. In addition to IM, gastric-type mucosa (GM) can be found in the esophagus, but this type is not regarded as premalignant. Recently, it has been suggested that replacement of normal squamous epithelium by IM could occur with GM as intermediate stage. As CDX2 is an early marker for intestinal differentiation, we investigated whether this marker was present in GM of the esophagus in patients with and without BE. Biopsies from the columnar epithelium of the esophagus from 61 patients were collected at two different gastroscopies. These patients were divided in three groups: 20 patients with in one set of biopsies only GM and in the other set IM (group 1), 23 patients with IM in both biopsy samples (group 2), and 18 patients with GM in both biopsy samples (group 3). Haematoxylin and eosin, alcian blue, and periodic acid-Schiff staining was performed to detect goblet cells as marker for IM. CDX2 was determined by immunohistochemistry. IM was present in 66/122 (54%) samples, whereas in 52/66 (79%) IM-positive samples also GM was observed. As expected, all 66 IM-positive samples were positive for CDX2. However, CDX2 expression was also observed in GM-glands adjacent to IM in 9/46 (20%) of samples of group 2 (IM in both biopsies), and in 10/23 (43%) of IM-positive samples of group 1 (the other biopsy negative for IM). The increased percentage of CDX2 expression in GM in group 1 suggests that these GM-glands are still differentiating into IM, while in group 2 this differentiation is more advanced.Strikingly, CDX2 was detected in 7/56 (13%) samples with only GM. In 6/7 (86%) CDX2-positive GM-samples IM was found in the other sample, which suggests that these GM-samples are differentiating into IM.
Conclusion: Our results suggest that GM could be an intermediate stage in formation of IM in columnar epithelium of the esophagus, and that CDX2 could be a predictive marker for the transition to IM. If true, this implicates that patients with only GM in columnar epithetilium of the esophagus and with CDX2 expression in these biopsies, need endoscopic surveillance to detect the transition to IM at an early stage.

**Postoperative chemoradiotherapy in gastric cancer - results of two parallel phase I-II studies of a fixed radiotherapy regimen with escalating doses of cisplatin and capecitabine**

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Background: The prospectively randomized Intergroup Study INT-0116 has demonstrated that postoperative 5FU-based chemoradiotherapy improves survival and locoregional control in gastric cancer. These results stimulated us to evaluate whether treatment outcome could be further improved using increasing doses of the radiosensitizing drugs cisplatin and capecitabine during radiotherapy.
Methods: Between December 2002 and May 2005, 70 patients with $T_{2-4}N_{0-3}M_0$ adenocarcinoma of the stomach or distal esophagus were enrolled in two parallel running phase I-II studies. Treatment started in both studies within 60 days after surgery with capecitabine 1000 mg/m$^2$ bid on days 1-14. Thereafter radiation started to a total dose of 45 Gy in 25 fractions of 1.8 Gy to the original tumor site, anastomoses and adjacent lymph nodes on weekdays during weeks 4 through 8. In the first study capecitabine given concurrently with radiation was escalated in groups of 20 patients per dose level from 600 to 900 mg/m$^2$ bid (planned maximum 1000 mg/m$^2$ bid). In the second study, both cisplatin and capecitabine were given concurrently with radiation. Cisplatin was administered in an escalating daily dose of 3 to 6 mg/m$^2$ (iv) and capecitabine was escalated from 250 to 650 mg/m$^2$ bid in groups of at least 3 patients per dose level.
Results: Up to May 2005, 47 patients have completed treatment with capecitabine only; one withdrew early due to anxiety. The full radiation and capecitabine dose were delivered to all patients. No grade III/IV toxicity was observed. In the cisplatin-capecitabine study 21 patients have completed treatment; one had to stop due to cisplatin allergy. Grade III toxicity consisted of neutropenia (n=1); dysphagia (n=1) and hand-foot syndrome (n=1). One patient developed grade IV leucopenia (cisplatin 5 mg/m$^2$; capecitabine 575 mg/m$^2$ bid). In 3 additional patients in this dose level, no other DLT's have occurred. In the next dose level (cisplatin 6 mg/m$^2$; capecitabine 650 mg/m$^2$ bid) one patient developed grade IV trombopenia, so 3 extra patients will be accrued. There were no toxicity-related deaths.
Conclusions: In these two ongoing dose escalating studies with capecitabine and cisplatin given concurrently with radiation in postoperative chemoradiotherapy in gastric cancer, no non-manageable acute toxicity was observed until so far. Final results of acute toxicity are anticipated in the near future.

**Palliation of patients with malignant gastric outlet obstruction with the WallFlex enteral stent: a retrospective multicentre study**

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Gastric outlet obstruction can be a late complication of advanced gastric, periampullary and duodenal carcinoma. Palliation of symptoms of obstruction is the primary aim of treatment in these patients. Self-expandable metal stents (SEMS) have emerged as a promising treatment option. The purpose of this study was to investigate the technical and short-term clinical success (30 days) of a new enteral stent made of nitinol (WallFlex, Boston Scientific) in a multicentre patient cohort.
The first 18 European centres having this stent were approached to collect data of every patient treated. Of these centres 15 returned their data. The collected data were: general patients' data, type of tumour, complaints, symptom-score (Gastric-Outlet-Obstruction-Scoring-System: a 4-point scoring system from 0 to 3) before and one week after stent placement, procedure related technical data and complications within 30 days. Descriptive statistics were used and the patients' improvement of GOOSS-score by two points was calculated using the Chi-square test. A GOOSS-score of 2 and 3 was considered a satisfactory quality of diet.
Case record forms of 62 patients (35 men, mean age 70 years) were completed. Obstruction was caused by cancer of the stomach in 9, periampullary in 47, duodenal in 1 and metastases in 5. All patients suffered from nausea, vomiting or inability to eat. The median length of the stenosis was 4 cm (1-15 cm). Sixty-six enteral stents (diameter 27/22 mm) were placed: 58 patients required one stent, 4 patients required two stents either because of the position (2) or the length of the stenosis (1) or due to migration (1). Before stent placement 49 patients had no oral intake or were only tolerating liquids (GOOSS-score 0 or 1). The GOOSS-score after one week was obtained from 57 patients (5 patients died): of these 8 were able to tolerate liquids only and 49 patients tolerated soft solids or a low-residue diet (GOOSS-score 2 or 3). A GOOSS-score improved by two points was significant (p=0.007). Oral intake was possible on average 1 day (0-8 days) after stent placement. Of the 62 patients enrolled the 30 days follow-up has so far been collected in 47. Complications within 30 days occurred in 10 patients: perforation (2), stent migration (2), tumour ingrowth (1), bleeding (1), aspiration pneumonia (2), cholangitis (1), sepsis of unknown origin (1). In total 10 patients (21%) died within 30 days.
WallFlex enteral stent placement for the palliative treatment of patients with malignant gastric outlet obstruction was technical successful in 58 out of 62 (94%) and allowed 49 out of 62 (79%) patients to resume a satisfactory quality of diet.

**Colonic function in slow transit constipation: motor and sensory disorder? (Final report Maag Lever Darm Stichting projectno. WS 01-40)**

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Chronic constipation is an invalidating disorder with a high prevalence among the population. It can be divided into three major groups on the basis of colonic transit studies and anorectal manometry. Slow Transit Constipation (STC) is characterized by delayed transit over all colonic segments in the absence of outlet obstruction. The pathophysiology of STC is poorly understood. Recent studies have pointed to the role of High-Amplitude Propagated Contractions (HAPC's) in colonic transport. However, little is known on HAPC's in STC. Apart from motor dysfunction of the colon, disturbed visceroperception may also contribute to the development of constipation. Aim of our project was to investigate colonic motor and sensory function and colorectal reflexes in STC-patients, patients with other causes of constipation and healthy volunteers.
Colonic motility was measured by 24-hr ambulatory colonic manometry. First we investigated the repeatability and validity of this method. Colonic pressure activity exhibits a diurnal pattern with a decrease in activity during the night. Colonic pressure activity increases upon meal ingestion. Compared to controls, patients with STC exhibit less overall colonic pressure activity during daytime, but not during the night. The number of HAPC's in STC-patients is diminished and HAPC's start more distally in the colon and propagate over a shorter distance in STC as compared to controls.
Concerning visceroperception, the perception for urge in STC-patients is diminished as compared with controls and patients with Normal Transit Constipation. However, pain perception in STC-patients is not diminished. Constipation frequently occurs after hysterectomy. The mechanism by which constipation in this group develops is not well understood. Therefore we investigated rectocolonic motor and sensory function in two groups of posthysterectomy patients (with and without constipation) and a group of healthy volunteers. In all posthysterectomy patients the perception for urge during rectal distensions was diminished. Reflexes between colon and rectum (intestinal cross-talk) were also altered in the posthysterectomy group. However, no differences were found between the posthysterectomy patients with and without constipation.
We conclude that different groups of constipation can be characterized with alterations not only in rectocolonic motility, but also in rectocolonic sensitivity.

## Conditional inactivation of the mouse glutamine synthetase gene in the liver

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Glutamine synthetase (GS), the enzyme that catalyzes the ATP-dependent conversion of ammonia and glutamate into glutamine, is expressed in a wide range of tissue and organs and in a tissue-specific and developmentally controlled manner. Cellular concentrations of GS differ >1,000-fold between GS-positive cells in different organs, with highest levels in the liver, where the enzyme is strictly confined to 2-3 hepatocytes surrounding the efferent central veins. With its high affinity for ammonia, GS is thought to play a key role in the detoxification of intestinal ammonia in the liver. The complete absence of the mouse GS gene ($GS^{LacZ/LacZ}$, conventional knockout of GS gene by in-frame replacement with the β-galactosidase reporter gene) results in early embryonic lethality. We, therefore, generated a conditional knockout (floxed) allele of this gene by flanking its entire coding sequence with loxP sites. Both $GS^{fl/LacZ}$ and $GS^{fl/fl}$ mice are phenotypically normal and fertile. This approach further allows a tissue-specific elimination of GS and, hence, a more definitive insight into the functional role of GS in different organs. In the liver, the floxed GS retains the typical pericentral expression pattern of unmodified GS. In this study, mice harboring the hepatocyte-specifically expressed Cre transgene (Alfp-cre) and modified GS alleles
($Cre^{+/-}$, $GS^{fl/lacZ}$) were generated after two generations of breeding among $GS^{fl/fl}$, $GS^{LacZ/+}$ and Alfp-Cre mice. In these mice, GS expression in the liver was completely eliminated, both at mRNA and protein level. Nevertheless, these mice did not have a phenotype. In contrast to prevailing predictions, our finding clearly demonstrates that the elimination of GS from the liver does not affect viability under normal conditions. Therefore, liver-GS most likely exerts a vital function under pathological situations only. The GS conditional knockout mouse described here will be a powerful tool to identify the functional roles of glutamine synthetase.

## The Role of Anion Exchanger 2 in Bile Secretion

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Background: Primary biliary cirrhosis (PBC) is an inflammatory autoimmune disease of the bile ducts of ill-defined etiology. PBC symptoms resemble those present in cholestasis, like jaundice, pruritus, fatigue, and scarring of the liver; but in many instances it can remain as a subclinical condition with milder symptoms and slow progression. However, PBC patients are eligible for liver transplantion when cirrhosis progresses into liver failure.
Anion exchanger 2 (AE2) is a chloride/bicarbonate antiporter present in the plasma membrane of several tissues. It is believed to participate in intracellular pH regulation and cellular volume regulation. In addition, AE2 is present in the apical membrane of hepatocytes and cholangiocytes and may play a role in bicarbonate secretion into bile, contributing to the bile acid independent fraction of bile flow. Medina et. al. (Hepatology; 25(1997):12-17) have shown that liver biopsies of PBC patients have decreased immunohistochemical staining for AE2 compared to healthy controls, suggesting that impaired bicarbonate secretion and bile flow could be linked to PBC pathogenesis. It was our aim to investigate the role of Ae2 in bile formation. We have generated mice in which the most important isoforms of Ae2 (Ae2a, Ae2b1 and Ae2b2) have been disrupted ($Ae2_{a,b}^{-/-}$ mice). These animals display several phenotypes including male infertility, osteopetrosis and impaired gastric acid secretion. In this study we used this knockout mouse model to investigate bile formation in the absence of this putative biliary bicarbonate transporter. Surprisingly, biliary bicarbonate secretion and bile flow were increased in $Ae2_{a,b}^{-/-}$ female mice compared to control $Ae2^{+/+}$ littermates. A concomitant increase in bile acid output in $Ae2_{a,b}^{-/-}$ mice was observed. These findings were related to the expression levels of several transporters involved in bile formation.
Conclusion: Our findings suggest that, in the mouse, either Ae2 does not play a major role in bicarbonate secretion into bile or its absence can be overcompensated by redundant bicarbonate transport pathways in hepatocytes and/or cholangiocytes.

**Metformin protects rat hepatocytes against bile acid and cytokine-induced apoptosis**

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Background: Metformin is a drug used in the treatment of diabetes mellitus type 2. In this disease, metformin improves the glucose tolerance. Furthermore, metformin treatment reduces liver injury in an experimental mouse model of non-alcoholic-fatty-liver disease (NAFLD), and in patients with NAFLD Thus, metformin is a hepatoprotective drug. The hepatoprotective mechanism of metformin has not been elucidated yet. Metformin is presumed to activate the 5'-AMP-activated protein kinase (AMPK). AMPK is a cellular energy sensor that is activated in conditions that increase the AMP:ATP ratio. The AMP:ATP ratio is important in regulating apoptosis. Therefore, metformin could modulate apoptosis of hepatocytes in chronic liver diseases. Aim of the present study was to examine whether metformin is hepatoprotective via inhibition of apoptosis.
Methods: Primary rat hepatocytes were exposed to the pro-apoptotic bile acid glychochenodeoxycholic acid (GCDCA: 50 μM) for 4 hrs or TNFα/Actinomycin D for 6 hrs to induce apoptosis. Metformin (0.1-2 mM) was added simultaneously. AMPK was inhibited using iodotubercidin (0.1–10 μm), and the AMPK-downstream target mammalian Target Of Rapamycin (mTOR) was inhibited using rapamycin (50-500 nM). Caspase-3 activation was measured with a fluorometric assay. Necrotic cell death was detected using the Sytox Green nuclear staining.
Results: Both GCDCA and TNFα/ActD induced caspase-3 activation was dose-dependently reduced by metformin (25% at 0.1mM, 58% at 0.5mM; 80% at 1mM). Furthermore, the anti-apoptic effect of metformin did not result in necrosis. Neither the AMPK-inhibitor iodotubercidin nor the mTOR inhibitor rapamycin had any effect on the protective effect of metformin on GCDCA induced caspase-3 activity.
Conclusion: Our results demonstrate a completely novel anti-apoptotic action of metformin. Metformin protects against various forms of apoptosis in primary rat hepatocytes. However, this protective effect is independent of AMPK or mTOR, suggesting the involvement of alternative mechanisms. Furthermore, our results suggest that the hepatoprotective effect of metformin in NAFLD, studied in animal models, could be based on reduction of apoptosis. In addition, metformin could be an important treatment in liver diseases in which apoptosis plays a major role.

**Oxidative stress induced apoptosis is inhibited by metformin via an ERK and Src dependent pathway in rat hepatocytes**

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Background: The majority of chronic liver diseases are accompanied by oxidative stress, which may induce apoptosis in hepatocytes and liver injury. Recent studies suggest that insulin resistance and oxidative stress are important in the pathogenesis of nonalcoholic fatty liver disease (NAFLD). Oxidative stress is also involved in the pathophysiology of diabetes complications. Metformin has recently been shown to be hepatoprotective in the insulin-resistant and leptin deficient ob/ob mouse model of NAFLD. However, the mechanism involved in the protective effect of metformin has not been elucidated yet.
Aim: To investigate the protective effect of metformin in an in vitro model of oxidative stress-induced apoptosis.
Methods: Primary hepatocytes were exposed to the superoxide anion donor menadione (50µM). The importance of survival pathways was studied using different survival pathway inhibitors: U0126 (10µM) for ERK MAP kinase; SB203580 (10µM) for the p38 MAP kinase; SP600125 (10µM) for the JNK MAP kinase; LY294002 (50µM) for the phosphatidylinositol-3 (PI-3)-kinase pathway and SU6656 (10µM) for the Src kinase pathway. Apoptosis was determined by measuring caspase-3 and -6 activity and PARP cleavage, and necrosis by Sytox Green nuclear staining.
Results: 1) Menadione induced apoptosis, peaking between 9-12 hrs, but not necrosis. Menadione-induced apoptosis is dependent on caspase activation and JNK activity. Activation of the ERK pathway attenuates menadione-induced apoptosis. 2) Metformin inhibits menadione-induced caspase-3 and -6 activation and apoptosis in a concentration dependent manner (50% inhibition of apoptosis at 0,1 mM metformin; >90% inhibition at 0,5 and 1 mM metformin). Inhibition of the ERK pathway and Src pathway (using SU6656) reversed the protective effect of metformin against menadione-induced apoptosis. Inhibition of p38 MAP kinase or PI-3-kinase does not abrogate the protective effect of metformin against apoptosis.
Conclusions: Metformin protects hepatocytes against superoxide anions-induced caspase activation and apoptosis. The anti-apoptotic effect of metformin is in part dependent on ERK and Src activation, but not on p38 or PI-3-kinase activation. Our results elucidate a completely novel protective mechanism of metformin and suggest that metformin could become an important candidate for treatment of oxidative stress-related liver diseases.

**Different inducers of oxidative stress have divergent effects on survival and proliferation of activated rat hepatic stellate cells**

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Background: In chronic liver injury, hepatic stellate cells (HSCs) proliferate and produce excessive amounts of connective tissue causing liver fibrosis and cirrhosis. Oxidative stress generated during liver injury has been implicated as a driving force of HSC activation and proliferation. However, opposite effects have been described with regard to the effects of oxidative stress on HSC proliferation, survival and the signaling pathways involved. Understanding these effects is important to develop anti-fibrotic therapies aimed to sensitize HSC cells to oxidative stress induced apoptosis.
Aim: To evaluate the effects of various inducers of oxidative stress on HSC proliferation, survival and signaling pathways.
Methods: Culture activated rat HSCs were exposed to oxidative stress induced by menadione (5-25μM: intracellular superoxide anion donor), xanthine oxidase (XO: 20μU/ml)/hypoxanthine (HX: 0.5mM) (extracellular superoxide anion donor) or hydrogen peroxide (0.2-5mM). Apoptosis and necrosis were determined by Acridine Orange and Sytox Green nuclear staining, respectively. HSC proliferation was determined by an ELISA-based detection of BrdU incorporation into DNA. mRNA expression of the oxidative stress responsive gene HO-1 was determined by quantitative RT-PCR. Activation of signaling pathways was determined by Western blot detection of phosphorylated forms of the MAP kinases JNK and p38.
Results: All 3 inducers of oxidative stress induced HO-1 mRNA levels at least > 6-fold. Menadione induced apoptosis in a concentration and time dependent manner. Hydrogen peroxide did not induce apoptosis and necrosis was only induced in HSC treated with extremely high concentrations (>5mM). XO/HX did not induce apoptosis or necrosis. XO/HX did block proliferation of HSC for more than 90%, whereas menadione and hydrogen peroxide had no effects on HSC proliferation.The signaling pathways activated upon menadione treatment were investigated in more detail. Menadione at the apoptotic concentration of 20μM activated JNK and p38. The specific inhibitor of JNK, SP600125, decreased apoptosis by approx. 75%, without affecting necrosis.
Conclusion: Different forms of oxidative stress induce divergent effects on HSC proliferation and survival. None of the inducers of oxidative stress stimulated proliferation, whereas extracellular superoxide anions (XO/HX) block proliferation. Only intracellular superoxide anions (menadione) induce apoptotic cell death of HSC via activation of JNK. Our results demonstrate that the pro-fibrogenic and proliferative effect of oxidative stress cannot be attributed to any of the reactive oxygen species investigated in this study.

**Ischemic-Type Biliary Lesions after Human Liver Transplantation are preceded by abnormal Bile Composition**

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Biliary complications are reported in 10 to 30% of the patients after orthotopic liver transplantation (OLT), representing a major cause of morbidity and mortality. Non-anastomic, ischemic-type biliary lesions (ITBL) are considered to be the most troublesome ones, leading to strictures and dilatations with episodes of cholangitis. The pathogenesis of ITBL remains largely unknown. The objective of this research was to study bile composition, physiology and gene expression of hepatobiliary transporters in patients with and without ITBL after OLT.Out of 105 consecutive adult patients undergoing OLT, 14 patients with ITBL were diagnosed in a 3.5-year time period. They were matched with a control group of 61 uncomplicated cases. Median diagnosis of ITBL, by cholangiogram, was at 4 months after OLT (range 1 – 27). For measurement of bile flow and bile sampling, a drain was inserted in the common bile duct. To maintain the enterohepatic circulation, bile was readministered to the patient via a feeding jejunostomy catheter. Bile samples were collected daily for the first 10 postoperative days, for measurement of bile acid (BA), cholesterol (CH) and phospholipid (PL) concentration. Liver needle biopsies were collected at the end of cold storage, approximately 3 hours after reperfusion and 1 week post-operatively. Biopsies were immediately snap frozen. Expression of bile transporters was quantified by measurement of RNA using real time RT PCR. There were no significant differences between the two groups in the initial clinical characteristics, liver enzymes, or bile flow during the first ten days after OLT. However, biliary BA, PH and CH secretion was significantly lower during this early period in patients who developed ITBL, compared to controls. In addition, gene expression of bile transporters NTCP, BSEP, MDR-3 and MRP-2 was lower at one week in the ITBL group.
Conclusion: Patients who develop ITBL are characterized by an abnormal bile composition during the very early postoperative period and long before clinical symptoms of ITBL develop. These findings suggest that defects in bile formation may play a role in the pathogenesis of ITBL.

**Several COMMD proteins interact with ATP7B; possible candidate genes for hepatic copper overload disorders with unknown etiology**

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Wilson Disease (WD), Indian Childhood Cirrhosis (ICC), Endemic Infantile Tyrolian Cirrhosis (ETIC) and Idiopathic Copper Toxicosis (ICT) are all disorders characterized by hepatic copper accumulation resulting in extensive liver damage. Copper Toxicosis in Bedlington Terriers, a canine model for these disorders, is caused by a deletion in the COMMD1 (previously MURR1) gene. COMMD1 interacts with ATP7B, the copper transporter defective in WD, suggesting that COMMD1 and ATP7B cooperate to regulate hepatic copper excretion. To gain further insight into the function of COMMD1, we previously identified COMMD6 as a novel interacting partner of COMMD1 using yeast two-hybrid analysis. The validity of this interaction was verified by co-immunoprecipitations, GST pull down assays and bimolecular fluorescence complementation. Interestingly, COMMD1 and COMMD6 share a novel conserved motif known as the copper metabolism gene MURR1 (COMM) domain, which is also present in 8 other human proteins that physically interact with COMMD1. Recently, we established that COMMD proteins constitute a family of inhibitors of the transcription factor NF-kappa-B. In addition, we established that ATP7B interacts with COMMD1, COMMD2, COMMD8 and COMMD10 but not with COMMD6. Whereas COMMD1 and COMMD10 bind to the N-terminal copper-binding region of ATP7B, COMMD2 and COMMD8 bind to other regions of this protein, suggesting that the latter bind to ATP7B independent of COMMD1. These data indicate that these COMMD family members can possibly act as regulators of copper homeostasis, and their genes can thus be regarded as excellent candidate genes for copper overload disorders with unknown etiology (ICC, ETIC and ICT), and as candidate modifier genes of disease presentation and severity in WD disease patients; mutation analysis is currently underway.

## A proteomics analysis of the cholestatic rat hepatocyte

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Cholestatic liver injury causes major changes in the relative abundance and function of the various liver-specific cell types. Some cellular mechanisms have been studied in detail, including bile formation, apoptosis and antioxidant mechanisms. Little is known about the most dominant effects of cholestatic conditions on the hepatocyte. The aim of this study is to determine the effect of obstructive cholestasis on the proteome of rat hepatocytes.
Male Wistar rats (220-250 g.) were subjected to bile duct ligation (BDL) and sacrificed after one week. Hepatocytes were isolated from BDL and normal rats, using collagenase perfusion of the liver followed by density centrifugation. 50 ug total hepatocyte protein extract was subjected to two-dimensional poly-acrylamide gel electrophoresis (2D PAGE) analysis, using a pH 3-10 linear gradient for isoelectric focusing and 10,5-20% Tris-HCl gels for the first and second dimension, respectively. Gels were stained using Coomassie Brilliant Blue or silver. Selected protein spots were analyzed by MALDI TOF mass spectrometry.
Approximately 420 of the most dominant protein spots were compared between hepatocytes isolated from BDL and normal rats. Approximately 130 of these proteins (30%) showed significant changes in expression levels in BDL-hepatocytes. Five proteins spots were selected for MALDI-TOF identification. Cu,Zn superoxide dismutase (Cu,Zn SOD) and Carbonic Anhydrase III (CA-III) are among the proteins that were strongly decreased in BDL-hepatocytes. Cu,Zn SOD and CA-III are part of the cellular antioxidant system and their decrease may lead to increased oxidative stress. Concomitantly, the glucose-regulated protein 94 (GRP94) is strongly increased in BDL-hepatocytes. GRP94 is a member of the HSP90 protein family involved in the unfolded protein response in the endoplasmic reticulum that becomes activated after cellular stress.
These results show that major changes in the BDL-hepatocyte proteome are associated with cellular and/or oxidative stress. Future research will be focused on the identification of additional proteins that are deregulated in the BDL-hepatocytes.

## Atp8b1 renders the canalicular membrane resistant to hydrophobic bile salts

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Progressive Familial Intrahepatic Cholestasis type 1 (PFIC1) or Byler disease is caused by mutations in *ATP8B1*. Patients with PFIC1 suffer from chronic intrahepatic cholestasis which progresses to severe liver disease, biochemically characterized by low biliary bile salt concentrations, elevated serum bile salt and bilirubin levels, low serum cholesterol levels and normal serum γ-glutamyltranspeptidase activity. Immunohistochemical analysis of liver biopsy specimens reveals a lack of canalicular ectoenzymes, including CD13. Electronmicroscopic (EM) analysis shows that canalicular bile has a 'coarsely granular' appearance as opposed to the amorphous bile observed in other forms of cholestasis. It is currently unknown how impaired ATP8B1 activity relates to cholestasis.
Bile formation was studied in wild type and *Atp8b1*$^{G308V/G308V}$ mutant mice, the mouse model for PFIC1 disease. Both in vivo and in isolated single-pass liver perfusions (ISPLP), bile was collected during infusion of different bile salts. Biliary concentrations of bile salt, phospholipid, cholesterol, and canalicular enzyme activities were determined. Livers were also analyzed by transmission EM.
Both in vivo and in ISPLP, biliary bile salt secretion was normal in mutant mice. However, accumulation of taurocholate (TC) was much greater in mutant than in wild type livers (22.6 ± 5.5% vs. 8.6 ± 1.1% of the administered dose, respectively). During TC infusion, hepatobiliary output of choline-containing lipids and cholesterol in mutants was elevated by 33% and 100%, respectively; biliary output of alkaline phosphatase and CD13 activities were 8- and 5-fold elevated, respectively, compared to wild types. EM analysis of mutant livers identified phenotypic similarities to PFIC1 patients in that canaliculi contained multiple multivesicular bodies that were absent in wild types. This phenotype depended on bile salt hydrophobicity, as infusion of tauroursodeoxycholate caused none of these effects.
We conclude that the canalicular membrane becomes unstable when Atp8b1 is lacking; this results in shedding of lipid vesicles containing apical ectoenzymes and in impaired bile salt transport. We hypothesize that Atp8b1 flips aminophospholipids from the outer to the inner leaflet of the canalicular membrane, which increases the relative content of sphingomyelin and cholesterol in the outer leaflet. In the absence of Atp8b1, the outer leaflet poorly resists high luminal concentrations of hydrophobic bile salts.

## Excess phospholipid excretion in Fic1 mutant mice is Mdr2 independent

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The canalicular membrane contains two translocators for phospholipids: ABCB4 is an ABC transporter that translocates phosphatidylcholine from the inner to the outer leaflet of the membrane (floppase). Conversely, ATP8B1 (FIC1) is a type 4 P-type ATPase that is thought to translocate aminophospholipids from the outer to the inner leaflet (flippase). Mutations in *ABCB4* cause Progressive Familial Intrahepatic Cholestasis (PFIC) type 3 while mutations in *ATP8B1* cause PFIC type 1. Both diseases resemble each other and progress to serious liver disease often requiring liver transplantation. It is currently unknown why the absence of the flippase function of ATP8B1 leads to cholestasis. A mouse model is available with a common PFIC1 mutation (G308V) in the murine orthologue *Atp8b1* (*Atp8b1*$^{G308V/G308V}$ mice). This mutation leads to the virtual absence of Atp8b1 protein.

We hypothesized that the absence of Atp8b1 affects phospholipid excretion process into bile. We have previously reported that phospholipid excretion in normal mice completely depends on Abcb4. We therefore analyzed biliary phospholipid excretion both in mice lacking Abcb4, mice lacking Atp8b1 as well as mice lacking both Atp8b1 and Abcb4 (double KO mice). Phospholipid excretion was analyzed during infusion of bile salt. As previously reported, *Abcb4*$^{-/-}$ mice excreted no detectable phospholipid into bile. *Atp8b1*$^{G308V/G308V}$ mice excreted about 10% more phospholipid into bile than wild type mice. Due to the absence of Abcb4, double KO mice were expected to excrete no detectable phospholipid. However, phospholipid excretion amounted to about 10% of wild type animals. These data indicate that the absence of Atp8b1 leads to phospholipid excretion that is independent of Abcb4.

We hypothesize that the increase in biliary PL in the absence of Atp8b1 is caused by uncontrolled extraction of phospholipid from the membrane by bile salts. Hence, Atp8b1 may flip aminophospholipids from the outer to the inner leaflet of the canalicular membrane, thereby increasing the relative content of sphingomyelin and cholesterol level of the outer leaflet, which will render the outer leaflet more resistant towards high luminal bile salt concentrations and prevent uncontrolled lipid extraction.

## Adherence to proton pump inhibitor therapy is low in daily clinical practice

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Proton pump inhibitors (PPIs) are usually prescribed as a daily therapy. It is suspected however, that a substantial number of PPI users do not comply with the prescribed dosage regimen but rather use PPIs 'on demand', upon the presence of symptoms. This study aimed to quantify the adherence to PPIs in the general population and to identify potential predictors of adherence.
We conducted a cohort study using the Integrated Primary Care Information database, a general practice research database containing the complete, longitudinal electronic medical records of more than 500,000 persons throughout the Netherlands. All incident PPI users were identified and the number of persistent users was estimated using Kaplan Meier survival analyses, with a 6-month non-use period as definition for end of therapy. Adherence was measured over the first year for persistent users as the 'proportion of days covered (PDC)' by dividing the number of PPI prescription days by the number of days of treatment. Patients with more than 80% of days covered with PPIs (PDC>0.8) were considered adherent. Between 1996 and 2003, 17,813 persons started PPI treatment. Of the total of 71,615 prescriptions, 68,739 (96%) were daily prescriptions and 2876 (4%) were 'on demand' prescriptions. One year after start of PPI treatment, persistent use was seen in 33% of patients, whereas after two years this was 25%. When divided by indication, persistence was highest in patients with oesophagitis or gastro-oesophageal reflux symptoms. Mean PDC (±SD) value was 0.61 (±0.30), indicating that PPIs are taken less than two thirds of the days. Overall, only one third (34.2%) of PPI users were adherent. Adherence was again highest in patients with oesophagitis or gastro-oesophageal reflux symptoms. Adherence increased with increasing age and increasing use of co-medication, but decreased with a higher number of tablets per day, and in patients living in areas with a lower socio-economic status.
Conclusions: The majority of patients starting a PPI stop within one year. In persistent users adherence was low. This suggests that, although almost all PPI prescriptions were prescribed as a daily therapy, the majority of patients use PPIs on an on-demand basis. The consequences of this are clinically less important if PPIs are prescribed for symptom control only, however may be more substantial if PPIs are used for particular indications, for example in Barrett's oesophagus or in combination with NSAIDs.

**Proton pump inhibitor use is associated with the development of sporadic fundic gland polyps**

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Fundic gland polyps (FGPs) are the most common gastric polyps, occurring in approximately 2% of the general population and in up to 84% of FAP patients. Proton pump inhibitors (PPIs) are widely used to inhibit gastric acid secretion. There is conflicting evidence regarding the association between the development of sporadic FGPs and use of proton-pump inhibitors (PPIs) and the influence of PPIs on the development of dysplasia in FGPs. FGPs are relatively rare making a case-control study the most feasible method to determine whether there is a relationship.
In this case-control study, the prevalence and duration of PPI use in a consecutive group of patients undergoing esophagogastroduodenoscopy were assessed. For each patient the sex and age were recorded as well as the length of PPI use. For each patient the number, localisation and size of FGPs, when present, were assessed during esophagogastroduodenoscopy. Biopsies were taken from FGPs and normal gastric mucosa. The biopsies were fixed in formalin, embedded in paraffin and stained for hematoxylin and eosin (H&E) for routine histology. Dysplasia was graded as negative, low grade or high grade.
The mean age of patients on PPI-therapy (n = 324, mean ± SD: 56 ± 13) was higher than patients not using PPIs (n = 275, mean ± SD: 51 ± 17). There was no difference in the sex distribution between the groups. Patients with histologically confirmed FGPs (n = 107) were older than patients without FGPs (n = 492, mean ± SD: 60 ± 12 vs. 52 ± 16 respectively, $P < 0.001$). PPI therapy was associated with an increased risk of FGPs (OR 2.3, 95% CI 1.5 - 3.6). Subgroup analysis showed that long term PPI use was associated with an increased risk of FGPs (1 - 4.9 years: OR 3.3, 95% CI 1.9 - 6.0 and > 5 years: OR 5.7, 95% CI 3.1 - 10.2) while short-term PPI therapy (< 1 year) was not (OR 1.0, 95% CI 0.5 - 1.8). Linear regression analysis showed that both age and PPI use were independent predictors of having FGPs ($P < 0.001$ for both). There were no differences in the localisation, size or number of FGPs found in patients with and without PPI use. No dysplasia was found in any of these FGPs.
In conclusion: Long-term use of proton-pump inhibitors is associated with an up to 6-fold increase in the risk of sporadic fundic gland polyps. The risk of dysplasia in these polyps is, however, negligible.

## Inappropriate prescription of proton pump inhibitors in a hospital setting

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Guidelines suggest that proton pump inhibitors (PPIs) should be used for the treatment of acid-related disorders and prevention of nonsteroidal anti-inflammatory drug (NSAID)-associated gastrointestinal symptoms. However, inappropriate use of PPIs in a general practitioner setting has been reported in the last few years. In this study, we assessed the number of patients on PPIs, and the reasons for use and dosing of PPIs on two pulmonary medicine wards, one from a university (240 patients) and one from a regional (60 patients) clinic.
On admission, 88/300 (29%) patients already used PPIs. During hospitalization, use of PPIs was terminated in 3 (1%) patients, whereas PPIs were initiated in 42 (14%) patients, resulting in 127 (42%) patients using PPIs. The reasons for PPI use were in 33/127 (26%) patients an acid-related disorder, in 75 (59%) patients the prevention of medication-associated complications (mainly complications of NSAIDs, corticosteroids and antibiotics) and in 19 (15%) patients another reason. In 72/127 (57%) patients on PPI therapy, PPIs were used for a registered indication, whereas in 55 (43%) patients, no registered indication for PPI use was present (overuse). The reasons for overuse were prevention of medication-associated complications in 41/55 (75%) patients and another reason in 14 (25%) patients. On the other hand, in 21 (7%) of all 300 patients, PPIs were not prescribed while a registered indication (i.e., acid-related disorder (mainly reflux esophagitis) and prevention of NSAID-associated gastrointestinal symptoms) was present (underuse). The recommended dose of PPIs was given in 27/127 (21%) patients, whereas in 100 (79%) patients the dose was higher as recommended. The most important differences found between the university and the regional clinic were the number of patients on PPI therapy on admission (31% vs. 22%, resp.) and during hospitalization (45% vs. 33%, resp.) and the number of patients during hospitalization with overuse (20% vs. 13%, resp.) or underuse (5% vs. 13%, resp.) of PPIs.
Conclusion: More than 40% of patients on pulmonary medicine wards are on PPIs. Of these, more than half, use PPIs for an unregistered indication, whereas more than three-quarter of patients use this medication in a higher than recommended dose. As it is likely that the same is true for other hospital departments, physicians in hospitals need to be educated on present guidelines for use of PPIs.

**E266K CARD4/NOD1 gene polymorphism increases the risk for peptic ulceration in Helicobacter pylori infected patients**

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CARD4/NOD1 is a member of the CATERPILLER (CLR) gene family and is involved in the recognition of enteroinvasive bacteria and the ensuing immune response. As a consequence, genetic variability could influence the gastric inflammatory response to Helicobacter pylori. We studied the frequency of the E266K CARD4/NOD1 gene polymorphism and its relation with Helicobacter pylori infection in a population referred for upper gastrointestinal endoscopy.
Consecutive patients visiting a Vietnamese general hospital for upper gastrointestinal endoscopy were eligible for the study. DNA was isolated from whole blood and polymerase chain reaction followed by restriction fragment length polymorphism procedure was used for genotyping. The association between endoscopy diagnosis, Helicobacter pylori infection and genotyped data was analysed using Pearson's Chi-square and regression analysis.
A total of 186 patients were studied (mean age 43 years with SD=12.6, 37% male). Helicobacter pylori infection rates did not differ between patients with different variants for E266K (wildtype (n=80) 54%, heterozygotes (n=71) 65% and homozygotes (n=35) 43%; p=0.09). In patients infected with Helicobacter pylori the prevalence of peptic ulcers during endoscopy were 19%, 17% and 47% for E266K wildtype, heterozygote and homozygote (p=0.049).
The E266K CARD4/NOD1 gene polymorphism carriers increases the risk for peptic ulceration in Helicobacter pylori infected patients.

**Evaluation of endoscopy outcomes a decade after nationwide introduction of protonpump inhibitors and guidelines for *Helicobacter pylori* eradication**

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In several studies incidences of different gastrointestinal diseases have been investigated among patients referred for open access upper gastrointestinal endoscopy. However, since the nationwide introduction of protonpump inhibitors and guidelines for *Helicobacter pylori* eradication over a decade ago, it is expected that incidences of endoscopic findings have changed. Therefore, the aim of this study was to compare current prevalences of different gastrointestinal diseases with prevalences of these diseases 15 years ago. Data about endoscopy outcomes of consecutive patients referred to a primary care hospital for open access endoscopy between January 2002 and December 2004 was collected from medical files. This was compared with data from three populations with similar characteristics described by Adang et al.[1], Numans et al.[2], and Schaap et al.[3] over a decade ago. Weighted mean prevalences were calculated and outcomes were compared with our data using chi-square.Our current study population consisted of 1298 subjects: 49% was male and mean age (range) was 54(18-91). The total historical population included 3004 subject, with a mean age (range) of 50(15-88) and 57% was male. The prevalences of peptic ulcer disease and duodenitis statistically significant decreased from 16.7% to 5.7%; $p<0.01$ and from 11.4% to 5.4%; $p<0.01$, respectively. The prevalence of oesophagitis statistically significant increased: from 15.4% to 21.9%; $p<0.01$, while gastroesophageal cancer is currently as frequent as it was in the historical population: 1.7% vs 1.8%; $p=0.87$.
Conclusion: The nationwide introduction of protonpump inhibitors and guidelines for *H. pylori* eradication has led to a decrease in prevalences of peptic ulcer disease and duodenitis. The increase of oesophagitis at endoscopy supports the hypothesis that *H. pylori* eradication might contribute to the development of oesophagitis.

1 Adang RP et al. Appropriateness of indications for diagnostic upper gastrointestinal endoscopy: association with relevant endoscopic disease. Gastrointest Endosc. 1995;42(5):390-7.
2 Numans ME et al. How useful is selection based on alarm symptoms in requesting gastroscopy? An evaluation of diagnostic determinants for gastro-oesophageal malign-nancy. Scand J Gastroenterol 2001;36(4):437-43.
3 Schaap NPM et al. Endoscopic studies of the digestive tract as a service for family practitioners; experience in the Eindhoven area. Ned Tijdschr Geneesk 1993;137(23): 1142-6.

**Colonic manometry as predictor of clinical success with cecostomy in children with defecation disorders***

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Cecostomy placement for antegrade colonic irrigation has been reported as a helpful surgical intervention for patients with intractable constipation and/or fecal incontinence. The value of diagnostic evaluations prior to cecostomy has never been well described. The purpose of this study was to define the predictive value of colonic manometry on clinical outcome of cecostomy in children.

Retrospective review was conducted on medical records, barium enema and colonic manometry studies of 32 consecutive children with severe defecation disorders who received a cecostomy. Diagnoses included idiopathic constipation (n=13), Hirschsprung's disease (n=2), cerebral palsy (n=1), imperforate anus (n=6), spinal abnormality (n=6), and anal with spinal abnormality (n=4). Barium enema studies evaluated colonic anatomy, location of the cecum and degree of colonic dilatation. Colonic manometry was considered normal when high amplitude propagating contractions (HAPC) occurred from the proximal to the distal colon. Clinical success of cecostomy was defined as normal bowel movement frequency (5/week - 3/day) with no - occasional soiling.

Colonic manometry was performed on all 32 patients and barium enema on 24 patients prior to cecostomy placement. Eight patients had normal colonic motility, 8 had HAPC only in the proximal colon and 9 had absence of HAPC in the entire colon. At follow-up, a median of 8 (range 1-21) months after cecostomy placement, 25 patients (78%) fulfilled the criteria for success. Absence of HAPC in the entire colon was related to unsuccessful outcome ($p$=0.03). A colonic response with normal HAPC after bisacodyl administration was predictive of success ($p$=0.04). Five out of 8 patients with a dilated distal colon showed motility abnormalities in the distal colon. Overall the presence of dilated colonic segments was not associated with colonic dysmotility ($p$=0.84). Colonic manometry is helpful in predicting the outcome with cecostomy. Patients with generalized colonic dysmotility are less likely to benefit from cecostomy. Normal response to colonic stimulants during motility studies is a predictor of favorable outcome.

**Prevalence and clinical presentation of constipation in a representative cohort of children with severe generalized cerebral palsy***

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Constipation is a common health problem in children with severe generalized cerebral palsy. Reported prevalence rates vary from 39 to 62%, however, these numbers are based on questionnaires or small studies. Their colonic transit time is delayed, even without symptoms of constipation. The etiology of constipation in such children is assumed to be multifactorial. Primary etiological factors appear to be altered intestinal muscle tone, innervation and neural modulation. Additional factors might be abdominal and perineal muscle tone, insufficient intake of fibers and fluids, immobility, medication use and reduced perception of urge to defecate.
We aim to determine the prevalence and clinical presentation of constipation in a representative cohort of children with severe motor ánd moderate to severe intellectual disabilities. Between 2002 and 2004, 115 children (2-18 years) were visited at their specialized day-care center or school. Mean age was 9.1 ± 4.0 years (59 girls). A structured parent interview was conducted for each child. In addition, parents and carers recorded children's defecation frequency, stool consistency and laxative use in a two-week diary. Simultaneously, food intake was recorded for a week. Finally, all children had a small physical examination. Constipation was defined according to the Rome-II criteria.In our cohort, 97.4% of children were incontinent. Despite the use of laxatives, 22 of 115 children (19.1%) had constipation. Most studied children used laxatives (60.9%), but with insufficient result in 31.4%. Constipation was diagnosed based on scybala on physical examination (6), hard stools (> 0.25 of time) combined with laxative use (15) or both (1). Median defecation frequency was 7.0 ± 5.5/week. Parent's appraisal of constipation did not correlate with ours. Daily intake of fibers was insufficient in 63.8% (37) of the children. An equal percentage was observed in the constipated children. In contrast to fluid intake, only daily fiber intake (corrected for age) was significantly correlated with defecation frequency (r=0.32; p=0.016).
This first population-based study on constipation in a representative cohort of children with severe generalized cerebral palsy found a high prevalence of constipation. This was unrelated to parents' appraisal. Defecation frequency was significantly correlated with fiber intake. Although many children were treated for constipation, it proved inadequate in a considerable number of children.

## Flow cytometry patterns specifying celiac disease in lymphocytic enteritis (MARSH I enteropathy): a pilot study

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In celiac disease, cytology changes seem to precede more structural damage in duodenal biopsy specimens. In an early phase of celiac disease, a cytological classification could be of help in its diagnostic process.
To study the lymphocytic characteristics of colour flowcytometry of duodenal biopsies in patients with chronic diarrhea, suspected for celiac disease, in comparison with biopsies from patients with non-ulcer dyspepsia (NUD), and ulcerative duodenitis due to peptic ulcer disease or Crohn's disease. Parallel to the histological examination, in 26 patients with classic seropositive celiac disease(gliadine, endomysium and/or transglutaminase antibodies), 9 patients with seronegative but HLADQ2/8 positive Marsh I enteropathy, 18 patients with NUD, as well as in 6 patients with ulcerative duodenitis, flow cytometry patterns were studied to characterize T cell subsets in clinically active disease. Patients with Helicobacter pylori gastritis were excluded from the analysis.
Results: Patients with Marsh I had a significantly higher % gdT cell and higher % Large Granular Lymphocytes (LGL), as compared to HLADQ2/8 negative lymphocytic enteritis patients. Patients with Marsh I had a significant higher % Natural Killer (NK) cells as compared to Marsh II-III celiacs. Patients with Marsh I also had a significant higher % gdT and % CD103 as compared to patients with NUD, as well as a significant higher % abT cells, % CD103 and % LGL, but lower CD4/8 ratio, as compared to patients with an ulcerative duodenitis. Both seronegative and seropositive celiacs had a significantly higher % gdT cells and a significantly lower % NK cells, as well as lower % CD3-CD7+ T cells, as compared to patients with NUD or ulcerative duodenitis.
Conclusion: The combination of a high % of gdT and CD103 positive cells, as well as a low NK and low CD3-CD7+ T cells, seems characteristic for celiac disease. In Marsh I celiacs, the % LGL seems raised as compared to HLADQ2/8 negative lymphocytic enteritis, or ulcerative duodenitis.

**Diagnostic value of measuring disaccharidase activities in duodenal biopsies of children***

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Endoscopically obtained duodenal biopsy specimens with normal histology from 181 children suspected of malabsorption were used to establish reference ranges for lactase, maltase, isomaltase and sucrase and were compared with biopsies of 143 children with (sub)total villous atrophy. The disaccharidase measurement is time-consuming and thus costly. We aimed to establish the additional diagnostic value. The histological and disaccharidase results of consecutively collected duodenal biopsies in 4 years were reviewed retrospectively and results were divided into 3 histologically separate groups: I normal (n=181 ; age 8 months to 17 yr), II subtotal (n=63 ) and III total villous atrophy (n=80 ). Disaccharidase assays were performed by Dahlqvist's method and expressed as U/g protein. Patients with an isolated disaccharidase deficiency were excluded from the data analysis. The reference range (95% confidence interval) in group I were 31 to 135 (median 73) for isomaltase; 11 to 62 (median 29) for lactase; 64 to 351 (median 171) for maltase and 24 to 146 (median 69) for sucrase. The results in group II (subtotal villous atrophy) were 13 to 95 (median 41) for isomaltase; 2 to 35 (median 8) for lactase; 21 to 217 (median 89) for maltase and 12 to 108 (median 35) for sucrase. In group III (total villous atrophy) results were 8 to 41 (median 21) for isomaltase; 1 to 13 (median 5) for lactase; 9 to 113 (median 50) for maltase and 2 to 47 (median 22) for sucrase.
All disaccharidase activities in biopsies with (sub)total villous atrophy were statistically significantly lower compared to biopsies with normal histology ($p < 0.05$). ROC curves were performed with area under the curve for isomaltase, lactase, maltase and sucrase of 0.966, 0.987, 0.952 and 0.943, respectively.
Conclusions: Measuring disaccharidase activities in biopsies that show (sub)villous atrophy does not add clinically significant information. There is only an indication for the routine measurement of the disaccharidase activity in patients with normal histology and selective enzyme deficiency is suspected. A considerable reduction of diagnostic costs can be achieved by this strategy.

**Prediction of response based on viral decline during PEG-interferon alpha-2b therapy in HBeAg-positive chronic hepatitis B**

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Treatment of chronic HBeAg-positive hepatitis B with pegylated interferon is effective in only 30-40% of the patients. The most important baseline predictors for response are HBV genotype, low baseline HBV-DNA and elevated ALT levels. Until now, there are no stopping rules for pegylated interferon treatment of chronic hepatitis B.
To investigate whether viral dynamics during pegylated interferon alpha-2b (PEG-IFN) monotherapy can predict response (defined as serum HBeAg loss 26 weeks post treatment), we analyzed 136 HBeAg-positive chronic hepatitis B patients who participated in a global randomised trial and who were treated with PEG-IFN 100 µg/week for 52 weeks. PEG-IFN dose was halved after 32 weeks of treatment. Serum HBV-DNA levels were measured monthly during therapy and 26 weeks post treatment by Taqman PCR assay.
At the end of follow-up, response was achieved in 49 of 136 patients (36%). During therapy responders exhibited a 4.23 log and non-responders a 1.22 log HBV-DNA decrease. Overall, early viral kinetics were not predictive for response. Only for patients with genotype A, responders had a pronounced decline of HBV-DNA whereas non-responders remained flat during the treatment period and follow-up. HBV-DNA at week 32 was available in 120 patients; 2 patients with genotypes G and F were not analyzed. One log decline of HBV-DNA at week 32 of treatment was highly predictive for response in genotype A; the area under the Receiver Operating Characteristic (ROC) curve was 0.961, with a sensitivity of 94%, a specificity of 92%, a positive predictive value of 89% and a negative predictive value of 96%. In the other genotypes, i.e. B, C and D, both responders and non-responders showed a decline in HBV-DNA during treatment followed by a post-treatment HBV-DNA rebound in non-responders and a sustained low HBV-DNA in responders; the area under the ROC curve for a 1 log decline at week 32 was 0.833, 0.708 and 0.594, respectively for genotypes B, C and D. In conclusion, for HBeAg-positive patients with genotype A, 1 log HBV-DNA decline after 32 weeks of PEG-IFN was highly predictive for sustained response. For the other HBV genotypes, on-treatment HBV-DNA decline did not predict response sufficiently to be used in clinical practice.

**Delayed viral decline is associated with highest sustained response rate during PEG-interferon treatment for HBeAg-positive chronic hepatitis B**

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Little is known about the patterns of HBV-DNA decline and their relation to response for chronic hepatitis B patients treated with alpha-interferon. To investigate different patterns in viral decline during treatment and follow-up, we analyzed 254 HBeAg-positive chronic hepatitis B patients treated with pegylated interferon alpha-2b (PEG-IFN) 100 µg/week for 52 weeks with or without lamivudine 100 mg/day. PEG-IFN dose was reduced to 50 µg/week after 32 weeks of treatment. Endpoints were HBeAg-negativity, HBV-DNA < 400 copies/ml and HBsAg negativity 26 weeks after therapy. The total trial population consisted of 266 patients. From 12 patients, insufficient HBV-DNA measurements were available to assess HBV-DNA patterns.In the patients treated with PEG-IFN monotherapy (n=124), 5 different patterns of viral decline could be recognized: a. early decline of at least 1 log during week 0-4 of therapy (n=23); b. delayed decline of at least 2 log from baseline HBV-DNA during week 4-32 (n=32); c. late decline of at least 2 log between week 32 and 52 (n=13); d. post-treatment decline of 2 log from baseline HBV-DNA after week 52 (n=11); e. no substantial decline at any time point (n=45). A delayed rather than early viral decline was associated with highest response rates at the end of follow-up (HBeAg response 63% vs. 52%, respectively). Patients with a late or post treatment decline pattern had lower response rates (HBeAg response 31% and 27%, respectively). Interestingly, 7 out of 8 patients (88%) with HBsAg loss and all patients with HBV-DNA <400 copies/ml at the end of follow-up exhibited a delayed HBV-DNA decline. In the patients treated with combination therapy (n=130), a similar biphasic pattern of decline in HBV-DNA was seen in nearly all patients with a fast decline in the first four weeks and a slower decline thereafter.In conclusion, different patterns in HBV-DNA decline were found during PEG-IFN mono-therapy. A delayed rather than early viral decline was associated with the highest response rate. This underlines the important immunomodulatory effect of PEG-IFN and the limited predictive value of early viral kinetics in PEG-IFN therapy for HBeAg-positive chronic hepatitis B.

**Relapse in HBeAg-positive chronic hepatitis B after Peg-Interferon alpha-2b therapy alone or in combination with lamivudine**

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Recurrence of serum HBeAg has been reported to be uncommon both after cessation of interferon and lamivudine therapy for HBeAg-positive chronic hepatitis B. We investigated the frequency of relapse after Peg-interferon alpha-2b therapy alone or in combination with lamivudine in chronic hepatitis B. A total of 266 chronic HBeAg-positive patients were treated with 100µg Peg-IFN weekly for 52 weeks combined with lamivudine (n = 130) 100 mg/day or placebo (n = 136). After week 32 the Peg-IFN dose was reduced to 50µg. The post-treatment follow-up lasted 26 weeks. At the end of treatment, HBeAg loss was seen in 44% in the combination group and 29% in the monotherapy group (p = 0.01). Overall, post-treatment HBeAg recurrence was seen in 27 patients (10%), but was strongly associated with treatment allocation. Twenty-two patients (17%) receiving the combination with lamivudine and 5 (4%: p < 0.0001) receiving Peg-IFN alone relapsed for HBeAg after treatment discontinuation. Except for treatment allocation, no other baseline variables could be identified as predictive factor for relapse. Among patients in the combination group post-treatment relapse occurred less frequently after HBeAg seroconversion (i.e. HBeAg clearance and development of anti-HBe) and was found in 8 (6%) patients versus 5(4%, p = 0.35) receiving Peg-IFN alone. Multivariate analysis showed combination therapy with lamivudine (RR 3.8 CI95% 1.2 to 12, p = 0.02) and absence of anti-HBe at week 52 (RR 6.3 CI95% 2.2 to 16.7, p < 0.0001) as independent predictors for relapse.
Conclusion: Recurrence of HBeAg is more often seen after combination of Peg-IFN with lamivudine than after Peg-IFN monotherapy. Treatment allocation and absence of anti-HBe at the end of therapy were the only independent predictors for post-treatment HBeAg relapse.

**Adefovir treatment of chronic hepatitis B patients recovers circulating numbers and function of myeloid dendritic cells**

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Dendritic cells (DC) play an important role in the induction of T-cell responses. Previously, we have shown that myeloid DC (mDC) of chronic hepatitis B virus (HBV) infected patients are impaired in the expression of costimulatory molecules, T-cell stimulatory capacity as well as TNF$\alpha$ production. In addition, we showed a reduced IFN$\alpha$ production by plasmacytoid DC (pDC) in chronic HBV patients compared to healthy controls. The question is whether the impaired DC function contributes to chronicity of HBV infection or whether the virus itself causes this dysfunction of DC. The aim of our study was to assess the effect of virus reduction by adefovir on function and absolute numbers of both mDC and pDC. In this study, 14 chronic HBV patients were treated with adefovir (10 mg od.) and followed during the first 6 months of treatment. The median serum HBV DNA decreased significantly from $1.1x10^8$ to $2.0x10^3$ copies/ml within the first 3 months and remained stable thereafter. The mean ALT levels decreased from 150 to 41 U/L after 6 months. As determined by flowcytometry, a significant increase in the absolute numbers of mDC, but not of pDC was found after 6 months of treatment compared to baseline. To determine DC function, mDC and pDC were isolated using magnetic cell sorting techniques. The T-cell stimulatory capacity of mDC increased significantly in 10 out of 14 patients after 3 months of treatment. The mean IL-12 as well as the TNF$\alpha$ production by mDC stimulated with poly (I:C) and IFN$\gamma$ significantly increased after 3 months of treatment in these 10 patients. In 4 out of 14 patients, the T-cell stimulatory capacity decreased after 3 months compared to baseline. At the same time, IL-12 and TNF$\alpha$ levels were reduced. Two of these patients started treatment at time of immune activation, i.e. ALT elevated 8 and 27 times the upper limit of normal. This indicates that their mDC might have been activated at baseline. The longitudinal expression of costimulatory molecules CD80, CD86 and CD40 concurred with T-cell stimulation results for all patients. The mean production of IFN$\alpha$ by isolated pDC stimulated with *Staphylococcus aureus* Cowan strain I antigen showed a trend towards reduction after 6 months of treatment compared to baseline.
Conclusion: Reducing the viral load by adefovir recovers circulating numbers and function of mDC, but not of pDC, suggesting that adefovir contributes to partial restoration of the immunological control to HBV infection.

**Switching lamivudine resistant chronic Hepatitis B patients from tenofovir to adefovir results in less potent HBV DNA suppression**

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The nucleotide analogue tenofovir disoproxil fumarate (tenofovir) inhibits viral replication of hepatitis B virus (HBV) by blocking the viral polymerase. Several small studies showed an excellent efficacy against HBV. In our department tenofovir was prescribed in addition to lamivudine for the treatment of lamivudine resistant chronic hepatitis B before the availability of adefovir dipivoxil (adefovir). After registration of adefovir these patients were switched to adefovir monotherapy. We studied the change in HBV DNA after this change. HBV DNA was measured using our in-house Taqman PCR (dynamic range 2.40 to 10 $\log_{10}$ copies/ml). A total of 10 patients were included. The median treatment duration with tenofovir was 77 weeks (range 51-94) resulting in a median viral load reduction of 5.71 $\log_{10}$ copies/ml (p=0,005, Wilcoxon test). Median follow-up after the initiation of adefovir was 27.5 (4.0-45.7) weeks. Switching to adefovir had no consequences in 7/10 patients. All these 7 patients had reached a viral load below 4 $\log_{10}$ copies/ml before the switch. However two patients relapsed after initiation of adefovir therapy and had an increase of the viral load above 4 $\log_{10}$ copies/ml. One subject was started on tenofovir + lamivudine combination therapy again with an excellent decline of HBV DNA to below 3 $\log_{10}$ copies/ml. During tenofovir therapy one patient showed phenotypic resistance with an increase of $\log_{10}$ 3.5 compared to the lowest load. After 5 months adefovir no decline occurred. After switching to tenofovir + lamivudine combination therapy a rapid decrease of the viral load below the limit of detection was documented.
Conclusion: Tenofovir therapy is very effective in treating lamivudine resistant HBV, clinical resistance occurred in 1/10 patients. Switching to adefovir results in 7/9 cases in a persistent suppression of HBV in case of low viral levels before initiation of adefovir. However 2/9 patients had viral breakthrough after switching to adefovir. No efficacy of adefovir was seen in case of viral rebound during tenofovir treatment. Switching back to tenofovir + lamivudine combination therapy resulted in an excellent viral suppression. Based on our findings we suggest that tenofovir appears to be a more potent antiviral agent than adefovir in patients with lamivudine resistant chronic HBV infection.

**Additional value of genetic fingerprinting in source and contact tracing of hepatitis B virus infection in the community**

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The additional value of genotyping and sequence comparison of HBV for regular source- and contact- tracing by a public health centre was evaluated in this study. For this purpose serum samples from both chronically infected and acute HBV patients reported to the municipal public health service (MHS) in Rotterdam were collected during a 3-year period (2002-2004). Genotyping and phylogenetic analyses were performed by sequence analyses of the HBsAg gene. Based on source and contact tracing and phylogenetic analyses, epidemiological and molecular clusters were defined respectively.Sera of 388 patients were collected at the MHS.
The HBV-DNA of 253 (31 acute, 222 chronic) of these patients was successfully genotyped. Genotypes A, B, C and D were identified both in acute and chronic patients while genotype E was identified in chronic patients only. The distribution pattern of genotypes of chronically infected patients differed from that of acute patients. Genotype A and D were frequently found within the acute infections. Genotype A was related to (mainly Dutch) men having sex with men (MSM). Genotype D was related to heterosexuals originating from the Mediterranean area or Dutch women having sexual contact with men originated from abroad (e.g. Turkey, Curacao). Phylogenetic analyses of 253 sequences resulted in twelve molecular clusters including genotypes A (4), C (1), D (6) and E (1). The clusters were related to MSM (A) or ethnicity; Chinese (C), Turkey (D) and Angola (E). Clusters containing both acute and chronic infections may lead to the identification of possible HBV sources in these clusters. For clusters consisting of chronic patients only, either the epidemiological relationship or molecular confirmation was difficult to define. Only six of the twelve clusters identified by molecular typing were also identified by regular source and contact tracing. On the other hand, source and contact tracing led to twelve supposedly epidemiological clusters, of which six could not be confirmed by molecular typing.
Conclusions: Phylogenetic analyses of HBV in both acute and chronic cases of HBV are necessary in the confirmation or denial of HBV-linkages found by source and contact-tracing. It adds considerably to insight in spread and mode of transmission of HBV that cannot be achieved by regular source and contact tracing only. Molecular epidemiology is therefore a mandatory tool for in public health control and should be introduced as standard technology.

## Non-invasive assessment of liver fibrosis in patients with hepatitis B and C. The first Dutch experience

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The liver biopsy is the gold standard to establish the amount of liver fibrosis and presence of cirrhosis in patients with chronic liver disease. Especially in viral hepatitis, presence and severity of liver fibrosis determines the indication for antiviral therapy. Liver biopsy has several important disadvantages including procedure-related morbidity and mortality, costs and sampling-error. Recently, a non-invasive technique (Fibroscan® - Echosens, France) based on the principles of elastography and ultrasound, to quantify the amount of liver fibrosis has been introduced. Recently reported data, particularly from France, suggest that elastography allows reliable quantification of liver fibrosis. Aim of the present study was to confirm these preliminary data in an independent patient cohort.
Methods: From January till May 2005, all consecutive patients with viral hepatitis referred for liver biopsy, were assessed with Fibroscan immediately prior to the procedure. Clinicians and pathologist were unaware of the histological (Metavir) and elastographic (expressed as kPa) results respectively. Fibroscan measurements were performed by two physicians according to a standard protocol; liver fibrosis was staged by one pathologist.
Results: 48 patients (mean age 39 [SD 11] years) could be evaluated, 17 with hepatitis B and 31 with hepatitis C. Mean length of the biopsies was 29 [SD 7] mm. Histologically, 7 cases had F0-stage of fibrosis, 21 F-1, 13 F-2, 3 F-3 and 4 F-4 (cirrhosis). Elastographic results (mean and 95% confidence interval) were as follows: F-0 5.13 kPa (3.71-6.55), F-1 6.63 kPa (5.21-8.05), F-2 9.10 kPa (6.68-11.52), F-3 15.3 kPa (9.12-21.48) and F-4 15.4 kPa (12.70-18.10). Liver elasticity was positively correlated to the fibrosis stage (tau beta of Kendall, 0.52; P=0.01). Fibroscan assessment took about 10 minutes and was completely free of any side-effect.
Conclusion: These results show that liver-elasticity measured with the Fibroscan increases with the amount of fibrosis assessed histologically according to the Metavir scoring system. In particular, the prediction of absence of fibrosis and the presence of severe fibrosis or liver cirrhosis was highly reliable. It is expected that in the near future Fibroscan-assessment of liver fibrosis will replace a significant number of liver biopsies in patients with viral hepatitis B or C.

**Decline in serum neopterin concentration correlates with HCV RNA decline during administration of VX-950, a Hepatitis C Virus Protease Inhibitor**

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Background: Neopterin is a guanosine triphosphate (GTP) derived compound that is produced by activated monocytes/macrophages. We followed neopterin levels during administration of VX-950, an orally administered inhibitor of the Hepatitis C virus (HCV) NS3•4A protease, in a multiple-dose study in 34 patients chronically infected with HCV genotype 1. Methods: VX-950 was administered for 14 days at doses of 450 mg or 750 mg every 8 hours, or 1250 mg every 12 hours, or placebo. Serum neopterin levels were measured by immunoassay at days -1, 7 and 14 of dosing, and day 10 of follow-up (day 24). HCV RNA was assessed by real-time PCR. Results: In patients with chronic HCV infection, VX-950 had substantial antiviral effects, with every patient demonstrating at least a 2-log drop in viral load in all dosing groups. In the 750 mg q8h dose group, there was a reduction in median HCV RNA of more than 3 log10 after 3 days, and of 4.4 log10 at the end of 14 days of dosing. In the 450 mg q8h and 1250 mg q12h dose groups, maximal effect was seen between 3 and 7 days of dosing followed by an increase in median viral load between days 7 and 14. Median viral loads increased in all groups between days 14-24 (post-dosing). Baseline neopterin levels were elevated in 23/34 patients (median 9.45 nmol/l; ULN 7.7 nmol/l). In the 750 mg q8h dose group, the changes from baseline in median neopterin level were -3.6, -3.8 and -1.3 nmol/l at days 7, 14 and 24 (figure). In the 450 q8h dose group, the changes were -1.8, -1.4 and +0.4 nmol/l at days 7, 14 and 24 (figure). In the 1250 q12h dose group, the changes were, +0.3, -0.2 and + 1.0 nmol/l at days 7, 14 and 24 (figure). In the placebo group, the changes from baseline in median neopterin level were -0.2, +0.5, and +0.4 nmol/l at days 7, 14 and 24. Median ALT levels, which were elevated at baseline, normalized during 14 days of dosing in all groups.
Discussion: Changes in median neopterin levels correlated with the decrease in HCV RNA and ALT during administration of VX-950. The maximal decrease in median neopterin level was in the 750 mg q8h dose group, which was also the dose group with maximal reductions in HCV RNA. These data suggest that inhibition of HCV replication by VX-950 abrogates inflammation.

**Viral kinetics in patients with hepatitis C genotype 1, treated with high-dose daily interferon, high-dose pegylated interferon and pegylated interferon combined with daily interferon**

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Background/aim: Results of peginterferon/ribavirin are suboptimal in genotype 1 (SVR 50-55%). Since it takes 3-4 weeks for peginterferon-α2a to reach stable maximal plasma levels, we hypothesized that mending this early peginterferon gap might improve response rates. Potential options are high-dose daily interferon that induces a strong decline in HCV-RNA (Bekkering 2002) and double-dose peginterferon-α2a, that improves Early Virological Response (EVR) and SVR (Diago 2004).
Method: To assess which regimen is most effective, we randomized 20 genotype 1 patients to high-dose daily interferon-α2a (HDDI: 9 MU/d 4 weeks → 6 MU/d 20 weeks → peginterferon-α2a 180 µg QW 48 weeks) or double-dose peginterferon-α2a (DDPeg: 360 µg QW 4 weeks → 270 µg QW 20 weeks → 180 µg QW 48 weeks). Thereafter, another 11 consecutive patients with similar entrycriteria were given the combination of peginterferon-α2a and daily interferon-α2a (Peg+DI: 180 µg QW *plus* 4½ MU/d, 4 weeks → peginterferon-α2a 180 µg QW 68 weeks). All patients received 1.0-1.2 gram ribavirin. Currently, all patients are on therapy for 12 weeks or more. All patients were "difficult-to-treat", i.e. had genotype 1 with high viral load, cirrhosis or unresponsive to previous interferon-based treatment.
Results: At week 4, 5/10 (50%) HDDI patients, 3/10 (30%) DDPeg patients and 4/11 (36%) Peg+DI patients were HCV-RNA negative. At week 12 these numbers were: 8/10 (80%) HDDI patients, 6/10 (60%) DDPeg patients and 9/11 (82%) Peg+DI patients.
At week 4, four DDPeg patients (40%), no HDDI patient (0%) and 2 Peg+DI patients (18%) were identified as non-responders, defined as < 0.5 Log drop. Non-responders at week 4 were also non-responder at week 12.
Conclusions: High-dose daily interferon-α is highly effective in inducing an EVR in genotype 1 patients; its initial efficacy appears higher than that of peginterferon-α2a. Combination of daily interferon with peginterferon approaches the efficacy of high-dose daily interferon and may mend the PK gap of peginterferon.

**Variant mannose-binding lectin gene alleles in donor livers constitute a major risk for severe infections after orthotopic transplantation**

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Infection is the primary cause of death after orthotopic liver transplantation (OLT). Mannose-binding lectin (MBL) is a recognition molecule of the lectin pathway of complement, involved in the innate immunity and infection, assumed to be produced by the liver. MBL-deficient/variant gene individuals show an increased risk for infections. The aims of the present study were to establish the role of the liver in the production of serum MBL and to evaluate the contribution of MBL to the defense against infections after liver transplantation.
We investigated 49 patients undergoing OLT. The presence of *mbl*2 exon 1 gene (A = wildtype; O = variant) and gene promoter polymorphisms was assessed in patients and their liver transplant donors. MBL serum concentration was determined before and during one year after transplantation, and clinically significant infections as bacteremia, peritonitis or pneumonia, during this period were assessed.
Transplantation of MBL-wildtype recipients with donor livers carrying MBL-variant alleles resulted in a rapid and pronounced decrease of serum MBL levels, and vice versa. MBL genotype-equivalent transplantations did not affect the MBL levels. The wildtype to variant serum conversion was associated with the disappearance of high molecular weight MBL. No indication for extrahepatic production of serum MBL could be obtained. One year after OLT MBL-wildtype recipients had a 40-fold ($p<0.0001$) higher MBL serum level than the MBL-variant recipients, with a similar liver function according to the serum cholinesterase levels. The presence of MBL variant alleles in the *mbl*2 gene of the donor liver, but not in the recipient, was associated with an almost 4-fold increased incidence of clinically significant infections following OLT (according to genotype: 12 % in A/A, 39 % in A/O and 67 % in recipients of O/O livers; $P = 0.01$). Polymorphisms in the gene promoter region were found to have no major impact on the serum MBL level and on clinical outcome after transplantation.
We conclude that serum MBL is produced by the human liver under strong genetic control. A variant MBL genotype of the donor liver constitutes a major risk factor for clinically significant and potentially life-threatening infections after OLT.

**Anastomotic biliary strictures after liver transplantation: prevalence, presentation, management and outcome**

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We retrospectively studied the prevalence, presentation, treatment modalities, results of treatment, short- and long-term outcome of clinically relevant anastomotic biliary strictures (AS) in 531 adult liver transplantations performed between 1979 and 2003. Clinical and laboratory information was obtained from the hospital files and all radiological images were re-evaluated.
Forty-seven grafts showed an anastomotic stricture; 42 in duct-to-duct anastomoses, and 5 in hepaticojejunal Roux-en-Y anastomoses. The cumulative risk of AS after 1, 5 and 10 years was 6.6%, 10.6% and 12.3% respectively. In grafts transplanted after November 1995 the prevalence was significantly higher, respectively 9.5 and 16.7 % at 1 and 5 years compared to before this median date. Most patients presented with increased liver enzymes. In 47% of cases, additional non-anastomotic strictures (NAS) were diagnosed, most of them mild. All patients were successfully treated by one or more treatment modalities. Non-operative treatment, endoscopic retrograde cholangiopancreaticography (ERCP) in duct-to-duct anastomosis and percutaneous transhepatic cholangiodrainage (PTCD) in Roux-en-Y anastomosis, was the preferred treatment modality. As primary treatment ERCP was successful in 24 of 36 (67%) cases; and PTCD in 4 of 11 (36%). In the end 15 patients (32%) were operated.
AS presenting more than 6 months after transplantation needed more episodes of stenting by ERCP and more stents per episode compared to those presenting within 6 months. In addition, the diameter of the AS correlated with the number of stenting episodes, with a narrower diameter of AS needing more stenting episodes. Recurrence of AS after ERCP-treatment occurred significantly more in the cases that presented with AS more than 6 months after transplantation compared to those within (50% versus 5%).
Liver tests had improved significantly within 6 months after end of therapy. In this respect there was no difference between AS and AS combined with NAS. Graft and patient survival were not impaired by AS.
Conclusion: We observe an increasing prevalence of clinically relevant AS after liver transplantation. A relatively high percentage has additional NAS. Primary endoscopic treatment is very successful when AS presents within 6 months, but less successful hereafter. AS do not affect patient or graft survival.

## Actual non-estimated ten-year survival in adults after liver transplantation (LT)

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The aim of the study was to assess predictive factors for actual longterm patient survival in a single center cohort of 161 adult liver transplant recipients, transplanted between March 1979 and December 1992. All patients had completed a minimum follow-up of ten years. The influence of donor, recipient, and transplant related variables on patient survival was examined.
Prospectively collected data were retrieved from a database. Univariate analysis was performed using chi square test, Kaplan-Meier survival analysis, log rank test, and the Mann-Whitney U-test, where appropriate. Variables from the univariate analysis with a p-value <0.1 were entered in the Cox regression for multivariate analysis. One-year and ten-year patient survival was 71% and 57%, respectively. 70 Patients died (43%). The majority of these patients (67%) died during the first year post-LT. Patients having survived one year post-LT had an 80% probability of surviving ten years. After multivariate analysis, per-operative blood loss, VVB, and recipient gender appeared to be significant predictors for both one-year and ten-year patient survival.
Conclusion: This is the first study in which actual (non-estimated) longterm survival figures post-LT are presented. Survival of the first year is an important predictor for ten-year survival. The fact that per-operative blood loss and VVB are significant determinants for both short and longterm survival implicates that surgical technique is the key predictor for survival post-LT. This study further suggests that survival analysis of this population may be performed with a relatively short one year actual follow-up.

## Early hepatocellular injury during liver surgery leads to systemic inflammation

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Intermittent hepatic inflow occlusion is often applied to reduce blood loss during liver surgery. This may give rise to ischemic liver injury and to intestinal injury by congestion of the portal circulation. Fatty Acid Binding Proteins (FABP's) are small semi tissue-specific cytosolic proteins that are emerging as sensitive and specific markers of organ damage. We hypothesized that intermittent hepatic inflow occlusion induces the release of liver (L-) and intestinal (I-) type FABP's as markers of hepatocyte and enterocyte injury. In addition we studied the relation of cell injury to postoperative inflammation.9 Patients undergoing surgery with a view to resect colorectal metastases in otherwise normal livers were studied. Arterial blood was drawn before laparotomy, before and after intermittent hepatic inflow occlusion and 1.5 hours postoperatively. AST and LDH were determined by routine clinical chemistry, L-FABP, I-FABP and IL-6 by ELISA.From 12 patients undergoing upper GI surgery, blood was drawn from an arterial line and from the portal, hepatic and renal vein to study organ specific IL-6 and L-FABP release.Markers of hepatocyte damage AST, LDH and L-FABP increased already significantly (4-fold, 1.8-fold, 45-fold respectively, all $p<0.01$) during liver mobilization. No further increase was observed during subsequent intermittent hepatic inflow occlusion. L-FABP concentrations decreased immediately postoperative ($p=0.037$) concomitant with its rapid renal clearance (fractional extraction 34%, $r^2=0.75$, $p<0.001$). L-FABP was significantly released from the liver during liver mobilization (hepatic venous-portal concentration difference (mean±SE) 419±103 ng/mL, $p=0.001$). Intestinal release of L-FABP (portal-arterial concentration difference -15±52 ng/mL, NS) was negligible and arterial I-FABP remained undetectable throughout the study. The postoperative IL-6 peak (322±53 pg/mL) was significantly correlated with hepatocyte injury assessed by the area under the plasma concentration curves of L-FABP ($\theta=0.78$, $p=0.017$) and AST ($\theta=0.77$, $p=0.021$). Organ balance analysis revealed that both liver and intestines were involved in IL-6 production.
Conclusion: Hepatocyte injury is an early event during liver surgery and seems unrelated to intermittent hepatic inflow occlusion. Intermittent inflow occlusion does not seem to compromise intestinal integrity. Hepatocyte injury seems to be related to the magnitude of the systemic inflammatory response following liver surgery.

## Characterization of mice with gut specific expression of IL-12 family genes

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Inflammatory bowel diseases (Crohn's disease and ulcerative colitis) are chronic inflammatory diseases leading to destruction of gastrointestinal tissue. In Crohn's disease, an increased expression of Th1 cytokines is observed in which IL-12 has a pivotal role. Recently a novel heterodimeric cytokine, IL-23 was described that shares the p40 subunit with IL-12. IL-12 and IL-23 have overlapping biological activities (Oppmann et al, 2000). IL-23 also drives human Th1 T-cell development, but compared to IL-12, IL-23 preferentially stimulates proliferation and cytokine production by memory T-cells. General over-expression of p19 by the beta-actin promoter induced a lethal inflammatory phenotype in transgenic mice (Wiekowski et al, 2001). IL-23 production was found in the terminal ileum of normal mice and it was found up-regulated in the inflamed intestinal mucosa of patients with IBD (Becker et al, 2003). To investigate the function of IL-12, IL-23 and p40 in the gut in vivo and their role in experimental colitis we generate transgenic mice with the IL-12, IL-23 and p40 subunit expressed under a gut specific promoter, the fatty acid binding protein promoter (pFABP). A 0.6 Kb fragment consisting of the short L-FABP was excised from pELFABP plasmid promoter and cloned into the BgI II/PstI expression vector pCI-CMV (Promega), replacing the CMV promoter with the short L-FABP promoter. PCR fragments for p40, IL-12 and IL-23 (subunit of IL-12 and Il-23 are linked together) were cloned into the pCI FABP vector. The transgenic mice are characterized using southern blot, PCR, ELISA, immunohistochemical and western blot techniques. The cells population in the gut-associated lymphoid tissue and their susceptibility to experimental colitis was analyzed. Transgenic mice develop normally and do not display any abnormalities. Macroscopical and histopathological examination of the organs showed no apparent abnormalities or signs of inflammation in LFABPp40, LFABP/IL-12L, LFABP/IL-23L transgenic mice up to the age of one year. However the LFABP/IL-23 transgenic mice have reduced offspring compared to the normal FVB mice. In addition, LFABP/IL-23 transgenic mice showed an increased production of IL-17 in isolated splenocytes, evaluated by intracellular flow cytometry, when compared to LFABP/WT, LFABP/IL-12 and LFABP/p40 transgenic mice. Preliminary analysis of the susceptibility to experimental colitis after oral administration of DSS demonstrated no major differences between the transgenic mice. These preliminary characterizations of transgenic mice with gut specific expression indicate that they are viable and demonstrate a specific phenotype.

## Probing the role of peroxisome proliferator-activated receptor alpha (PPARα) in the small intestine

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Besides their role as energy providing molecules, fatty acids are molecules that regulate gene expression. It has become clear that fatty acid-dependent gene regulation is partially mediated by the nuclear receptor peroxisome proliferator-activated receptor alpha (PPARα). PPARα is a ligand-activated transcription factor and is expressed in the small intestine. Upon binding its ligand (e.g. long chain poly-unsaturated fatty acids), the PPARα/RXRα heterodimer induces the expression of various target genes, which are involved in different metabolic pathways. In this study we examined the role of PPARα in the small intestine.
Wild-type and PPARα-null mice were fed a diet supplemented with a PPARα ligand (0.1% WY14643) for 5 days (n=6 mice/group). RNA was extracted from the small intestine and hybridized to Affymetrix MOE430A mouse arrays. Statistical analyses of the arrays were performed with ArrayAssist software, using quantile normalization (GC-RMA). Differentially expressed transcripts were selected based on fold change (>|1.5|) and p-value (<0.01). To distinguish between the different cell types along the crypt-villus axis, fractions enriched in villus or crypt cells were isolated and analyzed with quantitative RT-PCR (qRT-PCR). In addition qRT-PCR was used to confirm microarray results.
QRT-PCR analyses showed that PPARα is expressed in the small intestine at levels comparable to those in liver. PPARα expression co-localized with that of villin, a marker for villus cells. Feeding studies identified 903 transcripts that were PPARα dependently regulated in the small intestine, including PPARα itself. Bioinformatics tools like GenMAPP, PathwayAssist and DAVID revealed that PPARα-regulated processes in the small intestine include β-oxidation, ketogenesis, glycolysis and biosynthesis of mitochondria and peroxisomes. In addition, the expression of lipases and peptidases involved in nutrient digestion were PPARα-dependently down-regulated.
Conclusion: In the small intestine PPARα controls expression of genes involved in metabolic pathways and signaling routes. PPARα and its target genes are predominantly expressed in villus cells of the jejunum. We conclude that PPARα is critical for the adaptive response of the small intestine to increased levels of nutritional or endogenous free fatty acids. By reprogramming the basal cellular functions of enterocytes, the activation of PPARα enhances the capacity to handle free fatty acids.

**Intestinal expression of cholesterol transporter genes and chylomicron formation are not affected by dietary phytosterols and stanols in rodents***

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Phytosterols and stanols have successfully been introduced in functional foods to lower plasma LDL levels by reducing cholesterol absorption. The precise mechanism by which phytosterols and stanols exert this action is still unknown. In this study we addressed the hypothesis that phytosterols en stanols effect intestinal gene expression of cholesterol transporter genes and thereby affect chylomicron formation. Wistar and Wistar Kyoto (WKY) rats, which hyperabsorb phytosterols and stanols due to an Abcg5 mutation, were fed with either semi-synthetic control diet, cholesterol diet (0.11 % w/w), cholesterol diet (0.11 % w/w) with phytosterols (0.46 % w/w) or cholesterol diet (0.11 % w/w) with phytostanols (0.48 % w/w) for 1 month (6 animals/group). The effects of the diets on fecal sterol loss and on expression levels of genes involved in cholesterol homeostasis in the intestine were measured. Additionally, the effects of the diets on chylomicron secretion into lymph were examined in Wistar rats only. Addition of phytosterols or stanols to the cholesterol-enriched diet resulted in a dramatic increase in fecal cholesterol loss (+100-220%) in both strains of rats. However, this occurred without any significant change in intestinal expression of cholesterol transporter genes, like *Npc1l1*, *Abca1*, *Abcg5* or *Sr-b1* and of genes involved in esterification or synthesis of cholesterol in Wistar and WKY rats. The cholesterol-enriched diet increased cholesterol secretion into lymph 2.9-fold compared to control diet. This increase was fully prevented by addition of phytosterols or stanols to the diet. However, during intestinal infusion of 4% Intralipid®, cholesterol secretion into lymph and chylomicron composition were similar in all groups.
Conclusions: Phytosterols and stanols strongly reduced cholesterol absorption efficiency without altering intestinal gene expression profiles in Wistar rats and in the phytosterol/stanol hyperabsorbing WKY rats. No sustained effects on chylomicron secretion were observed when phytosterols/stanols were added to the diet. Our data strongly indicate that phytosterols and stanols act at the level of the intestinal lumen, probably by mechanisms such as interference with mixed micelle formation.

**Cytoprotective role of Multidrug Resistance associated Protein (MRP1) in severe intestinal inflammation: involvement of the leukotriene pathway**

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Multidrug resistance associated proteins (MRPs) are well known for their cytoprotective role against cytostatic drugs. Additionally, they may also protect cells during disease condition since DSS-induced colitis is aggrevated in Mrp1-/- mice. In this study we investigated whether MRP1 is regulated during intestinal inflammation and is involved in the protection against cytokine and/or anti-Fas-induced cell death.
Inflamed and non-inflamed colon tissue from patients with Crohn's disease (CD; n=20) or Ulcerative colitis (UC; n=15) were analysed by real-time PCR and immunohistochemistry for MRP1 expression and compared to healthy controls (n=10). DLD-1 colon carcinoma cells with or without stable-overexpression of MRP1-GFP were treated with anti-Fas or TNF-alfa in the presence or absence of the MRP inhibitor MK571. The 5-lipoxygenase inhibitor AA861 was used in combination with anti-Fas/MK571 treated cells to determine whether the leukotriene pathway is involved in the cytoprotective function of MRP1. Apoptosis was measured by caspase-3 activity assays and PARP-cleavage Western blotting.
MRP1 mRNA levels are upregulated in inflamed colon tissue of UC and CD patients. In addition, MRP1 protein expression was strongly induced in the intestinal crypts in severe colitis. In vitro, anti-Fas or TNF-alfa increased caspase-3 activity and PARP cleavage in DLD-1 cells, which was markedly enhanced by MK571-treatment. Incubation with AA861 slightly increased anti-Fas-induced caspase-3 activity. Cotreatment of DLD-1 cells with anti-Fas, MK571 and AA861 resulted in caspase-3 activities comparable to anti-Fas/AA861-treated cells. DLD-1 MRP1-GFP cells showed lower caspase-3 activity levels compared to DLD-1 cells after stimulation with anti-Fas.
Conclusion: Our data suggest that MRP1 is involved in the cytoprotection of the intestinal crypt cells during inflammation. Most likeley, it exports proapoptotic compounds from the cysteinyl leukotriene biosynthesis pathway since 1) inhibition of the leukotriene pathway decreases apoptosis and 2) LTC4 is a high affinity substrate for MRP1, which is the only MRP upregulated in the inflamed colon.

**Chemokine production by buccal epithelium as a distinctive feature of pediatric Crohn's disease***

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Objectives: Inflammatory Bowel Diseases (IBD) represent an aberrant immune response by the mucosal immune system to luminal bacteria. Since the oral mucosa harbors the first epithelial cells that interact with microorganisms, we assessed the immunological activity of buccal epithelium in children with IBD and adults with Crohn's disease.
Methods: Buccal epithelial cells were obtained from 17 children and 14 adults with Crohn's disease, 18 children with ulcerative colitis, and 40 controls. Cells were cultured with and without microbial stimulation. Chemokine levels were determined in culture-supernatants by cytometric bead array and ELISA. CXCL-8 production was studied by immuno-histochemical analysis of these cells. CXCL-8 production by lipopolysaccharide stimulated monocyte-derived dendritic cells from these patients was determined.
Results: Compared to controls, pediatric ulcerative colitis patients, and adult Crohn's disease patients, only in children with Crohn's disease buccal epithelial cells exhibited enhanced production of CXCL-8, CXCL-9, and CXCL-10. *In vitro* stimulation with lipopolysaccharide or zymosan resulted in a further increase of chemokine-levels only in cells from pediatric Crohn's disease patients. CXCL-8 production by stimulated monocyte-derived dendritic cells from children with Crohn's disease was equal to that of children with ulcerative colitis.
Conclusions: Buccal epithelium of children with Crohn's disease is immunologically active, even in the absence of oral lesions. The enhanced chemokine production is associated with pediatric Crohn's disease and seems restricted to cells derived from the epithelial barrier. Assessment of chemokine production by buccal epithelial cells may become a new, rapid, non-invasive test for screening and classification of IBD in children.

**Regulation of murine *Muc5ac* mucin gene expression by GATA-6 and HNF-4α transcription factors during stomach development***

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During embryonic and foetal development of the gastrointestinal tract, the *MUC5AC* mucin gene has a spatio-temporal pattern of expression restricted to the stomach and the small intestine. In the adult, MUC5AC is only expressed in the surface epithelial cells of the stomach. In order to better understand the molecular mechanisms responsible for this restricted pattern of expression, we studied its expression in stomach during mouse development by immunohistochemistry and correlated it to that of transcription factors known to be involved in cell differentiation programs during development. We also studied its transcriptional regulation by these factors in a murine cell line by co-transfection experiments and identified cis-elements within the promoter by gel shift assays. Our results indicate that GATA-4, HNF-1α, and HNF-3β were already expressed in the pseudostratified epithelium of the hindstomach at embryonic day (ED)14.5 and remained expressed at EDs 17.5/18.5, post natal day (PND)1.5 and in adults. GATA-6 and HNF-4α started to be expressed in the stomach as of ED17.5, concomitant with the outgrowth of the hindstomach into a glandular stomach. Their expression remained weak until ED18.5 but increased substantially after birth. As of ED17.5, all the transcription factors studied were expressed in epithelial cells from the bottom to the top of the glands. Interestingly, at PND1.5 we observed an increase in GATA-6 and HNF-4α expression concomitant with the induction of Muc5ac. The implication of GATA-6 and HNF-4α in *Muc5ac* up-regulation during gastric differentiation was confirmed in *in vitro* studies where we showed up-regulation of promoter activity by these two factors and binding of HNF-4α to two cis-elements in the promoter. In conclusion, these results indicate that GATA-6 and HNF-4α play an important role in mediating the spatio-temporal pattern of expression of Muc5ac mucin in the stomach.

**The Neutrophil Activating Protein (HP-NAP) of Helicobacter pylori plays a role in adherence to gastric epithelial cells**

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Adherence of Helicobacter pylori to the gastric epithelium is believed to be an important step in the induction of active inflammation of the mucosal layer. Several specific adhesins, like SabA, BabA2, AlpA and AlpB, have already been identified, but other factors may well be involved. The aim of this study was to assess the role of the Neutrophil Activating Protein (HP-NAP) in adherence of H. pylori to epithelial cells. The napA gene of H. pylori strain ATCC43504 was disrupted by insertion of a kanamycin cassette. Strain ATCC43504 and the isogenic napA mutant were tested for their ability to adhere to Hela cells and gastric tissue sections. In addition, the binding of HP-NAP to mucin and sulfated oligo-saccharides was studied by ELISA. Wild-type H. pylori ATCC43504 displayed strong binding to Hela cells, but mutation of the napA gene resulted in a 10-fold reduction of this binding. The difference in adherence between the wild-type strain and the isogenic napA-mutant was also observed when using gastric tissue sections. Adhesion of the wild-type strain was primarily towards the mucus layer covering the epithelial cells. When compared to the wild-type strain, the isogenic napA-mutant displayed strongly reduced binding to purified mucin and sulfated oligosaccharides, suggesting that HP-NAP is indeed involved in binding to sulfated mucins.
Conclusion: HP-NAP is a multifunctional protein that plays an important role in the chronicity of H. pylori infection through binding to the gastric epithelium. The cell surface receptor involved in the interaction with HP-NAP is still unknown, but probably contains sulfated oligosaccharides.

**Evaluation of small bowel bacterial overgrowth in human duodenal biopsies by FISH**

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Background: There are several methods to diagnose small bowel bacterial overgrowth (BO) which include breath tests, culture of small bowel aspirate and culture of mucosal biopsy specimens. All have specific disadvantages. Therefore, we developed a new method to characterize and quantify individual bacteria in biopsy sections using fluorescence in situ hybridization (FISH). The following questions were addressed: 1.) Is it possible to quantify bacteria with FISH in sections of duodenal biopsies? 2.) What is the spatial relation of the bacteria to the epithelium? 3.) Do patients with an increased risk of BO have a quantitative or qualitative different bacterial population in the biopsies compared to normal controls?
Methods: Duodenal biopsies (3/participant) were taken from: healthy controls < 70 years (n = 19), liver cirrhosis (n = 17), >< 70 years with gastric pH > 5 (n = 8), > 70 years with pH < 5 (n = 7), and participants > 70 years with pH > 5 (n = 13). Each section was hybridised with two different 16SrRNA-targeted probes: one probe that hybridises with all bacteria and one of three different probes specific for bifidobacteria, enterobacteriacae or streptococci. Fluorescent bacteria were counted, using a fluorescence microscope.
Results: Bacteria were only located at the luminal side of the epithelium. Cirrhosis patients showed more bacteria (median: 59/section) than normal controls (median: 30/section; $p<0.04$). Compared to normal controls (36%), cirrhosis patients (6%, p><0.001), and the group >70yr with pH>5 (7%, $p<0.05$) had low percentages of Streptococci. Bifidobacteria and Enterobacteriacae could only be observed sporadically in all groups (>< 1% of all bacteria).
Conclusions: Using FISH, bacteria can be detected in duodenal biopsy sections at the luminal side of the epithelium. Patients with an increased risk of BO have quantitative and qualitative differences in their duodenal bacterial populations compared to normal controls. The increase in number of bacteria in these patients was smaller than we had expected.

## TRAIL and Fas are not responsible for apoptosis of crypt epithelial cells in intestinal transplant rejection

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Background: Acute allograft rejection (AR) is the major cause of graft failure after intestinal transplantation. Increased apoptosis of crypt epithelial cells is the key diagnostic feature of AR. The mechanism causing increased crypt cell apoptosis in AR is not known. Tumor Necrosis Factor Related Ligand (TRAIL) and Fas-Ligand (Fas-L) are capable of inducing apoptosis by binding death receptors 4/5 (DR4, DR5) and Fas respectively. Hypothesis: TRAIL and/ or Fas-L pathways are responsible for apoptosis of crypt epithelial cells in intestinal allograft rejection.
Methods: Immunohistochemical staining of TRAIL, DR4, DR5, Fas, Fas-L and active caspase-3 was performed on paraffin embedded biopsies from intestinal transplant recipients with AR, chronic rejection, non-specific enteritis, viral infection, indeterminate for rejection, normal histology and normal non-transplanted ileum. The number of apoptotic bodies and caspase-3 positive cells per 10 crypts, the percentage and intensity of epithelial staining and the number of positive cells in the lamina propria was scored.
Results: In the normal non-transplanted and transplanted small bowel a typical gradient expression in epithelial cells from the middle to the top of the villous was observed for Fas, Fas-L, TRAIL and DR4.DR5 was expressed in epithelial cells along the whole crypt-villus axis. During AR there was an increase in caspase-3 positive crypt epithelial cells (mean 7 per 10 crypts) which was consistent with apoptotic body count on hematoxylin-eosin staining. During AR the expression patterns for Fas, Fas-L, TRAIL and DR4 did not change in epithelial cells but in the lamina propria DR4 positive cells were increased. During AR a significant decrease of DR5 expression was observed in crypt epithelial cells.
Conclusion: TRAIL, Fas-L and the death receptors DR4, DR5 and Fas are not up-regulated in crypt epithelium cells during acute allograft rejection. The decreased DR5 expression in crypt epithelial cells suggests that crypt epithelial cells are less sensitive for TRAIL mediated apoptosis during rejection.

**5-Aminosalicylic acid inhibits colorectal cancer cell growth by inducing mitotic arrest and apoptosis**

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Epidemiological studies suggest that 5-aminosalicylic acid (5-ASA) has cancer-chemo-preventive properties in vivo, e.g., 5-ASA medication in ulcerative colitis is associated with a reduced colorectal cancer incidence. Due to its few and relative mild side effects 5-ASA is therefore an attractive candidate in a chemopreventive/treatment strategy to colorectal cancer. Inhibition of proliferation and induction of apoptosis are two widely recognized mechanisms of action of such agents. Our studies aimed to evaluate 5-ASA's potential on these features to colorectal cancer cells in vitro, within a physiological achievable range. 5-ASA was found to inhibit cell growth at low concentrations (<35mM), and reduced cell numbers to even below starting values at higher concentrations (>35 mM) in HT29, Colo205 and Caco2 cells (IC50 range 17-34 mM). Interestingly, 5-ASA enemas
(2 and 4 gram) had similar effects at the same 5-ASA concentration range (IC50 27-32 mM). Microscopic examination showed that 5-ASA induced cell arrest in the mitotic phase, with condensed chromosomes dispersed throughout the cytoplasm. This mitotic arrest did lead to a significant cell growth inhibition after 72 hours. Replating 5-ASA-treated cells in control medium revealed that this arrest was fully reversible, i.e., similar growth as non-treated cells. Isolating the mitotic cells and replating in 5-ASA showed that all mitotic phases are increased (also binucleated cells) and that there is no block at a particular mitotic phase. Features of mitotic catastrophe, i.e., giant cells containing micronuclei or multilobular nuclei, were frequently seen after arresting the cells in mitosis for longer time at higher dose. A dose-dependent increase in apoptosis by 5-ASA was also found, as assessed by immunohistochemical detection of active caspase-3 and a caspase-cleavage product of cytokeratin 18 (M30), in addition to the classical morphological features of apotosis, like chromatin condensation and cytoplasmic schrinkage.
These observations clearly indicate that prolonged exposure to higher doses of 5-ASA, also in enema formulation, induces mitotic arrest, slower growth, apoptosis and cell death in colorectal cancer cells *in vitro*.

**Important role for Mucin 2 in colonic protection against Dextran Sulfate Sodium***

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Mucin 2 (MUC2) is the major mucin of the mucus layer covering the colonic epithelium. The expression of MUC2 is lowered in inflammatory bowel disease (IBD), and therefore MUC2 might play a role in the onset and/or perpetuation of IBD. Our aim was to obtain insight in the role of Muc2 in epithelial protection against noxious agents present in the intestinal lumen. Therefore, we treated Muc2 knockout (KO), heterozygous (HZ), and wild type (WT) mice with 2.5% dextran sulfate sodium (DSS) in their drinking water for 5 days. The mice were scored daily for weight loss, stool consistency, occult faecal blood and general appearance to obtain a disease activity index (DAI). Before, during and after DSS treatment, mice were sacrificed and intestinal segments collected for histological analysis. Histological damage was determined as alterations in goblet cell numbers, mucosa thickening, cell infiltration, mucosal ulceration, crypt abscesses and destruction of colonic architecture. Before DSS treatment, HZ mice were indistinguishable from WT mice, whereas KO mice already showed signs of mild colitis. At day 2 of DSS treatment, the histological score was higher in HZ than in WT mice ($p<0.001$), but the DAI was not significantly different. At day 7 and 8, both the DAI ($p<0.05$ and $p=0.0504$ respectively) and the histological score were elevated ($p<0.05$, day 8) in the HZ compared to WT mice. The KO mice had a higher DAI at each time point investigated ($p<0.001$ vs. WT). Interestingly, the DSS-induced damage in the KO was different from the damage seen in the HZ and WT mice. Specifically, in KO mice many crypt abscesses were seen, instead of mucosal ulcerations (vs. WT and HZ mice).
Conclusions: Muc2 is of utmost importance for epithelial protection against DSS. Not only the Muc2 KO are very sensitive to colitis, also the HZ are more susceptible to DSS treatment than WT mice, which might be caused by a lower Muc2 production.

**Protection against experimental colitis by Filamentous Hemagglutinine A of Bordetella Pertussis is not exclusively mediated by IL-10**

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Filamentous haemagglutinin A (FHA) membrane antigen of *B. pertussis* causes immune evasion of this bacterium by inducing regulatory immune responses, that include induction of IL-10. Our previous experiments revealed that FHA is able to ameliorate disease activity in a chronic T-cell mediated model of colitis by the induction of regulatory immune responses. We have now further explored whether IL-10 critically mediates the protective effects of FHA in experimental colitis.
Materials and methods: The CD4+CD45RB$^{high}$ transfer model of colitis was adopted by using IL10 KO CD4+CD45RB$^{high}$ T-cells to induce colitis. Three groups of six mice were injected with CD4+CD45RB$^{high}$ T cells; the first group was also injected with CD4+CD45RB$^{low}$ T cells. The second and third group were injected with respectively 10 mg FHA and 0.9% NaCl, subcutaneous, every two weeks. Group four was injected with IL-10 KO CD4+CD45RBhigh T cells and treated with FHA as previously described. After eight weeks, mice were sacrificed and the colon was taken out for histological assessment using routing H&E staining.
Results: Mice treated with FHA showed less weight loss compared to control mice during the entire course of the experiment ($p<0.01$), the colon weight was significantly lower (140 +/- 1 mg. vs. 343 +/- 2 mg., $p<0.01$), the colon length significantly longer (10.1 +/- 0.6 cm. vs. 7.9 +/- 0.3 cm., $p<0.01$) and histological comparison showed a reduced inflammatory score (1.7 +/- 0.3 vs. 3 +/- 0.5, $p<0.01$) after treatment with FHA. There was no significant difference between the wt and IL10 KO CD4+CD45RB$^{high}$ treated with FHA. Both were protected from the development of wasting disease.
Conclusion: FHA of pathogenic *B. pertussis* is able to ameliorate disease activity in a chronic, T cell mediated model of colitis. The observation that also IL-10 KO T cells are able to prevent the development of colitis after injection of FHA indicate that IL-10 is dispensable for the induction of immune regulation by FHA.

**Methotrexate-induced mucositis in mucin 2 deficient mice***

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Chemotherapy-induced mucositis is one of the most frequent and severe side effects of cytostatic drug treatment. The intestinal mucus layer plays an important role in epithelial protection against luminal pathogens and noxes. As the structural component of this protective mucus layer, the mucin Muc2, is up-regulated after cytostatic drug treatment, increasing Muc2 synthesis could be a target in mucositis prevention or reduction The aim of our study was to determine the role of Muc2 during Methotrexate (MTX)-induced mucositis. Muc2 knockout (KO) and wild type (WT) littermates were injected intraperitoneally with MTX using dosages of 50 and 25 mg/kg body weight respectively on day –1 and 0. Animals were weighed daily, sacrificed at day 2, 3, 4 and 6 after final MTX injection and segments of the jejunum were collected for histological, protein and RNA analysis. Up to day 3 there were no differences in percentage loss of weight compared to initial body weight between WT and KO mice. Thereafter, WT mice showed a trend towards regaining their initial body weight, whereas KO mice continued to lose weight. Before MTX treatment, the small intestine of WT and KO mice were similar with respect to epithelial morphology and epithelial proliferation, apart from lack of recognizable goblet cells in the KO mice. Moreover, WT and KO mice both showed similar protein expression patterns for Sucrase-Isomaltase and Trefoil Factor 3, two important intestinal markers. Surprisingly, MTX-induced morphological damage in the intestine of KO mice was almost comparable to the damage seen in WT mice. The intestine of KO mice even showed epithelial regeneration at day 4 vs. day 5 of WT mice. Absence of Muc2 in KO mice probably leads to a continuous exposure to luminal antigens, which might have resulted in an immune response that protects the intestine to MTX-induced damage. This was confirmed by an up regulation in tumor necrosis factor (non-significant) and Interleukin-10 (IL10; p=0.0026) mRNA.
Conclusion: In contrast to our expectations, we saw no major differences in histological pathology or protein expression in the small intestine during MTX-induced mucositis in both groups. We therefore suggest that damage caused by MTX treatment may be tempered by triggering the immune system to release IL-10 prior to receiving MTX. This mechanism however, needs further investigation.

## Colonic application of tacrolimus inhibits local mucosal immune responses in mice

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Systemic treatment with the calcineurin inhibitor tacrolimus is effective in ameliorating inflammatory bowel diseases. A proposed underlying mechanism of action is inhibition of T cell proliferation. However, side effects resulting from general immune suppression limit its use. Local application of tacrolimus has been proven to be effective in several dermatological disorders and is associated with minimal side-effects. Possibly, intestinal inflammation is also perceptive to local treatment with tacrolimus. However, not much is known about the direct effect of tacrolimus on intestinal mucosa.The aim of this study was to investigate whether local application of tacrolimus in the colon affects mucosal immune responses in mice.

C57/BL6 mice were treated with a single 150 μl PBS enema, containing 1 mg/ml, 0.1 mg/ml or 0 mg/ml tacrolimus. After 24 h, lymphocytes were isolated from lamina propria, gut-draining lymph nodes, control lymphnodes and spleen. Isolated lymphocytes were stimulated ex vivo for 72 h by means of CD3- and CD28 antibodies. Interleukin (IL)-2 levels were measured in the supernatant after 48 and 72 h.

Tacrolimus treatment caused a dose-dependent inhibition of IL-2 production by lamina propria lymphocytes. Local treatment with 0.1 mg/ml tacrolimus caused an inhibition in LPL derived IL-2 production of 21% whereas treatment with 1 mg/ml further increased this inhibition to 54% compared to release by LPL from untreated mice. Interestingly, no differences in IL-2 production by mesenteric lymph node - or spleen derived lymphocytes from treated versus untreated mice were observed. This indicates that colonic administration of tacrolimus suppresses local immune responses in the gut, whereas systemic immunity remains unaffected.

Conclusion: Mucosal immune responses in the colon are inhibited upon intrarectal administration of a single tacrolimus enema in mice. On the other hand, systemic immune responses remain intact. These results indicate that tacrolimus enemas may be effective as local treatment for left-sided colitis.

**Mucosal delivery of Ovalbumin by the genetically modified L.lactis suppresses inflammatory T-cell responses in DO11.10 mice**

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Introduction: Genetically modified Lactococcus lactis (LI) can be used for local delivery of immunomodulatory proteins at the intestinal mucosa. We adopted this strategy to study the role of local and systemic immune responses after mucosal delivery of ovalbumin. For this purpose we genetically engineered ovalbumin (OVA) secreting LI and we evaluated the immune response in OVA-TCR transgenic mice (DO11.10) after oral supplementation.
Methods: A PCR amplified OVA cDNA fragment was ligated in the erythromycin resistant pT1NX vector. LI and LIOVA were grown overnight at 37ºC in erythromycin supplemented medium. Bacteria were diluted 1:50 and grown for three hours and harvested at exponential growth phase (approximately $1x10^7$ bacteria). Quantification of the secreted OVA was evaluated by ELISA and western blot. Mice were sensitised with 10 ug OVA s.c. and fed for 10 days with $1x10^9$ bacteria LI or LIOVA/day. After 10 days, mouse ears were injected with 10ug OVA, and ear thickness was measured after 24 hours. At day 12, the intestinal tract was isolated for histology. Bulk spleen and bulk MLN cells were used for FACS analysis on T-cell expression markers (CD4+OVATCR+ specific: CD45RB, CD69, CD44, CD62L and CD25) and restimulated with OVA (0.5mg/ml). Supernatants were used for cytokine measurements. (Bulk spleen cells)
Results: Sequence analysis confirmed the cloning strategy of the vector. In the exponential growth phase LIOVA produced 50 ng/ml OVA measured by ELISA. Daily intragastric administration of LIOVA led to a significant decrease of ear thickness compared to the sensitized LI treated mice ($3.3x10^{-2}$ mm vs $9.5x10^{-2}$ mm). A decrease in IFN-g production in bulk spleen cells and an increase in IL-10 production in MLN cells was seen in LIOVA compared to the LI treated mice (67.8 vs 278.9 pg/ml and 41.4 vs 9.25 pg/ml).
Conclusion: The mucosal delivery of OVA by genetically modified L.lactis induces suppression of local and systemic inflammatory OVA specific T-cell response in DO11.10 mice. These date suggest that the mode of mucosal delivery of an antigen to the mucosal immune system critically determines immune activation.

## Can probiotics inhibit the growth of antibiotic resistant micro-organisms? In-vitro study

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Resistant micro-organisms continue to consume increasing healthcare resources and are the cause of significant morbidity and even mortality. Recent studies have shown that probiotic bacteria can inhibit the growth of pathogens *in-vitro* and reduce incidence of infectious complications in both liver transplantation and acute pancreatitis patients *in-vivo.*
Ecologic 641 (E641) is a multispecies probiotic product designed to prevent bacterial translocation in acute pancreatitis patients. In this study the effect E641 against 3 strains of vancomycin resistant enterococcus (VRE) and 11 strains of methicillin resistant staphylococcus aureus (MRSA) that caused outbreaks amongst patients or employees in our hospital was assessed. Using the well-diffusion method we measured the effect of both E641 and of the six individual components of E641 (Lactobacillus acidophilus (LA), Lactobacillus casei (LC), Lactobacillus salivarius (LS), Lactococcus lactis (LL), Bifidobacterium bifidum (BB) and Bifidobacterium infantis (BI)) on the growth of the antibiotic resistant strains. From each individual probiotic strain and the E641 mixture we tested the overnight culture, the filter sterilized supernatant and the washed probiotic cells.
All 3 VRE strains were inhibited by overnight culture, supernatant and resuspended cells solution of E641, LC and LS. The overnight culture of BB inhibited all VRE strains whereas its sterilized supernatant inhibited 2 out of 3 VRE.
All MRSA strains were inhibited by the overnight solution of E641. Nine of the MRSA strains were also inhibited by the washed bacterial cells. The supernatant inhibited 8 of the MRSA strains. Of the individual probiotic components only LC and LS inhibited the growth of MRSA. The overnight culture of both LC and LS inhibited all MRSA. The filter sterilized supernatant of LC inhibited 7 MRSA strains and the washed cells inhibited 9. The supernatant of LS inhibited all MRSA, in addition the washed cells inhibited 7.
Conclusions: Selected probiotics inhibit growth of MRSA and VRE *in-vitro*. The observed effect is strain specific. Probiotics should be considered as an alternative strategy in future studies on prevention and/or treatment of resistant micro-organisms colonisation.

**Activation induced unresponsiveness to peptidoglycan of intestinal epithelial cells upon short term exposure to Lactobacillus casei***

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Food allergy represents an exaggerated immune-response to harmless food-antigens by the mucosal immune-system. Supplementation of probiotics has been shown to have beneficial effects in allergic disorders through restoration of mucosal tolerance. The intestinal epithelial cells (IEC) are among the first immuno-competent cells that are able to respond to these probiotic bacteria. We hypothesize that IEC from allergic patients are highly responsive to innate triggers, and that probiotic bacteria may render these cells hypo-responsive. An IEC cell-line caco-2 was incubated with Lactobacillus casei (LC) at 1x107 cfu/ml or peptidoglycan (PG) at 10 ug/ml for 24 hours. Subsequently, cells were washed twice and cultured in penicillin/streptomycin containing medium. At day 6, cells were re-stimulated with PG for 24 hours and cells and supernatants were collected. CXCL-8 levels were determined in the cell-culture supernatants by ELISA. Levels of the NF-kB related transcription factors P50 and P65 were determined in cell-lysates by using a chemiluminescent transfactor kit.No induction of CXCL-8 production was found upon culture with LC. In contrast, 24 hrs of PG stimulation led to CXCL-8 induction (201 pg/ml) by IEC.
Restimulation at day 6 with PG lead to a significant (p=0.000) diminution of the CXCL-8 production (from 201 to 61,1 pg/ml) for the cells that were originally stimulated with PG. Restimulation at day 6 with PG only lead to minimal induction of the CXCL-8 production (42,0 pg/ml) for the cells that were originally stimulated with LC (p=0,000 compared to cells pretreated with medium alone). In comparison to vehicle (medium) controls, both PG and LC-pretreated IEC exhibited a 2,1-2,6 fold increase of P50 and P65 monomers upon restimulation with PG. Culturing caco-2 cells with Lactobacillus casei or peptidoglycan for 24hr alters the responsiveness of these cells to a subsequent stimulation with peptido-glycan. CXCL-8 production is inhibited while the levels of the transcription factors P50 and P65 are increased.
These data suggest that probiotic bacteria may contribute to the restoration of a physio-logical state of unresponsiveness to microbial stimulation that is characteristic for the innate immune-sytem of the intestinal mucosa. The concomitant increase of NFkB family members suggest that this regulation occurs in an active manner.

**Host-microbe interactions in vivo in the human small intestine; the effects of lactobacillus plantarumWCFS1 using functional transcriptomics and proteomics**

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Entry of probiotics in the gut is proposed to affect host physiology and biochemistry, but effects on intestinal (human) host gene expression regulation is largely unexplored.Seven healthy volunteers were recruited to participate in this randomized cross-over study. After an overnight fast, an intraduodenal feeding catheter was placed orogastrically. After positioning of the catheter (tube tip positioned 5-10 cm distally from the pylorus), a test solution containing in total $10^{12}$ Lactobacillus plantarum WCFS1 and 10 g/L glucose in saline or, on another test day, only the glucose in saline, was injected continuously at 6.7 ml/min for 6 h. After this 6-h period, a tissue sample at 20 cm distally from the pylorus, was obtained by standard gastroduodenoscopy. In these tissue samples, gene expression levels were measured using genome-wide microarrays (Affymetrix). In duplicate tissue samples, differential proteome analyses were performed using the CyDIGE method (2D gel-electrophoresis with minimal fluorescent labelling). Differentially scored protein spots were subjected to identification using MALDI-TOF. The L. plantarum WCFS1 challenge significantly mediated the expression of 424 gene reporters; 383 were upregulated, 41 were downregulated. Most observed effects were relatively small but in all cases consistent for all volunteers. Biological interpretation of the gene expression results showed that fatty acid metabolism/beta oxidation, the electron transport chain, glycolysis, antigen processing, the TCA-cycle, mitochondrion, microsome, MHC class I and class II receptor activity, digestion and the cytoskeleton related processes were importantly mediated by L. plantarum.
The proteome analyses showed that two proteins differed consistently in all volunteers. One of these spots was identified as a specific microsomal protein, while this analysis did not allow identification of the second spot.
This study shows that L. plantarum WCFS1 triggers a specific, consistent transcriptional response of the small intestinal epithelium. Especially processes associated with oxidation, electron transport, antigen binding (suggesting effects on immune function) and also microsomes seem to be mediated. Overall, the findings suggest that L. plantarum administration in the human gut can serve to enhance general gut metabolism and immune function and possibly improves the integrity of intestinal epithelium.

**Increased portal blood flow maintains hepatic ammonia clearance capacity following major liver resection in man**

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Depts of Surgery, University Hospital Maastricht[1], The Netherlands, Royal Infirmary Edinburgh[2], Scotland, Free University Medical Center[3], Amsterdam, The Netherlands

We have demonstrated previously that whole body nitrogen homeostasis is maintained early after major liver resection but the underlying mechanism remains unclarified. We hypothesized that hepatic ammonia clearance per gram liver increases following major hepatectomy and that this is due to an increased blood flow per gram liver.
To test this hypothesis we studied 7 patients undergoing major hepatectomy (≥ 3 segments) for benign or malignant tumors in otherwise normal livers. Blood was sampled from an arterial line and from the portal, hepatic and right renal vein during laparotomy, prior to and after liver resection. Ammonia concentrations were measured enzymatically by spectrophotometry. Hepatic blood flow before and after major hepatectomy was measured in 6 other patients using ultrasonic flow probes. Arteriovenous concentration differences were multiplied by blood flow to quantify metabolic flux. Results are expressed as mean (SEM) and non-parametric tests were used for statistical analysis.
Intestinal ammonia production (baseline 22.1 (5.2) µmol/min) did not change significantly following major hepatectomy (31.6 (8.9) µmol/min) (p=0.38). Hepatic ammonia uptake (22.3 (5.8) µmol/min) was fully preserved and did not change following major hepatectomy (31.2 (10.2) µmol/min) (p=0.69). Total splanchnic ammonia production (hepatic venous – arterial concentration difference times splanchnic flow) was insignificant before (-0.2 (3.1) µmol/min) and after resection (0.4 (1.7) µmol/min). This means that hepatic ammonia uptake per gram liver increased proportionate to the resected volume. Mean (SEM) portal blood flow did not change significantly (925 (54) ml/min before resection and 828 (127) ml/min after resection, p>0.05). Hepatic arterial blood flow decreased from 404 (113) ml/min before resection to 216 (97) ml/min after resection (p=0.01). Portal blood flow per gram liver increased from 0.62 (0.04) ml/min/g liver to 1.18 (0.18) ml/min/g liver (p=0.028) and hepatic arterial blood flow per gram liver remained unchanged (0.35 (0.08) ml/min/g liver before and 0.33 (0.08) ml/min/g liver after major hepatectomy, p>0.05).
Conclusions. The hepatic capacity of ammonia clearance is maintained following major hepatectomy in patients with an otherwise normal liver. This means that there is an increased ammonia clearance per gram liver which may be facilitated by an increased portal blood supply per gram liver.

**Albumin synthesis in preterm ventilated infants on the first day of life, studied with [1-$^{13}$C]leucine***

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Plasma albumin is the main transport protein. Albumin levels can change considerably in the postnatal period and between individual infants and are often low. Plasma levels are not only sensitive to changes in plasma volume and transcapillary escape but also reflect the balance between albumin synthesis and degradation. We studied albumin synthesis using stable isotopes on the first day of life in order to determine the synthetic capacity. Ventilated preterm received within 6 hours after birth a constant infusion of [1-$^{13}$C]leucine (1 mg/kg/u) for 24 hours. Plasma enrichments of $\alpha$-keto-isocaproic acid (KICA) and incorporation of $^{13}$C into albumin were measured by gas chromatography mass spectrometry. During the study infants did not receive any lipids or aminoacids and were fed with only glucose intravenously. All data are presented as average±standard error of the mean. Twenty-three infants were included (gestational age 28.6±0.4 weeks, birth weight 1100±75 grams). Six infants were small fo gestational age, ten infants received a full course prenatal corticosteroids to induce lung maturation. Plasma KICA enrichments were in steady state in all patients, and $^{13}$C enrichment of plasma albumin increased linearly. The fractional synthesis rate was 14±2.7% per day and the absolute synthesis rate 270±28 mg/kg/d. The absolute synthesis rate tended to be lower in small for gestational age infants compared with adequate for gestational age (171±58 and 303±27 mg/kg/d, respectively. Prenatal corticosteroids did not influence albumin synthesis.
Conclusion. The turnover of plasma albumin in ill preterm infants is ±14% per day. Even in the catabolic state while receiving glucose only, the albumin synthesis rate is approximately 2 times higher than in healthy adults. The synthesis of albumin in small for gestational age infants tends to be lower compared to infants who are adequate for gestational age.

**Reduced circulating TNF receptor p75/p80 after enriching enteral nutrition with glutamine and antioxidants following major upper GI surgery**

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Major surgery is responsible for a depletion of important nutrients such as glutamine, vitamin C, Zinc, Selenium. Previously, we showed that a glutamine-enriched nutrition reduced the incidence of infectious complications and blunted the inflammatory response in severe trauma patients. In this study, we evaluated the effect of enteral nutrition enriched with glutamine and antioxidants on inflammatory markers following major upper GI surgery.

Twenty patients were randomised to receive a standard enteral nutrition (Sondalis-ISO)(C) versus an enteral nutrition enriched with glutamine, cysteine, zinc, selenium, $\alpha$-tocoferol, $\beta$-carotene and vitamine C (Study (S)). Included were all patients operated for at least 3 hours and postoperatively fed by jejunostomy. CRP, IL-6, IL-1, IL-8 and soluble TNF receptor p75/p80 were measured before surgery and postoperatively on day 1, 3, 5, and 7. A GEE population-averaged model was used for statistical analysis, corrected for baseline values. $P<0.05$ was considered significant.

No differences were seen for age, body weight and length among the groups. C-reactive protein reduced in the first 5 days ($p=0.084$) and in the first 7 days ($p<0.05$) in the study arm (S) (D1 C 90 ± 13; S 76 ± 10; D3 C 148 ± 34; S 121 ± 20; D5 C 117 ± 27; S 76 ± 17; D7 C 86 ± 21; S 45 ± 13). Soluble p75 TNF receptor significantly decreased in the first three days ($p<0.05$) and showed a trend in the first 5 days ($p=0.072$) in the study arm as compared to the control group. IL-1and IL-6 did not show significant differences between the two groups.

Conclusion: Enteral nutrition enriched with glutamine and antioxidants, by means of a modular mixing device, in patients after major upper GI surgery blunted the inflammatory response (CRP and sTNF p75/p80).

**Cachectic tumor (MAC16) induces poor post-operative response with arginine deficiency**

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We recently described that cancer patients are arginine deficient. Since arginine is important in post-operative repair, abnormalities in arginine metabolism could lead to a poor post-operative response in cancer. To investigate this hypothesis, we studied the post-operative response in normal and in cachectic tumor-bearing mice (10-15% bw loss) and related this to post-operative arginine metabolism.
All groups were studied before and 24h after laparotomy. Plasma concentrations of serum amyloid protein (SAP) were measured by ELISA to assess the acute-phase response. The stable isotopes 15N Cit and 15N Gln were used to measure disposal (D) and production (P) rates of Cit and Gln across the gut. Blood was sampled from the carotid artery and portal vein. Amino acid concentrations were determined by HPLC, enrichments by LC-MS. Significant differences in post-operative effects between controls and tumor-bearing mice were tested with Mann-Whitney U ($p < 0.05$).
In tumor-bearing mice, post-operative [SAP] did not increase to a similar extent as in controls (con 4175% ± 1556, tum 177 ± 43% versus pre-op, $p<0.05$). Moreover, tumor-bearing mice decreased plasma [Arg] after surgery (con 105 ± 5%, tum 89 ± 3% versus pre-op, $p<0.05$). Control mice maintained gut Gln D (98 ± 10% versus pre-op) and Cit P after surgery (100 ± 25% versus pre-op), whereas tumor mice showed decreased gut Gln D (71 ± 8% versus pre-op, $p<0.05$) and Cit P post-operatively (33 ± 19% versus pre-op, $p<0.05$).
Conclusions: Cachectic MAC16 tumor-bearing mice had a poor post-operative response, concomitant with Arg deficiency. This was related to low intestinal Cit production, suggesting that conversion of Cit to Arg was compromised and pointing out the gut as an important organ in maintenance of normal post-operative response. These results deserve further studies investigating whether supplementation of Arg or Cit can normalize the deviant response in the presence of tumor.

**Exposure to bacterial DNA before hemorrhagic shock leads to massive liver damage and apoptosis via an IFN-gamma dependent route**

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Acute liver failure is associated with sepsis and multi-organ failure (MOF) after major surgery. Although the exact underlying mechanisms are still elusive, a post-surgery inflammatory response, which involves IFN-gamma activity, seems to be pivotal. Recently, we have shown that preexposure to bacterial DNA (CpG-ODN) resulted in an enhanced inflammatory response and deteriorated intestinal barrier function following hemorrhagic shock (HS). This led us to investigate the role of bacterial DNA in liver damage after systemic hypoperfusion.
Rats were divided into several groups; rats were exposed to CpG-ODN or not (control) and sacrificed after 18h (n=7 per group). Next, a group of control and CpG-exposed rats were subjected to hemorrhagic shock and sacrificed at 4h after shock. The role of IFN-gamma in rats subjected to CpG-ODN before hemorrhagic shock was also studied using an intervention with anti-IFN-gamma antibodies. Liver damage was detected by determination of the small cytosolic protein Liver-Fatty Acid Binding Protein (L-FABP) and filamentous (F)-actin (structural protein) via immunofluorescence. Next, the extent of hepatocyte apoptosis was reflected by fragmented DNA amplified by ligase-mediated polymerase chain reaction and by immunofluorescence assessed intracellular caspase 7 activation.
Exposure to CPG-ODN (without HS) caused gross liver damage (L-FABP intensity loss of 47% compared to control, p=0.01). Administration of CpG-ODN prior to hemorrhagic shock significantly augmented liver damage (63% depletion of L-FABP vs control, p=0.01). Apoptosis, measured as DNA fragmentation and active caspase 7, was increased in liver tissue after exposure to CpG-ODN before hemorrhagic shock. Interestingly, anti-IFN-gamma abrogated liver damage to 79% of control values (p=0.01 vs CpG-ODN before HS) and reduced parameters of apoptosis (DNA-laddering and caspase 7 activity) to near control values.
Conclusion: Exposure to bacterial DNA prior to hemorrhagic shock leads to augmented liver damage via increased apoptosis. This effect is mediated via an IFN-gamma dependent route. These data suggest that bacterial DNA may play a role in pathogenesis of acute liver failure in patients undergoing major surgery.

**Natural history of early gallbladder stones: a follow-up study in patients with colorectal carcinoma**

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Yearly 1-2% of patients with gallbladder stones develop symptoms. However, it has been suggested that in the early stage of cholecystolithiasis symptomatology is more common than with longer duration. This is based on studies of populations with known stone disease that describe the interval between stone detection and the development of symptoms. However, no data are available on the interval after stone formation. Our aim was to evaluate the symptomatology in the first period after gallstone development.
To that purpose, all patients that entered our standard follow-up protocol for colorectal carcinoma between 1993 and 2001 patients were evaluated for cholecystolithiasis on ultrasound. Inclusion criteria were a gallbladder in situ, no stones on ultrasound and participation in the follow-up protocol. Patients with gallstones on follow-up ultrasound, preceded by a normal ultrasound with no stones, were evaluated for symptoms and interval to symptoms. End points were biliary colic, cholecystitis, cholangitis/pancreatitis or cholecystectomy, death, end of or lost to follow up. All data were put into a data base and life table analysis was performed.
In total, 593 patients met the inclusion criteria. Median follow-up was 5,2 (0.5 – 12,5) years. Sixty six patients developed cholecystolithiasis after operation (11%) and 8 of them became symptomatic. Biliary colic was present in 4 patients, cholecystitis in 3 and cholangitis in 1. Women did not develop gallstones more frequently than men, but did develop symptoms more frequently (4:1). Symptoms occurred early, all within 4 years after the first ultrasound positive for cholecystolithiasis.
Conclusion: Symptoms occur more frequently in the early phase of gallbladder stone development. Longitudinal studies should include patients without stones to determine the true incidence of symptoms.

**Laser capture microdissection is compatible with 16S rDNA-based genomic technologies in bowel biopsies from Crohn's disease patients**

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The intestinal bacterial flora may play an important role in the etiology of Crohn's disease (CD). Recent studies have analysed the composition of the bacterial flora in faecal material and whole bowel biopsies from CD patients using 16S rDNA-based genomic technologies, permitting highly accurate identification of all bacterial species present in a sample. As a next step, it is of interest to localize bacterial species in specific histological compartments or lesions. This assumes capability of combining microdissection of tissues from bowel biopsies with 16S rDNA-based genomic technologies, an assumption that has not yet been verified.We aimed to examine the feasibility of combining laser capture microdissection with 16S rDNA-based genomic technologies. Seven types of histological compartments or lesions were microdissected out of snap-frozen biopsies taken from surgical bowel specimens from 5 CD patients. Extracted DNA was used as template in a 2-round PCR using universal primers for the 16S rDNA gene. PCR products were purified, subcloned, sequenced and subjected to a BLAST-search against the GenBank-, EMBL- and RDP-databases.
PCR and subsequent cloning of 16S rDNA was successful in 15 microdissected locations (from all 5 patients), namely: normal mucosa (4); mucosa with disturbed architecture (3); mucosa-associated lymphoid tissue (MALT) (2); submucosal lymphoid aggregates in diseased bowel parts (1); ulcers (3); mesenteric lymph node (1) and epithelioid granulomas (1). Characterization of the microflora by sequence analysis revealed a larger diversity in the colon vs. the small bowel; in intact mucosa vs. ulcers; in MALT and submucosal lymphoid aggregates vs. mesenteric lymph nodes. Both aerobic and anaerobic species were recovered.
Conclusions: Microdissection of several tissue types from surgical bowel biopsies is compatible with 16S rDNA-based genomic technologies. We demonstrated the feasibility to detect and characterize bacterial DNA at the species level in specific histological compartments or lesions. Granulomas and MALT may be target lesions for microdissection in future studies.

## Alfabetische lijst van standhouders najaarscongres 2005 - B = Beneluxhal K = Kempenhal

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|---|---|
| Altana Pharma B.V. | B 14 |
| Alvleeskliervereniging | M 7 |
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