

**NEDERLANDSE VERENIGING 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**

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## VOORWOORD

Hierbij treft u het volledige programma aan van de komende najaarsvergadering te Veldhoven. Het programma zal ook dit najaar wat eerder van start gaan, namelijk om 13.00 uur. Op vrijdag zijn de tijden eveneens enigszins gewijzigd, zie hiervoor het programma.

De Nederlandse Vereniging voor Hepatologie start op donderdagochtend om 10.30 uur met basale voordrachten en vervolgt na de lunch het middagprogramma met vrije voordrachten en het symposium 'The immune system of the liver'.

Op donderdagmiddag heeft de NVGIC naast vrije voordrachten de minibattle, getiteld: 'Conservatieve en operatieve behandeling van colitis ulcerosa'. Vrije voordrachten zijn er verder van de Nederlandse Vereniging voor Gastroenterologie, gevolgd door een drietal projectpresentaties van de Maag Lever Darm Stichting. Om 17.00 uur is in de Brabantzaal de lezing van Dr. F. Koning, titel: 'Immunotherapie voor coeliakie: fact or fallacy?', dit in het kader van de Guido Tytgat lecture, aangeboden door Tramedico/Falk.

Tijdens de plenaire avondsessie, die om 20.00 uur van start gaat met de President Selection, zal de Altana lecture worden verzorgd, ditmaal door Dr. J. Cohn. Zijn voordracht handelt over Chronic Inharitable Pancreatitis. Ook wordt deze avond de AstraZeneca Gastrointestinale Research prijs 2004 uitgereikt. Alle leden worden van harte uitgenodigd hierbij aanwezig te zijn!

Op vrijdag zijn er sessies met vrije voordrachten van de Sectie Gastrointestinale Endoscopie, de Nederlandse Vereniging voor Gastroenterologie en de Sectie Experimentele Gastroenterologie. Tijdens de International Teaching Session spreekt Prof. M. Alison: 'Stem cells in hepato-gastrointestinal disorders: molecular pathways and therapeutic perspectives'. Voorts zijn er symposia over obstipatie en cystic fibrosis en wordt er daarnaast aandacht besteed aan de richtlijn acute pancreatitis.

In de Diezezaal wordt een programma verzorgd door Sectie Endoscopie Assistenten en Verpleegkundigen en in het Auditorium een sessie van de Vereniging 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 7 oktober 2004

DONDERDAG	BRABANTZAAK	BARONIEZAAL	PARKZAAL	AUDITORIUM	DIEZEZAAL
10.30	Geen ochtendprogramma in deze zaal.	Geen ochtendprogramma in deze zaal.	Vrije voordrachten (basaal) Nederlandse Vereniging voor Hepatologie  p.	Op donderdag 7 oktober geen programma in deze zaal.	Op donderdag 7 oktober geen programma in deze zaal.
12.00			Lunchbuffet Genderhal	-	-
13.00	Vrije voordrachten Nederlandse vereniging voor Gastrointestinale Chirurgie  p.	<b>Mini-symposium: 'Immuunsuppressiva bij inflammatoire darmziekten.'</b>  p.	Vrije voordrachten Nederlandse Vereniging voor Hepatologie  p.	-	-
15.00	Theepauze	Theepauze	Theepauze en ledenvergadering	-	-
15.30	<b>Mini-battle: 'Conservatieve en operatieve behandeling van de colitis ulcerosa'</b>  p.	Vrije voordrachten Nederlandse Vereniging voor Gastroenterologie, gevolgd door eindpresentaties MLDS  p.	Vervolg vrije voordrachten Nederlandse vereniging voor Hepatologie gevolgd door <b>Symposium: 'The immune system of the liver.'</b>	-	-
17.00	<b>Guido-Tytgat Lecture: 'Immunotherapie voor coeliakie: fact or fallacy?'</b>	Einde programma in deze zaal.	Vervolg symposium NVH	-	-
17.30	Congresborrel in expositiehal	Congresborrel in expositiehal	Congresborrel in expositiehal	-	-
18.00	Dinerbuffet in de Genderhal	Dinerbuffet in de Genderhal	Dinerbuffet in de Genderhal	-	-

20.00	<b>Presidential Selection</b> p.	-	-	-	-
21.00	Uitreiking eerste exemplaren nieuwe chirurgische GE-boek. p.	-	-	-	-
21.15	<b>Uitreiking AstraZeneca Gastrointestinale Research Prijs 2004</b> p.	-	-	-	-
21.35	<b>Altana Lecture, verzorgd door Dr. J.A. Cohn, V.S.</b> p.	-	-	-	-
22.05	Ledenvergadering Nederlandse Vereniging voor Gastroenterologie p.	-	-	-	-
22.30	Congresborrel in de Dommelpoort aangeboden door AstraZeneca p.	-	-	-	-

## Programma vrijdag 8 oktober 2004

VRIJDAG	BRABANTZAAL	BARONIEZAAL	PARKZAAL	AUDITORIUM	DIEZEZAAL
08.30	Casuïstiek voor de klinikus, vrije voordrachten Sectie Gastrointestinale Chirurgie  p.	<b>Symposium 'Cystic fibrosis'</b>  p.	Vrije voordrachten Nederlandse Vereniging voor Hepatologie  p.	09.30 Ontvangst leden Vereniging voor Maag Darm Lever Verpleegkundigen	
10.00	koffiepauze	Koffiepauze	Koffiepauze	Aanvang sessie VMDLV	
10.30	<b>Symposium 'Acute Pancreatitis'</b>  p.	Vrije voordrachten Nederlandse Vereniging voor Gastroenterologie  p.	Vrije voordrachten Sectie Experimentele Gastroenterologie  p.	Vervolg programma  p.	Ontvangst Sectie Endoscopie Verpleegkundigen en Assistenten  p.
11.00	Vervolg symposium, gevolgd door presentaties van een tweetal lopende studies naar 'Acute Pancreatitis'.  p.	Vervolg vrije voordrachten Nederlandse Vereniging voor Gastroenterologie  p.	<b>International Teaching Session verzorgd door Prof. M. Alison, UK</b>  p.	Koffiepauze	Aanvang sessie SEVA  p.
12.30	Lunchbuffet in expositiehal	Lunchbuffet in expositiehal	Lunchbuffet in expositiehal	Lunchbuffet in expositiehal	Lunchbuffet in expositiehal
13.30		Vrije voordrachten Nederlandse Vereniging voor Gastroenterologie  p.	Vrije voordrachten Sectie Experimentele Gastroenterologie  p.	Vervolg programma Vereniging Maag Darm Lever Verpleegkundigen  p.	Vervolg programma, met om 14.30 ledenvergadering SEVA  p.
15.00	Theepauze/einde programma	Theepauze/einde programma	Theepauze/einde programma	Theepauze/einde programma	Theepauze/einde programma

Cursuscommissie: Prof. dr. C.J.J. Mulder (voorzitter) (MDL, VUMC)  
Dr. H.M. van Dullemen (MDL, AZG)  
Dr. I.A.M. Gisbertz (MDL i.o., UMCN)  
Dr. C.M.F. Kneepkens (KA, VUMC)  
Dr. C.J.H.M. van Laarhoven (chirurg, Elisabeth Tilburg)  
Dr. M.E. van Leerdam (MDL i.o., Erasmus MC)  
Drs. N.C. Talstra (MDL i.o., Rijnstate)  
Dr. B.P.L. Wijnhoven (AGIO Heelkunde, Erasmus MC)

**Woensdag 6 oktober 2004**

20.30 - 21.00 uur NHL van de maag: diagnostiek en therapie  
Dr. H. Boot, MDL, AvL

21.00 - 21.20 uur Coeliakie: genetica/serologie bij case-finding en bij screening high risk groups  
Dr. M.L. Mearin, Kinder-MDL, LUMC/VUmc

21.20 – 21.50 uur Coeliakie zonder vlokatrofie  
Dr. P.J. Wahab, MDL, Rijnstate

21.50 - 22.20 uur Refractaire coeliakie en Enteropathy-Associated T-cell Lymfomen  
Prof. dr. C.J.J. Mulder, MDL, VUmc

**Donderdag 7 oktober 2004**

08.00 – 08.30 uur Slokdarm- /maagcarcinoom; workup voor endoscopische versus chirurgische therapie.  
*Dr. J.J.G.H.M. Bergman, MDL, AMC*

08.30 – 09.00 uur Transhiataal versus transthoracale oesophagus resectie. Wie, wat, wanneer?  
*Drs. J. Hulscher, Heelkunde, AMC*

09.00 – 09.30 uur Laparoscopische slokdarmresecties, een mogelijkheid voor de risicopatiënt?  
*Prof. dr. M.A. Cuesta, Heelkunde, VUmc*

09.30 – 10.00 uur Wat te doen bij intestinale metaplasie van de maag, verdwijnt maagkanker in Nederland?  
*Prof. dr. E.J. Kuipers, MDL, Erasmus MC*

10.00 – 10.30 uur koffiepauze

10.30 – 11.00 uur Chemo- / radiotherapie maag / slokdarm.  
*Dr. A. Cats, MDL, AvL*

11.00 - 11.30 uur Endoscopische curatieve therapie bij Barrett/maag dysplasie/carcinoom: worden we Japanners?  
*Drs. J. Haringsma, MDL, Erasmus MC*

11.30 - 12.00 uur Palliatieve therapie bij slokdarmkanker.  
*Dr. P.D. Siersema, MDL, Erasmus MC*

De cursuscommissie verwacht van de MDL-artsen i.o. dat ze de Cursus Klinische Hepatologie (NVH) éénmaal bezoeken in de eerste drie jaar van de opleiding en minimaal één maal in het tweede deel van de MDL-opleiding.



10.00 Ontvangst, inschrijving, koffie

**Voorzitters:** L. Klomp en R.P.J. Oude Elferink

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

10.30 Caspase-6 is involved in bile acid induced apoptosis  
T.E. Vrenken, L. Conde de la Rosa, M. Buist-Homan and H. Moshage

10.45 Early timing of vena porta embolization prior to partial hepatectomy increases activation of TNF- $\alpha$  /IL-6 pathway and leads to improved remnant liver regeneration  
R. Veteläinen, S. Dinant, A.K. van Vliet, T.M. van Gulik. Dept of Surgery (Surgical laboratory), Academic Medical Center, Amsterdam, The Netherlands

11.00 Large numbers of immature dendritic cells detach from the human donor liver pre-transplantation  
B.M. Bosma<sup>1</sup>, H.J. Metselaar<sup>1</sup>, S. Mancham<sup>1</sup>, P.P.C. Boor<sup>1</sup>, J.G. Kusters<sup>1</sup>, G. Kazemier<sup>2</sup>, H.W. Tilanus<sup>2</sup>, E.J. Kuipers<sup>1</sup>, J. Kwekkeboom<sup>1</sup>. Dept. of Gastroenterology and Hepatology<sup>1</sup>, and Surgery<sup>2</sup>, ErasmusMC-University Medical Center, Rotterdam, The Netherlands

11.15 Identification of new PPAR $\alpha$  target genes support its anti-inflammatory function in liver  
R. Stienstra, E. Lichtenauer-Kaligis, M. Muller. Nutrition, Metabolism and Genomics Dept of Human Nutrition, Wageningen University, The Netherlands

11.30 Low retinol levels potentiate bile acid-induced expression of the bile salt export pump in vitro and in vivo  
J.R.M. Plass, M.O. Hoeke, M. Geuken, J. Heegsma, D. van Rijsbergen, J.F.W. Baller, F. Kuipers, P.L.M. Jansen and K.N. Faber. Center for Liver, Digestive and Metabolic Diseases, Groningen, The Netherlands

11.45 Regulatory T cells contribute to immunologic hyporesponsiveness in chronic HBV patients  
J.N. Stoop<sup>1</sup>, R.G. van der Molen<sup>1</sup>, C.C. Baan<sup>2</sup>, L.J.W. van der Laan<sup>3</sup>, E.J. Kuipers<sup>1</sup>, S.W. Schalm<sup>1</sup>, J.G. Kusters<sup>1</sup>, H.L.A. Janssen<sup>1</sup>. Dept of Gastroenterology and Hepatology<sup>1</sup>, Dept of Internal Medicine<sup>2</sup>, Dept of Surgery<sup>3</sup>, ErasmusMC, Rotterdam, The Netherlands

12.00 Lunch

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12.30 Inschrijving, koffie

**Voorzitters:** O.R. Busch en J. Maring

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

13.00 Neoadjuvant chemoradiotherapy with consecutive surgery in patients with resectable esophageal cancer  
L. van de Schoot<sup>1</sup>, M. van der Sangen<sup>2</sup>, G.J. Creemers<sup>3</sup>, O.J. Repelaer van Driel<sup>4</sup>, H.J.T. Rutten<sup>1</sup>, G.A.P. Nieuwenhuijzen<sup>1,5</sup>. Depts of Surgery<sup>1</sup>, Radiotherapy<sup>2</sup> and Internal Medicine<sup>3</sup>,

Catharina Ziekenhuis, Eindhoven, Dept of Surgery<sup>4</sup>, Maxima Medisch Centrum, Eindhoven, on behalf of the collaborating hospitals, region South-East of the IKZ<sup>5</sup>

- 13.10 Impact of hospital volume on staging procedures for esophageal cancer  
E.P.M. van Vliet<sup>1</sup>, M.J.C. Eijkemans<sup>2</sup>, J.J. Hermans<sup>3</sup>, E.W. Steyerberg<sup>2</sup>, H.W. Tilanus<sup>4</sup>, A. van der Gaast<sup>5</sup>, G.P. Krestin<sup>3</sup>, E.J. Kuipers<sup>1</sup>, P.D. Siersema<sup>1</sup>. Depts of Gastroenterology<sup>1</sup>, Epidemiology and Biostatistics<sup>2</sup>, Radiology<sup>3</sup>, Surgery<sup>4</sup>, Oncology<sup>5</sup>, Erasmus MC, Rotterdam, The Netherlands
- 13.20 Clinical Relevance of FDG-PET in Staging Esophageal Cancer Regarding the Standardized Uptake Value (SUV) and Detection of Unexpected Primary Neoplasia  
H.L. van Westreenen<sup>1</sup>, H.M. van Dullemen<sup>2</sup>, P.L. Jager<sup>3</sup>, Th. Wiggers<sup>1</sup>, J.Th.M. Plukker<sup>1</sup>. Dept of Surgery<sup>1</sup>, Gastroenterology<sup>2</sup>, Nuclear Medicine/PET-center<sup>3</sup>, Groningen University Medical Center, Groningen, The Netherlands
- 13.30 Quality of life in adults after correction of oesophageal atresia  
J.A. Deurloo<sup>1</sup>, S. Ekkelkamp<sup>1</sup>, E.E. Hartman<sup>2</sup>, M.A.G. Sprangers<sup>2</sup>, D.C. Aronson<sup>1</sup>. Pediatric Surgical Center of Amsterdam<sup>1</sup> (Emma Children's Hospital/AMC and VU Medical Center), Dept of Medical Psychology<sup>2</sup>, Academic Medical Center, Amsterdam, The Netherlands
- 13.40 Prevention of secondary pancreatic infections with probiotics; in-vitro studies.  
B.U. Ridwan<sup>1</sup>, M.G.H. Besselink<sup>2</sup>, C. Koning<sup>3</sup>, H.M. Timmerman<sup>2</sup>, J. Verhoef<sup>1</sup>, H.G. Gooszen<sup>2</sup>, L.M.A. Akkermans<sup>2</sup>. Dept of Medical Microbiology<sup>1</sup>, Dept of Surgery<sup>2</sup>, UMC Utrecht, Dept of Medical Microbiology<sup>3</sup>, Academic Hospital Maastricht, The Netherlands
- 13.50 SELDI-TOF mass spectrometry for the identification of biliary tract malignancy in bile fluid.  
J.B. Tuynman<sup>1</sup>, B.Groen<sup>2</sup>, O.M. van Delden<sup>3</sup>, D.J. Richel<sup>1</sup>, J.M. Aerts<sup>4</sup>, D.J. Gouma<sup>5</sup>, T.M. van Gulik<sup>5</sup>. Dept of Medical Oncology<sup>1</sup>, Hepatology<sup>2</sup>, Radiology<sup>3</sup>, Biochemistry<sup>4</sup> and Surgery<sup>5</sup>, Academic Medical Center, Amsterdam, The Netherlands
- 14.00 Hepatectomy for colorectal cancer liver metastases: effects of multi-disciplinary regional collaboration and extended indications.  
R.M. van Dam<sup>1</sup>, M.C.G. van de Poll<sup>1</sup>, J.W.M. Greve<sup>1</sup>, R.L.H. Jansen<sup>2</sup>, R.G.H. Beets-Tan<sup>3</sup>, J. van der Bijl<sup>4</sup>, P.B. Soeters<sup>1</sup>, M.H.A. Bemelmans<sup>1</sup>, C.H.C. Dejong<sup>1</sup>. Dept of Surgery<sup>1</sup>, Dept of Oncology<sup>2</sup>, Dept of Radiology<sup>3</sup>, Academic Hospital Maastricht and Dept of Surgery<sup>4</sup>, Atrium Medical Center Heerlen, The Netherlands
- 14.10 Ten years experience with the Ileal Pouch-Anal Anastomosis without a diverting ileostomy; safe or sorry?  
A.T.P.M. Claassen<sup>1</sup>, S.A. Mollema<sup>1</sup>, P.W. Teunissen<sup>1</sup>, R.A. van Hogezaand<sup>2</sup>, W.A. Bemelman<sup>3</sup>, A. Gerritsen van der Hoop<sup>1</sup>. Dept. of Surgery<sup>1</sup> and Gastroenterology<sup>2</sup>, Leids University Medical Center, Leiden and Dept. of Surgery<sup>3</sup>, Academic Medical Center, Amsterdam, The Netherlands.
- 14.20 Integrity of the anal sphincters after pouch-anal anastomosis: evaluation with three-dimensional endoanal ultrasonography  
M.P. Gosselink<sup>1</sup>, R.L. West<sup>2</sup>, E.J. Kuipers<sup>2</sup>, B.E. Hansen<sup>2</sup>, W.R. Schouten<sup>1</sup>. Depts of Surgery<sup>1</sup> and Gastroenterology and Hepatology<sup>2</sup>, Erasmus MC, Rotterdam, The Netherlands
- 14.30 Defects and atrophy of the external anal sphincter in patients with faecal incontinence: comparison between 3 dimensional-anal endosonography and endoanal magnetic resonance imaging  
M. Cazemier<sup>1</sup>, M.P. Terra<sup>2</sup>, J. Stoker<sup>2</sup>, E.S.M. de Lange<sup>3</sup>, G.E.E. Boeckxstaens<sup>4</sup>, R.J.F. Felt-Bersma<sup>1</sup>. Dept of Gastroenterology and Hepatology<sup>1</sup>, Dept of Epidemiology and Biostatistics<sup>3</sup>, VU Medical Center, Dept of Radiology<sup>2</sup>, Dept of Gastroenterology and Hepatology<sup>4</sup>, Academic Medical Center, Amsterdam, The Netherlands
- 14.40 Inflammation and structural changes in donor intestine and liver after brain death induction  
L.G. Koudstaal<sup>1,2</sup>, N.A. 't Hart<sup>1,2</sup>, R.J. Ploeg<sup>1</sup>, H. van Goor<sup>2</sup>, H.G.D. Leuvenink<sup>1</sup>. Surgery Research Laboratory, Dept of Surgery<sup>1</sup> and Dept of Pathology and Laboratory Medicine<sup>2</sup>, University Hospital Groningen, The Netherlands

14.50 Perianal fistulas in Crohn's disease are predominantly colonized by skin flora: implications for antibiotic treatment?  
R.L. West<sup>1</sup>, C.J. van der Woude<sup>1</sup>, H.Ph. Endtz<sup>2</sup>, B.E. Hansen<sup>1</sup>, M. Ouwendijk<sup>1</sup>, H.A.M. Boelens<sup>2</sup>, J.G. Kusters<sup>1</sup>, E.J. Kuipers<sup>1</sup>. Depts of Gastroenterology and Hepatology<sup>1</sup> and Medical Microbiology and Infectious Diseases<sup>2</sup>, Erasmus MC/ University Medical Center Rotterdam, The Netherlands

15.00 Theepauze

**MINIBATTLE**

**'Conservatieve en operatieve behandeling van colitis ulcerosa'**

**Voorzitter: Dr. C.J.H.M. van Laarhoven**

15.30 Medicamenteuze behandeling van acute colitis ulcerosa.  
Drs. R.K. Weersma, mdl-arts in opleiding, Academisch Ziekenhuis Groningen

16.00 Operatieve behandeling van acute colitis ulcerosa.  
Drs. R.M. van Dam, chirurg in opleiding, Academisch Ziekenhuis Maastricht

16.30 Commentaar en discussie  
Dr. H.M. van Dullemen, mdl-arts, Academisch Ziekenhuis Groningen  
Prof. dr. P.B. Soeters, chirurg, Academisch Ziekenhuis Maastricht

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**Nederlandse Vereniging voor Gastroenterologie**

**Brabantzaal**

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**Voorzitter:**

17.00 **Guido Tytgat Lecture**  
Immunotherapie voor coeliakie: fact or fallacy?  
Dr. F. Koning, Afdeling Immunohematologie, Leids Universitair Medisch Centrum.

17.30 Congresborrel in expositiehal

18.00 Diner in de Genderhal

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**Nederlandse Vereniging voor Gastroenterologie**

**Baroniezaal**

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**Voorzitters:** C.J.J. Mulder en D.W. Hommes

**MINI SYMPOSIUM**

**Immuunsuppressiva bij inflammatoire darmziekten**

13.00 Opening  
Prof. dr. C.J.J. Mulder, VUMC, Amsterdam

13.05 Dutch IBD Research Group

- Dr. D.W. Hommes, AMC, Amsterdam
- 13.15 Azathioprine  
Drs. D.J. de Jong, UMCN, Nijmegen
- 13.35 Methotrexate  
Drs. C.J. van der Woude, EMC, Rotterdam
- 13.55 Infliximab  
Dr. A.A.M. van Bodegraven, VUMC, Amsterdam
- 14.15 Keynote lecture: problems in current approach  
Prof. dr. W. Reinisch, Vienna, Austria  
6-thioguanine: safe and effective alternative?
- 14.40 Panel discussion  
Chair: Dr. D.W. Hommes  
- Prof. dr. W. Reinisch (Vienna)  
- Prof. dr. C.J.J. Mulder (VUMC, Amsterdam)  
- Prof. dr. S.J. van Deventer (AMC, Amsterdam)  
- Dr. H.P.M. Festen (GZG, Den Bosch)  
- Dr. M. Russel (MST, Enschede)
- 15.00 End of programme, teabreak

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**Nederlandse Vereniging voor Gastroenterologie**

**Baroniezaal**

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**Voorzitters:** J.Ph. Drenth en J.B.M.J. Jansen

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

- 15.30 A genome-wide screen in a four-generation Dutch family with coeliac disease: evidence for linkage to chromosomes 6 and 9  
A.J. Monsuur, M.J. van Belzen, M.M. Vrolijk, C. Wijmenga. Dept of Biomedical Genetics, University Medical Centre, Utrecht, The Netherlands
- 15.40 Screening Patients with Hashimoto's Thyroiditis for Celiac Disease and Vice Versa  
M. Hadithi, H. de Boer, L. Verschoor, J.B.A. Crusius, A.S. Peña, J. Kerckhaert, P. Wahab and C.J.J. Mulder. Rijnstate Hospital, Arnhem and VU University Medical Center, Amsterdam, The Netherlands
- 15.50 Can 18F-fluoro-deoxy-glucose Positron Emission Tomography be Useful in Detecting Enteropathy-associated T-cell Lymphoma in Refractory Coeliac Disease?  
M. Hadithi, E. Comans, R. Heijmans, A. van Schie, M. von Blomberg, J. Oudejans, O. Hoekstra and C. Mulder. Free University Medical Centre. Amsterdam, The Netherlands.
- 16.00 Double-balloon enteroscopy: practical experience in 28 patients  
C.J. Mulder, M. Hadithi, A. van Bodegraven, A. Al. Toma, M. Jacobs, D. Heine. Dept of Gastroenterology, Free University Medical Center, Amsterdam, The Netherlands
- 16.10 Role of beta-interferon in the treatment of human pancreatic cancer.  
G. Vitale<sup>1</sup>, P.M. van Koetsveld<sup>1</sup>, A. Colao<sup>2</sup>, K. van der Wansem<sup>1</sup>, J.I. Erdmann<sup>1, 3</sup>, S.W.J. Lamberts<sup>1</sup>, C.H. van Eijck<sup>1</sup>, L.J. Hofland<sup>1</sup>. Dept of Internal Medicine<sup>1</sup>, Erasmus MC, Rotterdam, The Netherlands, Dept of Molecular & Clinical Endocrinology and Oncology<sup>2</sup>, "Federico II" University of Naples, Naples, Italy, Dept of Surgery<sup>3</sup>, Erasmus MC, Rotterdam, The Netherlands

- 16.20 Systemic rate of appearance of starch derived glucose and the secretion of gastrointestinal hormones\*  
R.E. Wachters-Hagedoorn<sup>1</sup>, M.G. Priebe<sup>1</sup>, J.A.J. Heimweg<sup>1</sup>, A.M. Heiner<sup>1</sup>, K.N. Englyst<sup>2</sup>, J.J. Holst<sup>3</sup>, F. Stellaard<sup>1</sup>, R.J. Vonk<sup>1</sup>. Dept of Pediatrics<sup>1</sup>, University Hospital Groningen, Groningen, The Netherlands, Englyst Carbohydrates – Research and Services<sup>2</sup>, Southampton, UK and Dept of Medical Physiology<sup>3</sup>, University of Copenhagen, Copenhagen, Denmark

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**Presentaties projecten Maag Lever Darm Stichting**

**Baroniezaal**

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

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

- 16.30 Novel insights in familial intrahepatic cholestasis syndromes (project WS 98-12)  
Dr. L.W.J. Klomp, UMC Utrecht, Wilhelmina Kinderziekenhuis
- 16.40 The role of the human gut microflora in Crohn's disease: towards a rapid molecular quantitative analysis (project WS 98-22)  
X.W. Huijsdens<sup>1</sup>, A. Catsburg<sup>1</sup>, R. Linskens<sup>2</sup>, SGM Meuwissen<sup>2</sup>, CMJE Vandenbroucke-Grauls<sup>1</sup>, PHM Savelkoul<sup>1</sup>. Departments of Medical Microbiology & Infection Control<sup>1</sup> and Gastroenterology<sup>2</sup>, VU University Medical Center, Amsterdam, The Netherlands
- 16.50 De interactie van vaatnieuwvorming en expressie van adhesiemoleculen bij metastasering van rectum carcinoom (project WS 00-51)  
I.D. Nagtegaal, J.H.J.M. van Krieken, Dept of Pathology, UMC St. Radboud, Nijmegen, The Netherlands
- 17.00 Einde programma in deze zaal.  
Voor de lezing van Dr. F. Koning kunt u zich begeven naar de Brabantzaal.

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**Nederlandse Vereniging voor Hepatologie**

**Parkzaal**

**Voorzitters:** B. van Hoek en H. Moshage

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

- 13.00 Successful treatment with Pegylated Interferon in HBV non-responders to standard interferon and lamivudine  
H.J. Flink<sup>1</sup>, B.E. Hansen<sup>2</sup>, M. van Zonneveld<sup>1</sup>, S.W. Schalm<sup>1</sup>, H.L.A. Janssen<sup>1</sup>, for the HBV 99-01 study group. Dept of Gastroenterology and Hepatology<sup>1</sup> and Dept. of Epidemiology and Biostatistics<sup>2</sup>, Erasmus MC, University Medical Center Rotterdam, The Netherlands
- 13.15 Preoperative estimation of postoperative remnant liver function using hepatobiliary scintigraphy  
B.J. Verwer<sup>1</sup>, S. Dinant<sup>1</sup>, R.J. Bennink<sup>2</sup>, O.R. Busch<sup>1</sup>, D.J. Gouma<sup>1</sup>, A.K. van Vliet<sup>1</sup> and T.M. van Gulik<sup>1</sup>. Depts of Surgery<sup>1</sup> and Nuclear Medicine<sup>2</sup> Academic Medical Center Amsterdam, The Netherlands
- 13.30 Sustained virological response virtually eliminates liver-related morbidity and mortality of hepatitis C cirrhosis

B.J. Veldt<sup>1</sup>, B.E. Hansen<sup>1,2</sup>, R.J. de Knegt<sup>1</sup>, S.W. Schalm<sup>1</sup>. Depts of Gastroenterology & Hepatology<sup>1</sup>, Epidemiology & Biostatistics<sup>2</sup>, Erasmus MC, Rotterdam, The Netherlands

13.45 HBsAg seroconversion in chronic HBV patients treated with pegylated interferon alpha-2b alone or in combination with lamivudine. The role of HBV genotype  
H.J. Flink<sup>1</sup>, M. van Zonneveld<sup>1</sup>, H.G.M. Niesters<sup>2</sup>, R.A. de Man<sup>1</sup>, S.W. Schalm<sup>1</sup>, H.L.A. Janssen<sup>1</sup> for the HBV 99-01 Study Group. Depts of Gastroenterology and Hepatology<sup>1</sup> and Virology<sup>2</sup>, Erasmus MC, University Medical Center Rotterdam, The Netherlands

14.00 Benign recurrent intrahepatic cholestasis type 2 is caused by mutations in ABCB11\*  
W.L. van der Woerd<sup>1,2</sup>, S.W.C. van Mil<sup>1,2</sup>, G. van der Brugge<sup>1</sup>, E. Sturm<sup>3</sup>, P.L.M. Jansen<sup>4</sup>, L.N. Bull<sup>5</sup>, I.E.T. van den Berg<sup>1</sup>, R. Berger<sup>1</sup>, R.H.J. Houwen<sup>2</sup>, L.W.J. Klomp<sup>1</sup>. Depts of Metabolic and Endocrine diseases<sup>1</sup> and Pediatric Gastroenterology<sup>2</sup>, University Medical Center, Utrecht, The Netherlands. Dept of Pediatrics<sup>3</sup> and Gastroenterology<sup>4</sup>, University Hospital Groningen, Groningen, The Netherlands. Liver Center Laboratory and Dept of Medicine<sup>5</sup>, San Francisco General Hospital, University of California San Francisco, California, USA

14.15 Enhanced lentiviral hepatocyte transduction by elimination of Kupffer cells  
N.P. van Til, D.M. Markusic, R. van der Rijt, C. Kunne, J. Hiralall, R.P.J. Oude-Elferink and J. Seppen. AMC Liver Center, Amsterdam, The Netherlands

14.30 MURR1/COMMD1 defines a novel protein family with a possible role in hepatic copper homeostasis and NF-κB signaling  
P. de Bie<sup>1</sup>, E. Burstein<sup>2</sup>, B. van de Sluis<sup>1</sup>, K. Duran<sup>1</sup>, C. Wijmenga<sup>1</sup>, C. Duckett<sup>2</sup>, L. Klomp<sup>1</sup>. Dept of Biomedical Genetics<sup>1</sup>, University Medical Center Utrecht, The Netherlands, Dept of Pathology<sup>2</sup>, University of Michigan Medical School, Ann Arbor, USA

14.45 Carbon monoxide protects hepatocytes against oxidative stress induced apoptosis  
L. Conde de la Rosa, M. Schoemaker, T. Vrenken, M. Homan, P.L.M. Jansen and H. Moshage. Dept of Gastroenterology and Hepatology, University Hospital Groningen, Groningen, The Netherlands

15.00 Theepauze, ledenvergadering.

**Voorzitters:** H. Moshage en J. Kwekkeboom

15.30 Analysis of intrahepatic immune response identifies four stages of chronic HBV infection  
D. Sprengers, R.G. van der Molen, J.G. Kusters, S.W. Schalm, H.L.A. Janssen. Dept of Gastroenterology and Hepatology, Erasmus MC, Rotterdam, The Netherlands

15.45 Altered disposition of acetaminophen in Mrp3 knockout mice  
J. Manautou<sup>1</sup>, R. de Waart<sup>1</sup>, N. Zelcer<sup>2</sup>, C. Kunne<sup>1</sup>, P. Borst<sup>2</sup>, R. Oude Elferink<sup>1</sup>

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**Nederlandse Vereniging voor Hepatologie**

**Parkzaal**

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**SYMPOSIUM:  
The immune system of the liver**

**Chairmen:** H. Moshage en J. Kwekkeboom

16.00 Immunological functions of liver sinusoidal endothelium.  
Dr. P.A. Knolle, Bonn, Germany

16.20 discussion

- 16.30 Interactions between the structural proteins of HBV and the human immune system – a delicate balance of stimulation and inhibition.  
Dr. G. Leroux-Roels, Ghent, Belgium
- 16.50 discussion
- 17.00 Regulation of lymphocyte migration into the diseased liver.  
Prof. dr. D.H. Adams, Birmingham, UK
- 17.20 discussion
- 17.30 Congresborrel en diner

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**Presidential Selection - plenaire sessie**

**Brabantzaal**

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

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

- 20.00 Selective COX-2 inhibition impairs ileal but not colonic experimental anastomotic healing in the early postoperative period  
I.H.J.T. de Hingh, H. van Goor, B.M. de Man, R.M.L.M. Lomme, R.P. Bleichrodt, T. Hendriks.  
Dept of Surgery, University Medical Center, Nijmegen, The Netherlands
- 20.15 The value of Narrow Band Imaging for the detection of dysplasia in longstanding ulcerative colitis  
E. Dekker<sup>1</sup>, S.J. van Deventer<sup>1</sup>, J. Hardwick<sup>1</sup>, J. Offerhaus<sup>2</sup>, P. Fockens<sup>1</sup>, D.W. Hommes<sup>1</sup>.  
Depts of Gastroenterology<sup>1</sup> and Pathology<sup>2</sup>, Academic Medical Center Amsterdam, The Netherlands
- 20.30 EMR improves staging of early neoplastic lesions in Barrett's esophagus  
I.M. Kerkhof, J. Haringsma<sup>1</sup>, H. van Dekken<sup>2</sup>, P. Blok<sup>3</sup>, P.D. Siersema<sup>1</sup>, E.J. Kuipers<sup>1</sup>. Depts of Gastroenterology and Hepatology<sup>1</sup> and Pathology<sup>2</sup>, Erasmus MC-University Medical Center, Rotterdam and Dept of Pathology<sup>3</sup>, Leyenburg Hospital, Den Haag, The Netherlands
- 20.45 The role of the intestine in reverse cholesterol transport  
A.E. van der Velde<sup>1</sup>, C. Kunne<sup>1</sup>, K. van den Oever<sup>1</sup>, F. Kuipers<sup>2</sup>, A.K. Groen<sup>1</sup>. AMC Liver Center<sup>1</sup>, Academic Medical Center, Amsterdam, Dept of Pediatrics<sup>2</sup>, University Hospital Groningen, Groningen, The Netherlands
- 21.00 Uitreiking eerste exemplaren van het nieuwe leerboek "Integrated Medical and Surgical Gastroenterology" aan de voorzitters van de Nederlandse Vereniging voor Heelkunde, de Nederlandse en Vlaamse Vereniging voor Gastroenterologie en de Nederlandse Vereniging voor Gastro-Intestinale Chirurgie.
- 21.15 **Uitreiking AstraZeneca Gastrointestinale Research Prijs 2004**  
door Prof. dr. I.H.M. Borel Rinkes, jurylid.  
Erevoordracht door de prijswinnaar
- 21.35 **Altana lecture**  
verzorgd door Dr. J. Cohn, Duke University Medical Center, Durham, U.S.A.  
Titel voordracht: 'Chronic Inheritable Pancreatitis'
- 22.05 Ledenvergadering Nederlandse Vereniging voor Gastroenterologie
- 22.30 Congresborrel in de Dommelpoort aangeboden door AstraZeneca

**Vanaf hier een koptekst: vrijdag 8 oktober 2004 (instructie voor Bijto)**  
**Graag op nieuwe pagina beginnen**

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**Casuïstiek voor de Klinikus**

**Brabantzaal**

**Voorzitter:** W. Hameeteman

08.30 uur Casuïstische presentaties

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**Sectie Gastrointestinale Endoscopie**

**Brabantzaal**

**Voorzitter:** Dr. W. Hameeteman

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

- 09.00 A new design esophageal stent (Niti-S stent) for the prevention of migration  
E.M.L. Verschuur<sup>1</sup>, M.Y.V. Homs<sup>1</sup>, E.W. Steyerberg<sup>2</sup>, P.J. Wahab<sup>3</sup>, J. Haringsma<sup>1</sup>, E.J. Kuipers<sup>1</sup>, P.D. Siersema<sup>1</sup>. Depts of Gastroenterology & Hepatology<sup>1</sup> and Public Health<sup>2</sup>, Erasmus MC/University Medical Center Rotterdam, and Dept of Gastroenterology & Hepatology<sup>3</sup> Rijnstate Hospital Arnhem, The Netherlands
- 09.10 Video Autofluorescence Imaging (AFI) Followed by Narrow Band Imaging (NBI) for Detection of High Grade Dysplasia (HGD) and Early Cancer (EC) in Barrett's Esophagus (BE)  
M. Kara<sup>1</sup>, F. Peters<sup>1</sup>, P. Fockens<sup>1</sup>, F. ten Kate<sup>2</sup>, S. van Deventer<sup>1</sup>, J. Bergman<sup>1</sup>. Depts of Gastroenterology & Hepatology<sup>1</sup>, and Pathology<sup>2</sup>, Academic Medical Center, Amsterdam, The Netherlands
- 09.20 Diagnostic value of videocapsule endoscopy in carcinoid tumours  
S.A.C. van Tuyl<sup>1</sup>, M.F.J. Stolk<sup>1</sup>, R. Timmer<sup>1</sup>, E.J. Kuipers<sup>2</sup>, B.G Taal<sup>3</sup>. Depts of Gastroenterology, St. Antonius Hospital Nieuwegein<sup>1</sup>, Erasmus Medical Center Rotterdam<sup>2</sup>, Netherlands Cancer Institute/Antoni van Leeuwenhoek Hospital, Amsterdam<sup>3</sup>, The Netherlands
- 09.30 Third generation autofluorescence endoscopy of the colon  
J. Haringsma, J.W. Poley, E.J. Kuipers. Dept of Gastroenterology and Hepatology, Erasmus MC-University Medical Center, Rotterdam, The Netherlands.
- 09.40 Clinical benefit from video capsule endoscopy - one year after  
K.F. Bruin, K.M.A.J. Tytgat, P. Fockens. Dept of Gastroenterology, Academic Medical Center, Amsterdam, The Netherlands.
- 09.50 Koffiepauze
- 10.30 **Symposium 'Acute Pancreatitis'**

*Bespreken van onderdelen uit de richtlijn Acute Pancreatitis, opgesteld namens Nederlandsche Internisten Vereeniging, het Nederlands Genootschap van Maag- Darm- Leverartsen en de Nederlandse Verenigingen voor Heelkunde en Radiologie.*

**Voorzitters:** Dr. R.J.Th. Ouwendijk, maag-darm-leverarts, voorzitter werkgroep richtlijn Acute Pancreatitis en Prof. dr. H. Gooszen, chirurg



Indicatie en timing van ERCP  
Dr. A.A.M. Masclee, Afd. Maag-, Darm- en Leverziekten , LUMC, Leiden

Radiologische diagnostiek en interventie  
Dr. M.S. van Leeuwen, radioloog, Afd. Radiologie UMCU , Utrecht

Antibiotica bij acute pancreatitis: is profylaxe geïndiceerd?  
Dr. L.G. Visser, internist, afdeling Infectieziekten, LUMC, Leiden

Chirurgische behandeling : welke opties en wanneer?  
Prof. dr. H.G. Gooszen , chirurg, Afd. Heelkunde, UMCU, Utrecht

Discussie

**Presentatie van een tweetal lopende studies naar 'Acute Pancreatitis'**

- 11.30 PROPATRIA: Probiotica bij acute pancreatitis  
M.G.H. Besselink, Afd. Heelkunde, UMCU, Utrecht
- 11.45 De EARL-studie: acute pancreatitis in Noord-Holland.  
M.J. Bruno, Afd. Maag-, Darm- en Leverziekten, AMC, Amsterdam
- 12.00 Lunch in de expositiehal

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**Nederlandse Vereniging voor Gastroenterologie**

**Baroniezaal**

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**Voorzitters:** M. Sinaasappel, H.J. Verkade

8.30 **SYMPOSIUM CYSTIC FIBROSIS**

**Pancreaticobiliary function in relation to malabsorption in CF.**

J. A. Cohn, Dept. Medicine & Cell Biology, Duke University Medical Center  
Durham, U.S.A.  
'Pancreatic insufficiency'

H.J. Verkade, pediatrician, Academic Hospital Groningen  
'Bile salt secretion in CF: role in fat malabsorption and liver disease'

**CFTR function in the intestinal tract a fundamental question for CF.**

M. Sinaasappel, pediatrician, Erasmus MC Rotterdam  
'Diagnostics in atypical CF'

H.R. de Jonge, UHD Dept Biochemistry, Erasmus MC Rotterdam  
Correction of CFTR in the intestine.

10.00 Koffiepauze

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**Nederlandse Vereniging voor Gastroenterologie**

**Baroniezaal**

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**Voorzitters:** E.C. Klinkenberg en P.D. Siersema

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

- 10.30 Interobserver variation in the diagnosis of dysplasia in Barrett's Esophagus  
M. Kerkhof<sup>1</sup>, A. de Bruine<sup>2</sup>, H. van Dekken<sup>3</sup>, A. Karrenbeld<sup>4</sup>, G.A. Meijer<sup>5</sup>, A.M. Mulder<sup>6</sup>, P. Krijnen<sup>7</sup>, J.G. Kusters<sup>1</sup>, E.J. Kuipers<sup>1</sup>, P.D. Siersema<sup>1</sup>, for the CBYAR-study group. Depts of Gastroenterology<sup>1</sup>, Pathology<sup>3</sup>, and Public Health<sup>7</sup>, Erasmus MC, Rotterdam, Dept of Pathology<sup>2</sup>, Academic Hospital Maastricht, Dept of Pathology<sup>4</sup>, Academic Hospital Groningen, Dept of Pathology<sup>5</sup>, VU Medical Center, Amsterdam and Dept of Pathology<sup>6</sup>, Rijnstate Hospital, Arnhem, The Netherlands
- 10.40 Characterization of Tissue Autofluorescence (AF) In Non-dysplastic (NDBE) And Dysplastic Barrett Esophagus (BE) By Confocal Fluorescence Microscopy (CFM)  
M. Kara<sup>1</sup>, R. DaCosta<sup>2</sup>, C. Streutker<sup>3</sup>, N. Marcon<sup>4</sup>, J. Bergman<sup>1</sup>, B. Wilson<sup>2</sup>. Dept of Gastroenterology and Hepatology<sup>1</sup>, Academic Medical Center, Amsterdam, The Netherlands, Dept of Medical Biophysics<sup>2</sup>, Princes Margaret Hospital, Toronto, Canada, Dept of Pathology<sup>3</sup>, St. Michael's Hospital, Toronto, Canada, Center for Therapeutic Endoscopy<sup>4</sup>, St. Michael's Hospital, Toronto, Canada
- 10.50 Barrett's esophagus is associated with reflux of secondary bile acids into the esophagus  
R.R. Sital<sup>1</sup>, F.W.M. de Rooij<sup>1</sup>, H. Geldof<sup>2</sup>, J.L.D. Wattimena<sup>3</sup>, J.G. Kusters<sup>1</sup>, E.J. Kuipers<sup>1</sup>, P.D. Siersema<sup>1</sup>. Depts of Gastroenterology & Hepatology and Internal Medicine, Erasmus MC<sup>1</sup>/University Medical Center Rotterdam<sup>3</sup> and IJsselland Hospital<sup>2</sup>, The Netherlands

**Voorzitters:** A. Cats en S.D.J. van der Werf

- 11.00 DNA copy number changes at 8q11-24 in metastasized colorectal cancer  
T.E. Buffart<sup>1</sup>, J. Coffa<sup>1</sup>, M.A.J.A. Hermsen<sup>1</sup>, B. Carvalho<sup>1</sup>, J.R.M. van der Sijp<sup>2</sup>, B. Ylstra<sup>3</sup>, G. Pals<sup>4</sup>, J.P. Schouten<sup>5</sup>, G.A. Meijer<sup>1</sup>. Depts of Pathology<sup>1</sup>, Surgery<sup>2</sup>, Micro-array Core Facility<sup>3</sup> and Clinical Human Genetics<sup>4</sup>, VU University Medical Center, Amsterdam, MRC-Holland<sup>5</sup>, Amsterdam, The Netherlands
- 11.10 Wnt pathway activation in colorectal tumors is associated with an increase in DR4 expression  
M. Jalving<sup>1,2</sup>, J.J. Koornstra<sup>1</sup>, W. Boersma-van Ek<sup>1</sup>, N. Zwart<sup>1,2</sup>, J. Wesseling<sup>3</sup>, E.G.E. de Vries<sup>2</sup>, S. de Jong<sup>2</sup>, J.H. Kleibeuker<sup>1</sup>. Depts of Gastroenterology and Hepatology<sup>1</sup>, Medical Oncology<sup>2</sup> and Pathology<sup>3</sup>, University Hospital Groningen, The Netherlands
- 11.20 CT colonography in colorectal screening. New perspectives by repeating a recent study in replicated samples of autopsy material from an asymptomatic Dutch population  
K.W. Geul<sup>1</sup>, J.D.F. Habbema<sup>2</sup>, C.W. Ting<sup>1</sup>, J.H.P. Wilson<sup>3</sup>, F.T. Bosman<sup>4</sup>, E.J. Kuipers<sup>1</sup>. Depts of Gastroenterology and Hepatology<sup>1</sup>, Public Health<sup>2</sup>, Internal Medicine<sup>3</sup> and Pathology<sup>4</sup>, Erasmus Medical Center Rotterdam, The Netherlands. (Currently: Institut Universitaire de Pathologie, Lausanne University, Switzerland)
- 11.30 Sulindac increases epithelial cell proliferative activity in the proximal colon of HNPCC-patients  
F.E.M. Rijcken, H. Hollema, T. van der Sluis, W. Boersma-Van Ek, J.H. Kleibeuker. Depts of Gastroenterology and Hepatology and Pathology, University Hospital Groningen, The Netherlands
- 11.40 Effects of sulindac on apoptotic activity and expression of DR5 and  $\beta$ -catenin in normal colon from patients with hereditary non-polyposis colorectal cancer and familial adenomatous polyposis  
C.N.A.M. Oldenhuis<sup>1</sup>, J.J. Koornstra<sup>1</sup>, F.E.M. Rijcken<sup>1</sup>, N. Zwart<sup>2</sup>, H. Hollema<sup>2</sup>, E.G.E. de Vries<sup>3</sup>, S. de Jong<sup>3</sup>, J.J. Keller<sup>4</sup>, G.J. Offerhaus<sup>4</sup>, F.M. Giardiello<sup>5</sup>, J.H. Kleibeuker<sup>1</sup>. Depts of Gastroenterology<sup>1</sup>, Pathology<sup>2</sup> and Oncology<sup>3</sup>, University Hospital Groningen, The Netherlands, Dept of Pathology<sup>4</sup>, Academic Medical Center, Amsterdam, The Netherlands, Dept of Medicine<sup>5</sup>, Johns Hopkins University, Baltimore USA

- 11.50 Abnormal neo-rectal contractility or 'irritable neo-rectum' after preoperative radiotherapy and rectal resection for rectal carcinoma  
R. Bakx<sup>1</sup>, J.F.M. Slors<sup>1</sup>, W.A. Bemelman<sup>1</sup>, J.J.B. van Lanschot<sup>1</sup>, G.E.E. Boeckxstaens<sup>2</sup>. Dept of Surgery<sup>1</sup>, Academic Medical Center, Amsterdam, Dept of Gastro-enterology<sup>2</sup>, Academic Medical Center, Amsterdam, The Netherlands
- 12.00 Mortality in Families with Hereditary Non Polyposis Colorectal Cancer  
A.E. de Jong<sup>1,2</sup>, S.Y. de Boer<sup>3</sup>, A. Cats<sup>4</sup>, G. Griffioen<sup>2</sup>, J.H. Kleibeuker<sup>5</sup>, F.M. Nagengast<sup>6</sup>, G.F.Nelis<sup>7</sup>, H.F.A.Vasen<sup>1,2</sup>. The Netherlands Foundation for the Detection of Hereditary Tumours<sup>1</sup>, Depts of Gastroenterology, Leiden University Medical Center<sup>2</sup>, Rijnstate Hospital Arnhem<sup>3</sup>, The Netherlands Cancer Institute<sup>4</sup>, Amsterdam, University Hospital Groningen<sup>5</sup>, University Medical Center Nijmegen<sup>6</sup>, Sophia Ziekenhuis Zwolle<sup>7</sup>, The Netherlands
- 12.10 Lunch in de expositiehal

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**Nederlandse Vereniging voor Hepatologie**

**Parkzaal**

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**Voorzitters:** K.J. van Erpecum en R.P.J. Oude Elferink

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

- 08.30 Human liver lymph nodes contain mature dendritic cells with high expression of Programmed Death Ligands  
E. Sen<sup>1</sup>, P.P.C. Boor<sup>1</sup>, B.M. Bosma<sup>1</sup>, R.G. van der Molen<sup>1</sup>, H.W. Tilanus<sup>1</sup>, H.A. Drexhage<sup>1</sup>, E.C. de Jong<sup>2</sup>, J.N.M. IJzermans<sup>1</sup>, H.J. Metselaar<sup>1</sup>, J. Kwekkeboom<sup>1</sup>. Dept of Gastroenterology and Hepatology<sup>1</sup>, ErasmusMC-University Medical Center, Rotterdam and Dept of Cell Biology and Histology<sup>2</sup>, Academic Medical Center, Amsterdam, The Netherlands
- 08.45 Cholestasis associated with acute liver rejection is characterised by selective down-regulation of hepatic bile salt transporters  
H. Blokzijl<sup>1</sup>, S. Vander Borgh<sup>2</sup>, L.I.H. Bok<sup>1</sup>, L. Libbrecht<sup>2</sup>, H. Moshage<sup>1</sup>, K.N. Faber<sup>1</sup>, T.A.D. Roskams<sup>2</sup>, P.L.M. Jansen<sup>1</sup>. Dept of Gastroenterology and Hepatology<sup>1</sup>, University Hospital Groningen, Groningen, The Netherlands, Lab of Morphology and Molecular Pathology<sup>2</sup>, University of Leuven, Leuven, Belgium
- 09.00 Mrp-type transporters protect activated hepatic stellate cells against cell death  
R. Hannivoort, M. Buist-Homan, K.N. Faber, H. Moshage
- 09.15 Preferential gene transfer to hepatocytes with baculovirus GP64 pseudotyped lentiviral vectors  
D. Markusic, R. Oude Elferink, J. Seppen. Academic Medical Center, Liver Center, Amsterdam, The Netherlands
- 09.30 Reduced incidence of acute rejection in HBV-infected liver transplant recipients may be due to suppression of dendritic cell function by polyclonal anti-HBs immunoglobulin  
J. Kwekkeboom<sup>1</sup>, W.M.W. Tra<sup>1</sup>, W. Hop<sup>2</sup>, J.G. Kusters<sup>1</sup>, R.A. de Man<sup>1</sup>, H.J. Metselaar<sup>1</sup>, Depts of Gastroenterology and Hepatology<sup>1</sup> and Biostatistics and Epidemiology<sup>2</sup>, Erasmus MC-University Medical Center, Rotterdam, The Netherlands
- 09.45 Role of hydrophobic bile salts, phospholipids and cholesterol crystals in a rat model of biliary pancreatitis  
L.P. van Minnen<sup>1</sup>, N.G. Venneman<sup>1</sup>, J.E. van Dijk<sup>2</sup>, H.G. Gooszen<sup>1</sup>, L.M.A. Akkermans<sup>1</sup>, K.J. van Erpecum<sup>1</sup>. Gastrointestinal Research Unit Depts of Gastroenterology and Surgery<sup>1</sup> University Medical Center, Utrecht and Dept of Veterinary Pathology, Faculty of Veterinary Medicine<sup>2</sup>, Utrecht University, Utrecht, The Netherlands.

10.00 Koffiepauze

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**Vrije voordrachten Sectie Experimentele Gastroenterologie**

**Parkzaal**

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**Voorzitter:** G. Dijkstra en H.W. Verspaget

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

10.30 The interferon-gamma gene in coeliac disease shows expression correlated with tissue transformation but no evidence for genetic susceptibility \*  
M.C. Wapenaar<sup>1</sup>, M.J. van Belzen<sup>1,2</sup>, R.H.J. Houwen<sup>2</sup>, J.W.R. Meijer<sup>3</sup>, C.J.J. Mulder<sup>4</sup>, and C. Wijmenga<sup>1</sup>. Depts of Biomedical Genetics<sup>1</sup> and Pediatric Gastroenterology<sup>2</sup>, University Medical Center Utrecht, Utrecht, Dept of Pathology<sup>3</sup>, Rijnstate Hospital, Arnhem and Dept of Gastroenterology<sup>4</sup>, VU Medical Center, Amsterdam, The Netherlands

10.40 The role of gene polymorphisms in the bacterial agonist recognizing TLR4-CD14 system in the susceptibility to and severity of CD and UC in Dutch Caucasian IBD patients  
S. Ouburg<sup>1,2</sup>, R. Mallant-Hent<sup>2</sup>, A.A. van Bodegraven<sup>2</sup>, C.J.J. Mulder<sup>2</sup>, J.B.A. Crusius<sup>1</sup>, R. Linskens<sup>3</sup>, A.S. Peña<sup>1,2</sup>, S.A. Morr  <sup>1</sup>. Lab of Immunogenetics<sup>1</sup> and Dept of Gastroenterology<sup>2</sup>, VU University Medical Centre, Amsterdam, Dept of Gastroenterology<sup>3</sup>, St Anna Ziekenhuis, Geldrop, The Netherlands

10.50 Clinical and functional significance of TNF- $\alpha$ , MMP-2 and MMP-9 gene promoter single nucleotide polymorphisms in inflammatory bowel disease  
M.J.W. Meijer, M.A.C. Mieremet-Ooms, W. van Duijn, R.A. van Hogezaand, C.B.H.W. Lamers, H.W. Verspaget. Leiden University Medical Center, Dept of Gastroenterology-Hepatology, Leiden, The Netherlands

11.00 **INTERNATIONAL TEACHING SESSION**

Stem cells in hepato-gastrointestinal disorders: molecular pathways and therapeutic perspectives  
*Prof. M. Alison, Dept of Histopathology, Hammersmith Hospital, London, U.K.*

12.00 Lunch

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**Sectie Neurogastroenterologie en motiliteit**

**Brabantzaal**

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**Voorzitters:** M. Samsom / G. Boeckstaens

13.30 **SYMPOSIUM:**  
**“Diagnosis and management of constipation”**

Pathophysiology of colorectal function

Clinical analysis of constipation : which diagnostic test and when?

Treatment of constipation : diet, drugs or biofeedback?

**Voorzitters:** D. de Jong en M. Sinaasappel

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

- 13.30 Prevalence of Helicobacter pylori antibiotic resistance in The Netherlands: Trends in time  
M.J.R. Janssen<sup>1</sup>, L. Hendrikse<sup>1</sup>, S.Y. de Boer<sup>2</sup>, W.A. de Boer<sup>3</sup>, J.B.M.J. Jansen<sup>1</sup>. Dept of Gastroenterology & Hepatology<sup>1</sup>, UMC St. Radboud, Nijmegen, Dept of Gastroenterology<sup>2</sup>, Slingeland Hospital, Doetinchem and Dept of Internal Medicine<sup>3</sup>, Bernhoven Hospital, Oss, The Netherlands
- 13.40 A specific COX-1 polymorphism (A842G/C50T) protects against peptic ulcer disease  
R.J.F. Laheij, E. de Kleine, M.I.A. Koetsier, R.H.M. te Morsche, M.G.H. van Oijen, J.P.H. Drenth, J.B.M.J. Jansen. Dept of Gastroenterology and Hepatology, University Medical Center St Radboud, Nijmegen, The Netherlands
- 13.50 Octreotide for Therapy-resistant Functional Dyspepsia  
E.A. van Hoboken, C. Penning, A.A.M. Masclee. Leiden University Medical Center, Leiden, The Netherlands
- 14.00 The effect of a multispecies probiotic on the intestinal flora and bowel habits in healthy volunteers treated with amoxicillin.  
C. Koning<sup>1</sup>, D. Jonkers<sup>1,2</sup>, E. Stobberingh<sup>2</sup>, R. Stockbrugger<sup>1</sup>. Depts of Gastroenterology<sup>1</sup> and Medical Microbiology<sup>2</sup>, University Hospital Maastricht, Maastricht, The Netherlands
- 14.10 The effect of the probiotic L. plantarum 299v on the faecal and mucosal bacterial flora of patients with inactive ulcerative colitis  
D. Goossens<sup>1</sup>, D. Jonkers<sup>1</sup>, E. Stobberingh<sup>2</sup>, R. Stockbrugger<sup>1</sup>. Dept of Gastroenterology<sup>1</sup> and Medical Microbiology<sup>2</sup>, University Hospital Maastricht, Netherlands
- 14.20 Liver histology in inflammatory bowel disease  
K.H.N. de Boer<sup>1</sup>, H. Tuynman<sup>2</sup>, C.M.J. van Nieuwkerk<sup>1</sup>, A.A. van Bodegraven<sup>1</sup> en J. Westerga<sup>2</sup>. VU medical center<sup>1</sup>, Amsterdam, Slotervaart Hospital<sup>2</sup>, Amsterdam, The Netherlands
- 14.30 Inflammatory bowel disease after liver transplantation: a role for cytomegalovirus infection  
R.C. Verdonk<sup>1</sup>, E.B. Haagsma<sup>1</sup>, A.P. van den Berg<sup>1</sup>, A. Karrenbeld<sup>2</sup>, M.J.H. Slooff<sup>3</sup>, J.H. Kleibeuker<sup>1</sup>, G. Dijkstra<sup>1</sup>. Depts of Gastroenterology and Hepatology<sup>1</sup>, Pathology<sup>2</sup> and Surgery<sup>3</sup>, University Hospital Groningen, The Netherlands
- 14.40 Clinical and endosonographic effect of ciprofloxacin on the treatment of perianal fistulas in Crohn's disease with infliximab: a double-blind placebo-controlled study  
R.L. West<sup>1</sup>, C.J. van der Woude<sup>1</sup>, B. E. Hansen<sup>1</sup>, R.J.F. Felt-Bersma<sup>1</sup>, A.J.P. van Tilburg<sup>2</sup>, J.A.G. Drapers<sup>3</sup>, E.J. Kuipers<sup>1</sup>. Dept of Gastroenterology and Hepatology<sup>1</sup>, Erasmus MC University Medical Center, Rotterdam, Dept of Internal Medicine<sup>2</sup>, St. Franciscus Gasthuis, Rotterdam and Dept of Internal Medicine<sup>3</sup>, Ziekenhuis Walcheren, Vlissingen, The Netherlands
- 14.50 Pharmacokinetic effect of discontinuation of mesalamine on 6-mercaptopurine metabolite levels in IBD patients  
L.P.L. Gilissen<sup>1</sup>, L.J.J. Derijks<sup>2</sup>, P.M. Hooymans<sup>3</sup>, L.P. Bos<sup>4</sup> and L.G.J.B. Engels<sup>4</sup>. Dept of Internal Medicine and Gastroenterology<sup>1</sup>, Academic Hospital Maastricht, Dept. of Clinical

Pharmacy<sup>2</sup>, Maxima Medical Centre, Veldhoven and Depts. of Clinical Pharmacy<sup>3</sup> and Gastroenterology<sup>4</sup>, Maasland Hospital, Sittard, The Netherlands

15.00 uur Theepauze

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**Sectie Experimentele Gastroenterologie**

**Parkzaal**

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**Voorzitter:** A.H.M. van Vliet en M.A.C. Meijssen

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

- 13.30 Dendritic Cells in Immunotherapy for Esophageal Adenocarcinoma: The Near Future  
F. Milano<sup>1</sup>, J.J.G.H.M. Bergman<sup>2</sup>, A.M. Lukuc<sup>1</sup>, J. van Baal<sup>1</sup>, S.J.H. van Deventer<sup>2</sup>, M. Peppelenbosch<sup>1</sup>, K.K.Krishnadath<sup>2</sup>. Dept of experimental medicine<sup>1</sup>, Dept of Gastroenterology and Hepatology<sup>2</sup>, Academic Medical Center, Amsterdam, The Netherlands
- 13.40 Fiber modified adenovirus vectors that target to EphrinA2 Receptor and Vascular Endothelial Growth Factor Receptor II reveal enhanced gene transfer to human pancreatic tumor cells  
M.A. van Geer<sup>1</sup>, P.J. Bosma<sup>1</sup>, K.F.D. Kuhlmann<sup>2</sup>, R.P.J. Oude Elferink<sup>1</sup>, D.J. Gouma<sup>2</sup>, J.G. Wesseling<sup>1</sup>. Experimental Hepatology, AMC Liver Center<sup>1</sup> and Dept of Surgery<sup>2</sup>, Academic Medical Center, Amsterdam, The Netherlands
- 13.50 Glutathione S-transferases and UDP-glucuronosyltransferases in duodenal mucosa of patients with Familial Adenomatous Polyposis and controls  
M. Berkhout, H.M.J. Roelofs, P. Friederich, F.M. Nagengast, W.H.M. Peters. University Medical Center Nijmegen, Nijmegen, The Netherlands
- 14.00 Specific kinase profiling in colon cancer predicts stage of disease and identifies potential molecular targets for therapy.  
J.B. Tuynman<sup>1</sup>, H.H. Versteeg<sup>1</sup>, H.H. Thygesen<sup>2</sup>, A.H. Zwinderman<sup>2</sup>, J. Joore<sup>1</sup>, G.J. Offerhaus<sup>3</sup>, M.P. Peppelenbosch<sup>1</sup>, D.J. Richel<sup>1</sup>. Dept of Medical Statistics<sup>2</sup>, Pathology<sup>3</sup> and Medical Oncology<sup>1</sup>, Academic Medical Center, Amsterdam, The Netherlands.
- 14.10 Kinome Analysis Reveals Lck and Fyn As Novel Targets Of Glucocorticoid Action In Activated T cells  
M. Löwenberg<sup>1,2</sup>, J. Tuynman<sup>2</sup>, J. Bilderbeek<sup>2</sup>, S.J.H. van Deventer<sup>1</sup>, M.P. Peppelenbosch<sup>2</sup>, D.W. Hommes<sup>1</sup>. Depts of Gastroenterology and Hepatology<sup>1</sup>, and Experimental Internal Medicine<sup>2</sup>, Academic Medical Center, Amsterdam, The Netherlands
- 14.20 Internalization of *Campylobacter jejuni* into Caco-2 cells is lipo-oligosaccharide class A/B dependent  
R.P.L. Louwen<sup>1</sup>, M.P. Bergman<sup>1</sup>, A.P. Heikema<sup>1</sup>, M. al-Patty<sup>1</sup>, H.P. Endtz<sup>1</sup>, E.E.S Nieuwenhuis<sup>2</sup> and A. van Belkum<sup>1</sup>
- 14.30 Bacterial DNA exacerbates the inflammatory response and gut barrier failure caused by systemic hypotension.  
M.D.P. Luyer<sup>1</sup>, W.A. Buurman<sup>1</sup>, M. Hadfoune<sup>1</sup>, C. van 't Veer<sup>3</sup>, J.A. Jacobs<sup>2</sup>, C.H.C. Dejong<sup>1</sup>, J.W.M. Greve<sup>1</sup>. Depts of Surgery<sup>1</sup> and Medical Microbiology<sup>2</sup>, University of Maastricht and University Hospital Maastricht, Lab for Experimental Internal Medicine<sup>3</sup>, Academic Medical Center Amsterdam, University of Amsterdam, The Netherlands
- 14.40 Regulation of the murine Muc2 mucin gene by GATA factors in intestinal cells \*  
M. van der Sluis<sup>1</sup>, M.H.M. Melis<sup>1</sup>, N. Jonckheere<sup>2</sup>, M-P. Ducourouble<sup>2</sup>, H.A. Büller<sup>1</sup>, I.B. Renes<sup>1</sup>, A.W.C. Einerhand<sup>1</sup> and I. van Seuningen<sup>2</sup>. Pediatric Gastroenterology and Nutrition, Dept of Paediatrics<sup>1</sup>, Erasmus MC and Sophia Children Hospital, Rotterdam, The Netherlands, Unité INSERM No560<sup>2</sup>, Place de Verdun, 59045 Lille Cedex, France

14.50 The NikR Protein Mediates Nickel-Responsive Induction of Helicobacter pylori Urease via Binding to the ureA Promoter  
F.D. Ernst, J.G. Kusters, R. Sarwari, A. Heijens, J. Stoof, C. Belzer, E.J. Kuipers, A.H.M. van Vliet. Dept of Gastroenterology and Hepatology, Erasmus MC-University Medical Center Rotterdam, The Netherlands

15.00 Theepauze/einde

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**Vereniging Maag Darm Lever Verpleegkundigen**

**Auditorium**

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09.30 Ontvangst en koffie

10.00 Welkomstwoord en inleiding door de voorzitter.  
Dhr. H. Welling, voorzitter VMDLV.

10.15 Anatomie en fysiologie van de pancreas  
acute en chronische pancreatitis; diagnose en behandeling  
Prof. dr. J.B.M.J. Jansen, maag-darm-leverarts, UMC St. Radboud Nijmegen

11.00 Koffiepauze

11.15 Chirurgische behandelmethoden:  
- Splanchnicusdenervatie  
- Whipple of Beger  
- Transplantatie  
Prof. dr. H.G. Gooszen, chirurg, UMC Utrecht

12.00 Lunchbuffet in de expositiehal

13.00 Voeding bij pancreatitis  
Mevr. Nieboer, hoofd dietetiek MMC

13.20 Psychosociale aspecten bij pancreatitis,  
verslavingsaspecten en manipulatie.

14.00 Het belang van een patiëntenvereniging  
K. Hof, voorzitter Alveeskliervereniging

14.20 Het verhaal van een patiënt.  
Ervaringsdeskundige

14.40 Afsluiting  
H. Welling, voorzitter VMDLV

15.00 Theepauze/einde

- 10.30            Inschrijving, ontvangst, koffie
- 11.00            Instructie en planning op de endoscopieafdeling  
Mevr. M. van Voorst, endoscopieverpleegkundige, Den Haag
- 11.20            Rapport 2004 "Desinfectie flexibele endoscopieunit"  
Mevr. Bilkert, Inspecteur Gezondheidszorg
- 11.40            Ontwikkeling nieuwe endoscopieunit  
Mevr. E. Ploeger en mevr. A. Thijssen, endoscopieverpleegkundigen, Amsterdam
- 12.00            Lunchbuffet in de expositiehal
- 13.30            Afhandeling calamiteit scopendesinfector  
Mevr. B. Meuser, endoscopieverpleegkundige, Alkmaar
- 13.50            Ervaring van de enteroscoop  
Drs. D. Heine, maag-darm-leverarts in opleiding, VU medisch centrum, Amsterdam
- 14.10            Dikkedarm kanker  
Dr. G.A. Meijer, patholoog-anatoom, VU medisch centrum, Amsterdam
- 14.30            Ledenvergadering SEVA
- 15.00            Koffie/thee, einde programma



## **Caspase-6 is involved in bile acid induced apoptosis**

T.E. Vrenken, L. Conde de la Rosa, M. Buist-Homan and H. Moshage

**Background:** In cholestatic liver diseases, hepatocytes are exposed to increased levels of bile acids. Some bile acids like glycochenodeoxycholic acid (GCDCA) induce apoptosis of hepatocytes, whereas others, like tauroursodeoxycholic acid (TUDCA) protect against bile acid induced apoptosis. GCDCA-induced apoptosis involves activation of the executioner caspase-3. The role of the executioner caspase-6 in bile acid induced apoptosis has not been investigated yet. **Aim:** To examine the role of caspase-6 in bile acid induced apoptosis. **Methods:** Primary rat hepatocytes were exposed to GCDCA (50  $\mu$ M), TUDCA (50 $\mu$ M) or both. Caspase-6 and caspase-3 activation was measured using an activity assay and Western blot analysis. The caspase-3 inhibitor Z-DEVD-FMK was used at 0.1  $\mu$ M and the caspase-6 inhibitor Z-VEID-FMK and caspase-9 inhibitor Z-LEHD-FMK were used at 0.2  $\mu$ M. **Results:** GCDCA induces caspase-6 activation, starting 45 minutes after bile acid exposure, peaking between 150 minutes and 6 hrs and returning to control level after 9 hrs. Caspase-3 activity followed a similar pattern. TUDCA does not activate caspase-6 and reduces GCDCA-induced caspase-6 activation by 50 %. Both GCDCA-induced caspase-6 and caspase-3 activation are completely blocked by caspase-9 inhibitor, indicating the importance of the mitochondrial pathway in bile acid induced apoptosis. Both the caspase-6 inhibitor and the caspase-3 inhibitor blocked both caspase-3 and caspase-6 activity. **Conclusion:** Caspase-6 is involved in GCDCA-induced apoptosis. Caspase-6 activation, like caspase-3 activation is caspase-9 dependent, indicating that the executioner caspases-3 and -6 act downstream from mitochondria. Caspase-3 and caspase-6 activate each other amplifying total executioner caspase activity leading to bile acid induced apoptosis. Therefore, caspase-6, in addition to caspase-3, is a target for intervention to prevent excessive hepatocyte apoptosis.

## **Early timing of vena porta embolization prior to partial hepatectomy increases activation of TNF- $\alpha$ /IL-6 pathway and leads to improved remnant liver regeneration**

R. Veteläinen, S. Dinant, A.K. van Vliet, T.M. van Gulik. Dept of Surgery (Surgical laboratory), Academic Medical Center, Amsterdam, The Netherlands

Vena porta embolization (VPE) is used to enable larger resections in case of insufficient remnant liver volume. Resection is performed 4-8 weeks following VPE while regenerative peak in hepatocytes has passed. VPE and resection are strong stimuli for regeneration and it is unknown if a closer timing leads to increased post-resection liver regeneration. The aim of this study was to evaluate the effect of resection as an extra stimulus to proliferation of hepatocytes in remnant liver regeneration. Wistar rats were divided into 5 groups (n= 4-6). In group IA, 70% portal vein ligation (VPL) was performed 2 days prior to 70% resection. In group IB, sham laparotomy was performed 2 days prior to resection. In group IIA and B, a VPL or sham laparotomy respectively was performed 14 days prior to 70% resection. Group III had only VPL. Rats were terminated at 2 and 14 days and blood was sampled after 6, 24, 48h and 14 days after resection. Parameters for liver proliferation and regeneration, hepatocellular damage and synthetic function and proinflammatory cytokine response (TNF- $\alpha$ , IL-6 and IL-1b) were assessed. Regeneration ratio at 14 days postresection was higher in group IA as compared to IB, IIA and IIB (p< 0.05). ALT and AST were higher in group IA, IB and IIB at 6h post-resection and in groups III and IIB 24 h post-resection (p< 0.05). There was no differences in bile production between groups. Prothrombin time was significantly prolonged and albumin production was decreased 6 h and 24 h post-resection in group IB and IIB compared to IA and IIA and remained prolonged till day 14 in group IIB (p< 0.05). At 6h post-resection in group IA TNF- $\alpha$ , IL-6 and IL-1b plasma levels were higher compared to other groups (p< 0.05). Vena porta ligation combined with early liver resection increases activation of TNF- $\alpha$  /IL-6 pathway and results in higher liver regeneration ratio than when combined with delayed resection. Vena porta ligation preserves synthetic function after resection.

## Large numbers of immature dendritic cells detach from the human donor liver pre-transplantation

B.M. Bosma<sup>1</sup>, H.J. Metselaar<sup>1</sup>, S. Manchan<sup>1</sup>, P.P.C. Boor<sup>1</sup>, J.G. Kusters<sup>1</sup>, G. Kazemier<sup>2</sup>, H.W. Tilanus<sup>2</sup>, E.J. Kuipers<sup>1</sup>, J. Kwekkeboom<sup>1</sup>. Dept of Gastroenterology and Hepatology<sup>1</sup>, and Surgery<sup>2</sup>, ErasmusMC-University Medical Center, Rotterdam, The Netherlands

It is a paradigm that after organ transplantation mature donor myeloid dendritic cells (MDC) are responsible for the initiation of rejection by stimulation of recipient T-cell responses against the graft. We determined whether MDC are present in and can detach from human donor livers pre-transplantation, and established the maturation-status of these MDC. MDC were immunohistochemically detected in donor liver cryo-sections (n=12) with CD1c antibody. Mononuclear cells (MNC) were isolated from donor liver tissue (n=5) and from donor liver perfusates (n=10). Perfusates were collected at the end of the pre-transplantation cold storage period by vascular perfusion of the graft with 200 ml albumin-solution. As controls, MNC were isolated from donor hepatic lymph nodes (LN) (n=10) and from blood of healthy volunteers (n=5) and multi-organ donors (n=3). MNC were immunophenotyped by flow cytometry. CD1c<sup>+</sup> MDC were mainly present in the portal fields of the donor livers. Donor liver MNC contained MDC (median 1.3%; range 0.3-2.2%) which were immature, having a low expression of CD80 (6.5±5.5%). The perfusates contained 64±38 x10<sup>6</sup> MNC. Their CD4/CD8 ratio differed significantly from blood (0.7±0.4 versus 3.6±1.9; p=0.002), but was comparable to liver MNC (0.6±0.1), indicating that cells in perfusates are liver-derived. Perfusates contained considerable numbers of CD1c<sup>+</sup> MDC (median 1.8%; range 0.6-6.6%) which proved to be immature: The percentages of MDC in perfusates and blood expressing CD80 were significantly lower than in LN (8.2±7.1% and 2.0±1.0% respectively, versus 35±22%; p< 0.002). Similarly MDC in perfusates and blood had a lower DC-LAMP expression than LN MDC (5.4±4.7% and 0.2±0.2% respectively, versus 64±24%; p= 0.008). Conclusions: Considerable numbers of immature MDC are present in and detach from human donor livers pre-transplantation. We postulate that these donor MDC migrate into the recipient and induce anti-donor T-cell reactivity.

## **Identification of new PPAR $\alpha$ target genes support its anti-inflammatory function in liver**

R. Stienstra, E. Lichtenauer-Kaligis, M. Muller. Nutrition, Metabolism and Genomics Dept of Human Nutrition, Wageningen University, The Netherlands

The Peroxisome Proliferator-Activated Receptors (PPARs) belong to the family of Nuclear Receptors and play important roles in numerous cellular processes including lipid metabolism, gluconeogenesis and inflammation. Ligand-activated PPARs bind to specific sequences in promoter regions which results in stimulation of expression of target genes. Besides activation of genes, PPARs are also able to suppress gene expression by mechanisms less well defined. The clearly emerged anti-inflammatory role of PPAR $\alpha$ , the isoform predominantly expressed in the liver, has mainly been attributed to its capacity to suppress pro-inflammatory genes. The objective of this study was to identify PPAR $\alpha$ -controlled inflammatory genes in liver as well as investigate mechanisms by which PPAR $\alpha$  mediates its anti-inflammatory effect. Hepatic gene expression levels of wildtype and PPAR $\alpha$  knockout mice treated with a synthetic ligand were analyzed using Affymetrix microarrays. Various genes linked to inflammation and the liver-specific Acute Phase Response were PPAR $\alpha$ -dependently decreased. These include Interleukin-18, Interleukin-1 Receptor Accessory Protein and the Leukemia Inhibitor Factor-Receptor. By contrast, the Interleukin-1 receptor antagonist (IL-1ra) was strongly induced after activation of PPAR $\alpha$  in the liver. Since this protein is able to inhibit the IL-1 signaling pathway it is an important anti-inflammatory agent. In addition to up regulation by pro-inflammatory compounds, promoter-reporter analysis demonstrates that IL-1ra is a direct PPAR $\alpha$  target. These results were confirmed in a human liver cell line. In conclusion, using microarray techniques, new possible PPAR $\alpha$  target genes related to inflammation were identified. Besides suppressing pro-inflammatory genes, PPAR $\alpha$  is also able to activate genes with a strong anti-inflammatory activity.

## **Low retinol levels potentiate bile acid-induced expression of the bile salt export pump in vitro and in vivo**

J.R.M. Plass, M.O. Hoeke, M. Geuken, J. Heegsma, D. van Rijsbergen, J.F.W. Baller, F. Kuipers, P.L.M. Jansen and K.N. Faber. Center for Liver, Digestive and Metabolic Diseases, Groningen, The Netherlands

**Background & aims:** The farnesoid X receptor (FXR) is the mammalian bile salt sensor that regulates transcription of key genes involved in bile salt homeostasis, including the Bile Salt Export Pump (BSEP) and Small Heterodimer Partner (SHP). It is crucial for preventing hepatocellular accumulation of potentially toxic bile salts. FXR functions as a heterodimer with the retinoid X receptor  $\alpha$  (RXR $\alpha$ ). FXR is activated by bile acids (e.g. chenodeoxycholic acid (CDCA)) and RXR $\alpha$  by retinoids (e.g. 9-cis retinoic acid (9cRA)). Previously, we showed that human BSEP is positively regulated by CDCA-activated FXR (Plass et al, 2002). Here, we evaluated the role of 9cRA in the expression of BSEP/Bsep and SHP/Shp in vitro and in vivo.**Methods:** Human BSEP and SHP expression was quantified by real time RT-PCR in HepG2-rNtcp cells with or without transfection of hFXR- and hRXR $\alpha$ -expression plasmids and cultured in the presence or absence of CDCA and/or 9cRA. BSEP promoter activity was measured by luciferase reporter assays and FXR/RXR $\alpha$  DNA binding by electrophoresis mobility shift assays. Vitamin A-depleted C57BL/6J mice were used to evaluate the effect on cholic acid-induced Bsep and Shp expression in vivo.**Results:** In vitro, 9cRA strongly antagonized the CDCA-dependent BSEP gene transcription, by inhibiting binding of the FXR/RXR heterodimer to the BSEP FXR response element. In contrast, 9cRA agonized SHP expression. In vivo, vitamin A depletion enhanced cholic acid-induced expression of Bsep mRNA and protein, while reducing Shp expression.**Conclusions:** 9cRA either agonizes (SHP) or antagonizes (BSEP) bile acid-activated transcription of FXR/RXR-target genes. Vitamin A is therefore an important determinant in regulation of bile acid transport and synthesis. In patients with obstructive cholestasis, vitamin A derivatives may be therapeutically useful to decrease BSEP expression, thereby reducing the hepatobiliary bile acid flux and pressure in the biliary tree.

## Regulatory T cells contribute to immunologic hyporesponsiveness in chronic HBV patients

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Chronic hepatitis B virus (HBV) infection is characterized by a weak immune response to HBV. Since, CD4<sup>+</sup> CD25<sup>+</sup> regulatory T cells (Treg) are capable of suppressing the function of dendritic cells, T cells and NK cells, we investigated whether Treg contribute to this impaired immune response. The percentage of Treg, in peripheral blood mononuclear cells (PBMC) from 50 patients, 23 healthy controls and 9 individuals with a resolved HBV infection, was determined using flowcytometry with specific antibodies (CD4, CD25, CD45RO and CTLA-4). The FoxP3 expression, which is a specific transcription factor expressed by Treg, was determined in PBMC by real time RT-PCR. Treg were depleted out of PBMC from HBV-infected patients using MACS beads. PBMC, Treg depleted PBMC and Treg depleted PBMC reconstituted with 10%, 20% or 30% Treg were stimulated with HBV core antigen and after 6 days their proliferation and IFN- $\chi$  production was determined. In chronic HBV infected patients, a higher percentage of Treg was found within the CD4<sup>+</sup> population in peripheral blood compared to healthy controls (4.1% vs. 2.6%, p= 0.002) and compared to individuals with a resolved HBV infection (4.1% vs. 1.5%, P<0.001). In a subgroup of 25 chronic HBV patients FoxP3 expression, corrected for the percentage of CD4<sup>+</sup> cells present in the sample, was increased as compared to 11 healthy controls (203 copies/ ng RNA vs. 96 copies / ng RNA, p= 0.001). Depletion of Treg from PBMC of HBV patients resulted in enhanced proliferation (n=12, p= 0.034) after stimulation with HBV core antigen, while reconstitution of Treg depleted PBMC with 10% 20% and 30% of Treg resulted in a dose dependent inhibition of the proliferation and IFN- $\chi$  production (n=6). In conclusion, patients with a chronic HBV infection have an increased percentage of Treg in peripheral blood. These Treg have a HBV-specific suppressive effect. The presence of Treg in chronic HBV patients may explain the inadequate immune response against the virus.

## Neoadjuvant chemoradiotherapy with consecutive surgery in patients with resectable esophageal cancer

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The outcome for patients with esophageal cancer undergoing surgical resection with curative intention is poor. We aimed to assess the feasibility and efficacy of a new treatment strategy, neoadjuvant chemoradiotherapy followed by surgery in patients with stage II-III esophageal cancer.

In the period from jan 2002 – april 2004, 35 patients with a potential resectable stage II-III esophageal cancer received chemotherapy with paclitaxel 175 mg/m<sup>2</sup> iv and carboplatin AUC 5 iv on day 1 and 22, 5-FU 200 mg/m<sup>2</sup> ci on day 1 to 42 in combination with radiotherapy 45 Gy in 25 fractions starting on day 1. Surgery followed 6-8 weeks after completion of neoadjuvant treatment.

35 patients have completed neoadjuvant therapy. Patient characteristics: M/F:31/4, median age 63 yrs (45-74), median WHO 1 (0-2), adenoca (n=30), squamous cell ca (n=5).

Toxicity: no treatment related deaths due to chemoradiotherapy, uncomplicated grade 3 leucopenia in 16 pts (46%). All patients experienced esophagitis, usually mild ( $\leq$  gr 2), however 4 pts needed nasogastric enteral feeding during therapy.

2 patients showed metastatic disease at surgery, hence 33 pts underwent surgery with a curative intention.

16 of 33 operated patients (48%) had a pathologic complete response (pCR). There were 4 post-operative deaths (12%), due to major anastomotic complications of the gastric tube (n=3) and a progressive chylothorax (n=1). Post-operative complications: anastomotic leakage (major n=5, minor n=8), pulmonary (n=12) and cardiac dysrhythmias (n=2).

As follow-up is short no data can be given of total- and disease free survival.

Conclusions: This novel combined-modality neoadjuvant approach for treatment of patients with stage II-III esophageal cancer is feasible and preliminary assessment of efficacy is encouraging, with 48% of the patients having a pCR. Follow-up data have to be awaited to obtain data on survival. Accrual will stop soon, since we will collaborate in the recently started national randomised study.

## Impact of hospital volume on staging procedures for esophageal cancer

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An inverse correlation between mortality and hospital volume for esophageal resection has been reported. We analyzed whether a relationship was also present between the number of patients undergoing staging procedures and the detection rate of metastases from esophageal cancer. In the period 1994-2003, 486 patients underwent staging procedures for esophageal cancer in one high-volume center ( $\geq 20$  patients/yr) as well as in one of 63 low-volume ( $< 20$  patients/yr) centers. The results of the staging procedures were compared and correlated with histologic confirmation as gold standard. The repeated investigations were chest X-ray (n=270), computed tomography (CT) (n=194), and ultrasound (US) neck (n=153) and abdomen (n=167). Comparison of high versus low-volume centers showed the detection of metastases by chest X-ray in 8/270 (3%) vs. 1/270 (0.5%) patients, corresponding with sensitivities of 64% and 9% resp. (p=0.031). CT detected metastases in 53/194 (27%) vs. 28/194 (14%) patients, corresponding with sensitivities of 44% and 84% resp. (p<0.001). Abdominal US detected celiac lymph nodes in 21/167 (13%) vs. 3/167 (2%) patients, corresponding with sensitivities of 6% vs. 44% resp. (p<0.001). Abdominal US detected liver metastases in 12/167 (7%) vs. 0/167 (0%) patients, corresponding with sensitivities of 6% and 71% resp. (p<0.001). US neck detected metastases in 16/153 (10%) vs. 5/153 (3%) patients, corresponding with sensitivities of 26 % and 84% resp. (p=0.001). The specificity of all staging procedures was  $\geq 95\%$ , both in high- and low-volume centers (p=NS). In total, in 93/486 (19%) patients, metastases were detected in a high-volume but not in a low-volume center.

**Conclusions:** There is a correlation between the number of patients undergoing staging procedures in a center and the detection rate of metastases from esophageal cancer. These results suggest that staging procedures for esophageal cancer should preferably be performed in a high-volume center.



## **Clinical Relevance of FDG-PET in Staging Esophageal Cancer Regarding the Standardized Uptake Value (SUV) and Detection of Unexpected Primary Neoplasia**

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Along with improvements in diagnostic technology, the detection of incidental synchronous other organ cancers in patients with esophageal cancer has increased during preoperative work-up. The aim of this study was to determine the rate and clinical importance of unexpected second primary neoplasia seen of FDG-PET in staging of esophageal cancer.

From January 1996 to December 2003, a total of 180 patients with biopsy-proven malignancy of the esophagus underwent FDG-PET for initial staging. This series of patients was retrospectively analyzed for the detection of synchronous primary neoplasia.

In this series of 180 FDG-PET scans, 11 synchronous primary neoplasia were identified in 11 patients (6.1%). Six neoplasia were localized in the colorectum and two in the kidney. The remaining 3 neoplasia were localized in the lung, thyroid and gingiva. The neoplasia in the thyroid and lung led to an incorrect upstaging of the esophageal tumor however, additional investigations revealed a synchronous carcinoma in these patients. Only the two neoplasia of the kidney were visualized on CT.

Conclusions: FDG-PET may detect unexpected primary neoplasia in patients with esophageal cancer. Because synchronous neoplasia can mimic metastases, they must be confirmed by additional investigation before the choice of treatment.

tweede abstract (let op de oorspronkelijke titel van abstract NVGE 1716 - zie cijferlijst) weer boven het abstract in het boekje plaatsen)

Clinical relevance of the standardized uptake value (SUV) in staging esophageal cancer with FDG-PET

Positron emission tomography (PET) with 18-F-fluorodeoxyglucose (FDG) has been accepted in staging esophageal cancer. The increased glucose metabolism is the rationale behind FDG uptake in malignant cells. The tissue glycolysis level can be quantified by the accumulation of FDG. The most commonly used parameter to assess FDG accumulation in clinical FDG-PET imaging is the standard uptake value (SUV). The aims of this study were to investigate the relation between SUV and stage of disease and whether SUV can predict resectability and survival in patients with esophageal cancer.

The studied population consisted of 40 patients with cancer of the esophagus or gastro-esophageal junction. Patients were included from January 2001 to December 2002. After measuring the SUV, patients were retrospectively divided into two groups using the median SUV as cutoff value. SUV values were evaluated according to the stage of disease, histology, age, sex, and resectability. Survival was analyzed by using the log-rank test and Cox regression analysis.

The median SUVmax was 6.7(range 1.8-19.2) and median SUVmean was 5.7(range 1.4-15.7). SUVmax and SUVmean differed significantly for tumor stage and resectability. The mean survival of patients with SUVmax  $\leq$ 6.7 and SUVmean  $\leq$ 5.7 was 613 days compared to 262 days for patients with SUVmax  $>$ 6.7 and SUVmean  $>$ 5.7 ( $p=0.016$ ). Cox regression analysis did not reveal a significant impact of SUV on survival independent of resectability.

Conclusions: SUV can be used to predict resectability, however SUV is not an independent factor to assess the survival in patients with esophageal cancer.

## Quality of life in adults after correction of oesophageal atresia

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Over the last decades, the mortality of patients with oesophageal atresia (OA) has decreased to approximately 5%. Little is known about the influence of OA and its long-term sequelae on the quality of life (QoL) of the surviving patients. The aim of our study was to investigate the generic and disease specific QoL after correction of OA in a large adult population, and to compare the generic QoL to that of a healthy population. All surviving patients treated for OA between 1947-1985 (n=119) were sent a questionnaire. The questionnaire assessed sociodemographic characteristics, generic (SF-36) and disease specific (GIQLI, EORTC-OES24) QoL. It also contained 3 open-ended questions about the daily consequences of the disease. Clinical characteristics were collected from patient charts. For reasons of multiple testing, the level of significance was defined as  $p < 0.01$ . We received 97/119 completed questionnaires (82% response rate). When comparing the generic QoL of OA patients to that of healthy subjects, we found no differences in the 'overall' physical and mental functioning. However, patients do report impaired QoL on 'general health' and 'vitality'. The type of atresia did not influence generic or disease specific QoL. The presence of concomitant congenital anomalies did not influence generic QoL, these patients only reported impaired QoL in the 'indigestion'-scale (EORTC-OES24). A third of the patients reported negative consequences of the OA in their daily life. Gastro-intestinal symptoms were mentioned most often (23%). These patients also reported impaired QoL on several domains of the questionnaires. Conclusion: In general, after correction of OA, patients perceive their generic and disease specific QoL to be good. The type of atresia or the presence of concomitant congenital anomalies does not influence generic QoL. However, a third of the patients report negative consequences of the disease.

## **Prevention of secondary pancreatic infections with probiotics; in-vitro studies.**

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Several randomised trials with a single probiotic strain have shown a significant reduction of infectious complications both in surgical and acute pancreatitis patients. We hypothesise however, that a combination of probiotic strains might be more effective. The goal of PROPATRIA (a randomised multicenter trial) is to reduce the overall number of infectious complications in severe pancreatitis with multispecies probiotics. The present in-vitro studies tried to quantify the antibacterial properties of the six different probiotic strains used in PROPATRIA (Ecologic 641: *Lactobacillus acidophilus* (LA), *Lactobacillus casei* (LC), *Lactobacillus salivarius* (LS), *Lactococcus lactis* (LL), *Bifidobacterium bifidum* and *Bifidobacterium infantis*). The lactobacilli and the lactococcus were tested against fourteen pathogens isolated from infected pancreatic necrosis and a vancomycin resistant enterococcus (VRE) using the well-diffusion method. Both overnight cultures and filter-sterilized supernatant of the different probiotics were tested against the pathogens. All tested probiotics and its supernatants inhibited the growth of streptococci, CNS, Enterobacteriaceae and *Pseudomonas* (inhibition zones mean 13, range 9-26 mm), with LC and LS producing the largest zones of inhibition (mean 15 versus 11 mm). VRE was inhibited by all except LA. Anaerobes were not inhibited by LS and LA. Enterococcus was not inhibited by LA, LL and LL-supernatant. *S. aureus* was inhibited only by LL (mean 9 mm), LC-supernatant and LS-supernatant (means 13 and 12 mm). The combination of both the probiotics and the probiotic supernatants inhibited the growth of all pathogens tested. Conclusions: These results indicate that multispecies probiotics preparations are to be preferred over monospecies preparations. In-vitro, Ecologic 641 is effective against pathogens, which cause infection of pancreatic necrosis and VRE.

## **SELDI-TOF mass spectrometry for the identification of biliary tract malignancy in bile fluid.**

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Differentiation of benign biliary strictures from malignant biliary strictures is difficult despite MRI cholangiography. Because of these limitations of preoperative evaluation, the incidence of benign lesions in patients resected for suspicious hilar obstruction is 15 per cent. A definitive diagnosis is highly desirable to make a decision about the extent of a surgical intervention. Until now the cytological analysis of bile has failed to accurately differentiate malignancy from benign causes of biliary tract strictures. Advances in proteomics like Surface-enhanced laser desorption/ionization-time of flight (seldi-tof) mass spectrometry could provide a powerful diagnostic tool for biliary strictures. In this study we aimed to differentiate between benign or malignant biliary stricture using seldi-tof analysis of bile fluids. Bile was collected from patients with a biliary tract malignancy or benign biliary disease by percutaneous drainage. After optimization we were able to extract reliable protein mass spectrometric profiles in bile in the presence of bilirubin and lipids in bile fluid using a optimized surface enhanced peptide binding chip. This allowed us to analyse the bile fluid without lipid and salt removal or filtration. We have analysed 20 bile samples with different underlying diseases. Remarkably, we could distinguish malignancy from benign disease including primary sclerosing cholangitis by the presence of specific peaks between 7.5 and 13.5 Kdalton. Proteins responsible for the observed differences are being identified using precise mass-spectrometry methods. We conclude that Seldi-tof analysis of bile has the capacity to detect malignancy in bile fluids. This novel technique could provide biomarkers of malignancy to improve diagnosis and management of patients presenting with a biliary stricture.

## **Hepatectomy for colorectal cancer liver metastases: effects of multi-disciplinary regional collaboration and extended indications**

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Partial hepatectomy is the only curative treatment for patients with colorectal cancer (CRC) liver metastases (LM). 10-15% of all CRC patients may eventually become candidate for hepatectomy. Incidence numbers of the Dutch national cancer registry (VIKC) show that yearly 60–90 patients in our region and over 1000 patients nationwide should be offered hepatectomy. In 2000 we initiated a regional multidisciplinary collaboration to increase the number of patients undergoing hepatectomy for CRC-LM. Simultaneously indications were extended according to growing insights from literature data. Former contra-indications such as the presence  $\geq 4$  metastases, size and synchronicity were abandoned and (neo-)adjuvant chemotherapy as well as local ablative therapies were introduced. Records of all patients undergoing liver resection for CRC-LM in our institution from 1991-2003 were analysed (prospectively from 2000). Distinction was made between the period 1991-1999 (n=32, group I) and 2000-2003 (n=49, group II) to evaluate the effect of intensified patient recruitment and selection on number of hepatectomies, mortality and long-term outcome. The median annual number of hepatectomies for CRC-LM increased from 5 (1-6) before 2000 to 14 (6-16) (p=0.003) thereafter. Mortality dropped from 6.5% in group I to 2.0% in group II (p=0.3). Overall 5-year survival following liver resection for CRC-LM was 48% (median follow-up 1.8 (0.1-11.9) years). Two-year survival was 83% for group I and 95% for group II (p=0.17). Conclusion: Mortality of partial hepatectomy is low. Regional collaboration and extension of indications can increase the number of patients undergoing hepatectomy for CRC-LM without decreasing long-term outcome, although still not all candidates are offered a hepatectomy. Improving awareness of the curative potential of hepatectomy in referring gastroenterologists and oncologists may further increase the number of patients eligible for resection.

## Ten years experience with the Ileal Pouch-Anal Anastomosis without a diverting ileostomy; safe or sorry?

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Restorative Proctocolectomy (RP) with Ileal Pouch-Anal Anastomosis (IPAA) has become the surgical treatment of choice for Ulcerative Colitis (UC) and Familial Adenomatous Polyposis (FAP). Historically temporary ileostomy was constructed to decrease the incidence of anastomotic complications. Today, technical modifications and increased experience have resulted in decreased morbidity and complications and routinely creation of a diverting ileostomy has therefore become controversial. We analyzed our IPAA procedures of the last decade and compared our results with literature. 98 patients were included, M:42/F:56, age 35 (14-67). IPAA was constructed for UC 81, FAP 12, HNPCC 4 and Crohn's disease 1. Preferably patients were operated in a single procedure: RP with IPAA without ileostomy. 47 patients underwent a proctectomy with IPAA after a previous subtotal colectomy for fulminant colitis. In 8 patients a diverting ileostomy was constructed because of intra-operative findings. There were no deaths. Mild and severe complications occurred in 37 patients, small bowel obstruction in 10 and clinical significant leakage in 8. 18 patients were re-operated of whom 9 patients received a diverting ileostomy. After takedown of the ileostomy in these patients functional results resemble those of the patients who received an ileostomy in the first procedure. Stool frequency is 7,4 dd average. 73 patients defecate no more than 6 times daily and twice at night. 87 patients are completely continent. Pouchitis occurred in 26 patients, inflammation of the rectal cuff "cuffitis" in 8. In 3 patients the pouch was lost due to pouchitis, cuffitis and fistula. Our results are similar to those reported in literature including those of series where diverting ileostomies were constructed routinely. This retrospective study suggests that it is safe to construct a diverting ileostomy only when intra-operative findings make it necessary or when clinical significant leakage occurs.

## **Integrity of the anal sphincters after pouch-anal anastomosis: evaluation with three-dimensional endoanal ultrasonography**

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The aim of the present study was to assess the integrity of the anal sphincters after handsewn pouch-anal anastomosis performed with the help of a Scott retractor. For this purpose the anal sphincters were visualized with 3D endoanal ultrasonography (EUS). Between October 2001 and October 2003, 36 pts underwent a pouch anal anastomosis. Before and six months after the procedure, the length and volume of the internal (IAS) and external (EAS) anal sphincter were assessed with 3D EUS and anal manometry was performed in 28 pts. Continence scores were determined using the Fecal Incontinence Severity Index (FISI). Fifteen pts with a colonic pouch-anal anastomosis (CPAA) and 13 pts with an ileal pouch-anal anastomosis (IPAA) were included. Six months after the procedure, 3D EUS showed significant alterations of the IAS in 8 pts with a CPAA (53%) and in 9 pts with an IPAA (73%). These alterations were characterized by asymmetry and variations in thickness along the circumference. Despite these changes none of the pts with a CPAA showed an IAS-defect. In two pts with IPAA a small defect in the IAS was found. In pts with a CPAA the volume of the IAS decreased significantly ( $p=0.009$ ). This was not observed in pts with an IPAA. In both groups the length of the IAS remained the same. Thickness, length and volume of the EAS did not change in both groups. Six months after the procedure a significant reduction of maximum anal resting pressure was found in both groups (CPAA:  $p<0.001$ , IPAA:  $p=0.001$ ). Maximum anal squeeze pressure was reduced in pts with an IPAA ( $p=0.006$ ). The observed alterations of the IAS and the manometric findings showed no correlation with the post-operative FISI scores. Conclusion: 3D EUS shows changes in the IAS in 62% of pts after a handsewn pouch-anal anastomosis performed with a Scott retractor. In only two pts an IAS-defect was found. No correlation was observed between these alterations and functional outcome.



## **Defects and atrophy of the external anal sphincter in patients with faecal incontinence : comparison between 3 dimensional-anal endosonography and endoanal magnetic resonance imaging**

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Anal endosonography (AE) and endoanal magnetic resonance imaging (MRI) are both endoluminal imaging techniques used for the detection of anal sphincter defects in patients with faecal incontinence. With MRI atrophy of the external anal sphincter (EAS) can be established. With AE this aspect has not been thoroughly investigated. The aim of this study was to compare 3 dimensional-AE (3D-AE) to endoanal MRI in the detection of EAS defects and EAS atrophy in patients with faecal incontinence. A defect was defined at AE by a hypoechogenic zone and at MRI as a discontinuity of the sphincteric ring and/or scar tissue. The 3D-AE-scores for EAS atrophy were depending on the distinction of the AES and its reflectivity. MRI-scores for EAS atrophy were depending on the amount of muscle and the presence of fat-infiltration. Atrophy score was defined as none (1), moderate (2) and severe (3). 18 patients were included (median age 58 years, range 27-80; 15 women and 3 men). 3D-AE detected 7 EAS defects and MRI 10 EAS defects. 3D-AE and MRI agreed in 13 of 18 patients (72%) for the detection of EAS defects. In 1 patient only 3D-AE showed a small sphincter defect and in 4 patients only MRI showed some scar tissue. 3D-AE demonstrated in 16 patients EAS atrophy, which was graded moderate in 12 patients and severe in 4 patients. MRI detected in 13 patients EAS atrophy, which was graded moderate in 8 patients and severe in 5 patients. 3D-AE agreed with MRI in 15 of 18 patients (83%) for the detection of EAS atrophy. Using the grading system 8 of the 18 patients (44%) scored the same grade. Conclusion Both endoanal techniques are comparable in detecting defects and atrophy of the EAS, although there is a substantial difference in grading of EAS atrophy. Discrepancy in grading atrophy is probably explained by the difficult visualization of the most distal part of the EAS with 3D-AE. Inconistency between the two methods needs to be evaluated with surgery and histology.

## **Inflammation and structural changes in donor intestine and liver after brain death induction**

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The majority of donor organs used for transplantation are derived from brain dead donors. The unphysiological state of brain death (BD) alters the hemodynamic and neurohormonal status of the donor. Previous studies showed that BD negatively affects the immunological and inflammatory status of both the liver and the kidney. In addition, hepatic graft survival from brain dead donors is inferior compared to living donors. Since intestinal transplantation is now feasible, we investigated the effect of BD on intestinal morphology and inflammation. BD was induced in rats by inflation of an epidural balloon catheter. During BD, normotension was maintained. Three groups (n=6) of male Wistar rats were compared: 1 h, 4 h BD and 0 h sham operated controls. Intestinal and liver morphology were assessed using PAS and HE staining. Intestinal injury was determined using the Park-score. Polymorphonuclear cells (PMN), reflecting inflammatory status, were counted in intestine and liver (200 x magnifications). The morphology of the intestine was compromised after 1 and 4 h BD. In BD rats, apical lifting of epithelial cells was clearly present, which resulted in higher Park-scores compared to controls ( $p < 0.05$ ). Liver morphology remained intact. In the intestine the PMNs increased from  $9.3 \pm 2.0$  (control) to  $15.3 \pm 2.4$  PMNs/ field after 1 h BD. Liver results showed an increase from  $11.8 \pm 1.3$  to  $33.0 \pm 6.7$ , respectively ( $p < 0.05$ ). After 4 h BD, the PMN count further increased to  $22.7 \pm 4.0$  in the intestine and  $59.7 \pm 5.1$  PMNs/ field in the liver ( $p < 0.05$ ). In conclusion, brain dead rats revealed an increase in PMNs, reflecting inflammatory changes in both liver and intestine. PMN influx was accompanied by marked intestinal injury. Future studies are warranted to elucidate on the factors involved and the possibly interrelated changes and effects on graft survival.

## **Perianal fistulas in Crohn's disease are predominantly colonized by skin flora: implications for antibiotic treatment?**

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Perianal fistulas can complicate Crohn's disease. Little is known about the microflora present in Crohn's fistulas nor is there much insight in the effect of antibiotics on these microorganisms. We aimed to determine which microorganisms are found in perianal fistulas in Crohn's disease and whether treatment with ciprofloxacin affects these microorganisms. Thirteen patients (M/F 7/6, median age 34 yrs, range 18-61) with perianal fistulizing Crohn's disease were treated with infliximab 5 mg/kg intravenously at week 6, 8 and 12. Patients were randomized to double blind treatment with ciprofloxacin 500 mg bd (n=6) or placebo (n=7) for 12 weeks. Samples from perianal fistulas were taken at baseline and after 6 and 18 weeks. In the ciprofloxacin group 10 different genera of microorganisms were identified, while 13 genera could be identified in the placebo group. In contrast to what is generally assumed gram-negative enteric flora were present in a small minority. The predominant genera found in the ciprofloxacin group were *Corynebacteria* spp., coagulase-negative staphylococci and *Arcanobacterium* spp. at baseline, *Corynebacteria* spp. and coagulase-negative staphylococci at week 6 and *Corynebacteria* spp. and streptococci at week 18. In the placebo group this was *Corynebacteria* spp., streptococci and *Escherichia coli* at baseline, *Corynebacteria* spp. and coagulase-negative staphylococci at week 6 and *Corynebacteria* spp. and coagulase-negative staphylococci at week 18. No significant differences in types of bacteria were found over time in the separate groups or between the groups. **Conclusions:** Gram-positive microorganisms predominantly colonize perianal fistulas in Crohn's disease. Therefore, antimicrobial treatment should be directed towards these microorganisms.

## **A genome-wide screen in a four-generation Dutch family with coeliac disease: evidence for linkage to chromosomes 6 and 9**

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Apart from HLA DQ2, most of the genes that probably contribute to coeliac disease (CD) have not yet been identified. Therefore, several genome-wide screens have been performed to locate these genes. Here we report a genome-wide screen in a four-generation family. 321 microsatellite markers were genotyped in 17 patients, who make up four generations of a Dutch family. Dominant, recessive and model-free linkage analyses were performed. The latter analysis was to allow for possible transmission of CD from either of the great-grandparents, who were not related. The great-grandmother was diagnosed with CD, while the great-grandfather had dermatitis herpetiformis and was also likely to be affected by CD. Besides linkage to the HLA region, a dominant locus was detected at 9p21-13; this locus was transmitted from the great-grandmother. Model-free analysis revealed a locus on 6q25.3, which was transmitted from the great-grandfather. Although these loci were not found in a Dutch genome-wide screen in affected sibpairs, the locus on 9p21-13 has been implicated in Scandinavian families. This genome-wide screen revealed two potential non-HLA loci. The locus on 9p21-13 replicated earlier findings in Scandinavian populations, suggesting a role for this locus in Caucasian populations.

## **Screening Patients with Hashimoto's Thyroiditis for Celiac Disease and Vice Versa**

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**Background and Aims:** The association between coeliac disease (CD) and autoimmune thyroid disease (ATD) has been suggested 30 years ago. Although variable, evidence of higher prevalence rates of ATD in patients with CD and of CD in ATD have been reported ranging from 12% to 29.7% for the former, and 3.2% to 7.8% for the latter. We attempted to investigate this association in a prospective manner in a group of patients with Hashimoto's thyroiditis and in another group of patients with CD. An interim analysis from an ongoing study is presented.

**Patients and Methods:** 61 adult HT outpatients (mean age 45; M:F=8:53), were enrolled in the study from 01-01-2001. Coeliac serology (EMA, tTG, and AGA-IgA) as well as HLA-DQ2/DQ8 were determined. Villous architecture was evaluated according to Marsh classification. Since 01-05-1998, thyroid function test (TSH, free T4) and serology (anti-TPO & -TG) were determined in 160 adult CD patients (mean age 53.7; M:F=28:124).

**Results:** Three patients with HT (4.9%) had positive CD serology; 2 patients were DQ2 heterozygous and one patient was DQ2 homozygote; and all had villous atrophy (Marsh IIIa in 2 & IIIc in 1). All three were overt hypothyroid requiring hormonal replacement. 68% from 47 HT patients carried one of the CD susceptibility haplotypes. Anti-TPO and/or anti-TG was present in 37 CD patients (23%). 14 (38%) were hypothyroid, 15 (40%) subclinical hypothyroid and 8 (22%) were euthyroid. Three had Graves disease. Twenty-seven were DQ2 heterozygous, 5 DQ2 homozygous, and 5 were heterozygous for both DQ2 and DQ8.

**Conclusion:** Both the prevalence of CD in HT as well as HT in CD establishes the concurrence of these autoimmune disorders. Reciprocal screening for these auto-immune diseases is warranted. A pronounced rate of carrying the characteristic HLA-DQ haplotypes strengthens their close immunogenetic relations.

## **Can 18F-fluoro-deoxy-glucose Positron Emission Tomography be Useful in Detecting Enteropathy-associated T-cell Lymphoma in Refractory Coeliac Disease?**

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**Background and aims:** A minority of unresponsive coeliac patients (refractory, RCD) to gluten free diet can evolve further into enteropathy associated T-cell lymphoma (EATL) of the intestine. A recent report suggested a promising role for 18F-fluoro-deoxy-glucose positron emission tomography (18F-FDG-PET) in diagnosing and imaging EATL. In a pilot study, we enrolled patients with RCD and EATL to assess the value of this technique in this clinical setting. **Patients and methods:** Non-responsive coeliacs referred to our tertiary referral hospital for further evaluation underwent 18F-FDG-imaging. During one year, 12 patients (mean age 58; F/M 5/7) were included. All had Marsh III villous atrophy (one submucosal collagenous band); serology was positive in 5; 8 were DQ2 heterozygous, 3 DQ2 homozygous, and one patient carried both DQ2 and DQ8 alleles; average percentage ( $\pm$ SD) of monoclonal T-cells in RCD & EATL was 40% ( $\pm$  30) & 58% ( $\pm$  31) respectively. **Results:** In RCD patients (n=6), although abdominal CT-scan showed lymphadenopathy in two patients, the 18F-FDG-PET was normal in 5 and showed diffuse moderate uptake in the small intestine in one. On the other hand, in EATL patients (n=6), CT-scan showed abnormalities in 5 patients (like lymphadenopathy, thickened intestinal wall or a tumor) and 18F-FDG-PET was abnormal in all patients (showing focally intense uptake in small intestine in 5 and diffuse moderate uptake in one). Histological analysis of the resected samples confirmed the diagnosis of EATL in all 6 patients. **Conclusion:** These preliminary results, despite the small number of subjects studied, indicate that 18F-FDG-PET is at least as sensitive for diagnosing and imaging EATL as conventional CT-scan analysis. Additional studies are needed to determine whether 18F-FDG-PET is more sensitive than CT-scan analysis for early detection of EATL in RCD.

## **Double-balloon enteroscopy: practical experience in 28 patients**

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Introduction: The small intestine, known as the black box is uneasy to evaluate due to the length and the sharp loops. Till now various means (including push enteroscopy) have been employed, however they were limited and invasive. A new insertion method of enteroscopy, a double-balloon method (Fuji Photo Optical Incorporated Company) has been previously reported which enables endoscopic scrutiny of the entire small bowel with intervention capabilities.

AIMS & METHODS: Between January and June 2004, 28 patients (F:M = 10:18; mean age 56.3; range 29-80) with occult gastrointestinal bleeding (n=11), refractory coeliac disease (n=7), Peutz-Jegher syndrome (n=5), Crohn's disease (n=1), radiation enteritis (n=1), lymphangectasis (n=2) en suspected intestinal melanoma (n=1) underwent the double-balloon technique. Preliminary results with this new technique are presented.

RESULTS: Antegrade (oral) or retrograde (anal) introduction occurred in 24 & 4 patients respectively. Although large extent of the small intestine could be inspected in majority of patients in oral introduction, the caecum was reached in only 2 patients (8%). Average intravenous sedoanalgesia consisted of  $75 \pm 25$  mcg fentanyl and  $12 \pm 2$  mg midazolam during  $85 \pm 35$  minutes ( $m \pm SD$ ). Anti-peristaltics were administered during interventions. Lesions detected included polyps, arterio-venous malformations and mucosal changes seen in coeliacs. Biopsy, argon plasma coagulation (20 watt; 0.2L/min), snare polypectomy and tattooing of small intestine could be performed without adverse effects. Only patient had post-procedural abdominal pain that resolved spontaneously within hours during observation.

CONCLUSION: Double-balloon enteroscopy is a new elegant endoscopical technique that seems promising as the endoscopist can reach undiscovered small bowel segments. Interventions in the small intestine are possible. Complications and invasiveness are minimal in this well-tolerated procedure.

## **Role of beta-interferon in the treatment of human pancreatic cancer.**

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Chemotherapy and radiotherapy have only a marginal role in the management of pancreatic adenocarcinoma. Therefore, novel therapeutic strategies are needed. The aim of the present study was to evaluate the role of type I interferons (IFNs) and their receptors in the regulation of cell growth in 3 human pancreatic adenocarcinoma cell lines (BxPC-3, MiaPaCa-2 and Panc-1). The mRNA expression of the two type I IFN receptors (IFNAR-1 and IFNAR-2, short and long form), as well as of IFN- $\beta$  mRNA, were evaluated by real time quantitative RT-PCR. All receptor subunits were expressed in the three cell lines, whereas IFN- $\beta$  mRNA was detected in BxPC3 only. The effects of recombinant IFN- $\alpha$ 2b (at the doses between 10-1000 IU/ml) and recombinant IFN- $\beta$ 1a (1 to 100 IU/ml) on cell proliferation were evaluated through DNA quantification. The treatment with IFN- $\beta$  showed a potent inhibitory effect on the proliferation of BxPC-3 (IC50: 11 IU/ml; maximal inhibition: 90-95% at the dose of 100 IU/ml) and MiaPaCa-2 (IC50: 71 IU/ml, maximal inhibition: 60-75% at the dose of 100 IU/ml) cells after 6 days. The inhibitory effect of IFN- $\beta$  was dose- and time dependent and significantly stronger compared with IFN- $\alpha$ . This antitumor effect seems to be mainly modulated by the induction of apoptosis, which was significantly higher following the treatment with IFN- $\beta$  than IFN- $\alpha$ . IFN- $\beta$  also inhibited Panc-1 cell proliferation (IC50: 44 IU/ml), although with a significantly lower maximal inhibition (30-45% at the dose of 100 IU/ml). Interestingly, IFN- $\beta$  did not stimulate apoptosis in Panc-1. Finally, a moderate anti-proliferative effect without an increase in apoptosis was observed in Panc-1 after the treatment with IFN- $\alpha$ . In conclusion, IFN- $\beta$  has a potent anti-proliferative effect on pancreatic cancer cell lines via inducing apoptosis. In more resistant cancers IFN- $\beta$  and IFN- $\alpha$  induce a moderate anti-tumor effect through a mechanism not related to the stimulation of apoptosis.



## **Systemic rate of appearance of starch derived glucose and the secretion of gastrointestinal hormones \***

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The various types of starchy foods differ considerably in the effects they exert on postprandial glucose response and might also possess different potencies to stimulate gastrointestinal hormone secretion. Glucagon-like peptide-1 (GLP-1), glucose-dependent insulintropic peptide (GIP) and peptide YY (PYY) play an important role in the regulation of postprandial glucose homeostasis, appetite and/or gut motility. The aim of this study was to assess the effect of glucose and two starchy foods, varying in their content of rapidly and slowly available glucose, on the secretion of GIP, GLP-1 and PYY in healthy volunteers and to establish whether the concentrations of those hormones are related to intestinal glucose absorption. In a crossover study 7 healthy male volunteers received a primed-continuous D-[6,6-<sup>2</sup>H]<sub>2</sub>glucose infusion and uncooked corn starch (UCCS), corn pasta (CP) or glucose as naturally <sup>13</sup>C-labeled test meals. During 6 h following consumption of the test meals insulin, GLP-1, GIP and PYY concentrations were monitored as well as the rate of appearance of exogenous glucose (RaE), which reflects intestinal glucose absorption. GLP-1 concentrations were significantly increased from 15 to 60 min after ingestion of glucose and from 180 to 300 min after ingestion of slowly available UCCS and corresponded with elevated RaE. There was a strong positive within-subject correlation between RaE and GIP concentrations ( $r=0.85$ ,  $p<0.01$ ). No increase in PYY concentration was observed after ingestion of the test meals used. These results provide evidence that the RaE of glucose affects the secretion of GIP and indicate that slowly available carbohydrates can induce late and prolonged GIP and GLP-1 responses. This might be of importance for the prevention of postprandial hyperglycemia of a *subsequent* meal.

**Novel insights in familial intrahepatic cholestasis syndromes (WS 98-12)**  
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## **The role of the human gut microflora in Crohn's disease: towards a rapid molecular quantitative analysis (Final report Maag Lever Darm Stichting project WS 98-22)**

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In 1989 we started a project to develop Real-Time PCR for the detection of microbial DNA of the human gutflora. The Real-time quantitative PCR is a valuable tool to study both the gastrointestinal microflora in faeces and of the adherent microflora to the colonic mucosa. We developed primers and probes, based on conserved DNA sequences, for the most prevalent groups of gut bacteria. Finally, a universal assay was developed for the estimation of the total bacterial load and to calculate the percentage of each bacterial species contributing to this total bacterial load. Total DNA was isolated from faeces and gut biopsy specimens and quantified by the developed assays. DNA was extracted with the DNeasy™ Tissue Kit. Primers and probes were mostly based on 16S rDNA sequences. Specificity of the different assays was tested with representative bacterial species and closely related species. The probes were labeled at the 5'-end with FAM (6-carboxyfluorescein) as the reporter dye and with TAMRA (6-carboxytetramethylrhodamine) as the quencher dye at the 3'-end. Primers and probes were used in a quantitative PCR on an ABI-Prism 7700. The assays showed a high sensitivity (1-50cfu) and specificity. The detection of bacterial genera and species was highly reproducible. The bacterial load was determined to be approximately 14.4% *B. vulgatus*, 5.4% Streptococci, 3.6% Bifidobacteria, 1.0% *E. coli*, and 0.4% Propionibacteria in healthy individuals. In contrast to what is usually found by culture, Fusobacteria were hardly detected in the gut samples neither were *Helicobacter* spp. and *Listeria* spp.. In conclusion, Real-Time PCR is a rapid and reliable technique to study the gastrointestinal microflora. Molecular analysis is a promising alternative to elucidate the microflora in faeces as well as the adherent microflora to the intestinal mucosa. Real-Time PCR cfu-equivalents may lead to new insights in the actual in vivo ratio's of the different bacterial species present in the gut of healthy persons and of patients suffering from inflammatory bowel diseases like Crohn's disease and ulcerative colitis. This in turn can lead to a better understanding of the effects of antibiotics and probiotics on the (adherent) gut microflora.

## **De interactie van vaatnieuwvorming en expressie van adhesiemoleculen bij metastasering van rectum carcinoom ((Final report Maag Lever Darm Stichting project WS 00-51)**

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The application of new surgical techniques in combination with preoperative radiotherapy has minimised the risk on local recurrence in rectal cancer. However, distant metastasis is still a serious problem after seemingly curative resection in patients with rectal cancer. The project aimed at identifying crucial factors that determine the occurrence of distant metastasis. Three different questions were addressed: 1. Can the TNM system be adopted so that better selection of patients with a high risk on distant recurrence is feasible? 2. What is the relation between

characteristics of tumour vasculature and the development of metastasis. 3. What is the role of EpCAM in the process of metastasis?

1. An analysis of pathological factors of Dutch patients with rectal cancer, included in the RT + TME trial (n = 1530) showed that nodal status, circumferential margin (CRM) and tumor depth are the most important prognostic factors. The status of the CRM and the lymph nodes formed the foundation for the new NCRM classification system which is superior to the TNM classification, giving pronounced spreading of prognosis for survival and a more favourable patient grouping.

2. Eighty-eight patients were selected from the same trial ensuring a relatively high percentage of metastasis. Vessel number, perimeter, and area were assessed at both the invasive front and intratumoral area. Hot spot and random selections were performed simultaneously. The data show that only vascular perimeter randomly assessed at the invasive front was associated with distant metastasis independent of TNM staging. Patients with a high score had a lower distant metastasis rate compared to patients with a low score (37% and 62%, respectively).

3. The presence of adhesion molecules is found to be important in tumor growth and spread. The distribution of EpCAM throughout tumors and its clinical relevance was studied since it is a target for immunotherapy. Immunofluorescence double staining clearly illustrated a partial loss the epithelial adhesion molecule. This was seen predominantly at the invasive front but also in other parts of the tumor. Decrease was correlated to a higher risk on local recurrence and overall recurrence. The results of the double staining with different antibodies give a strong indication of cleavage of the protein. Since the presence of nuclear beta catenin is accompanied by a decrease of Ber-ep4 staining, beta catenin target genes may be responsible for the EpCAM cleavage. If selective cleavage of EpCAM actually takes place

in tumors, it would change the approach for EpCAM as a target for immune therapy.

In conclusion, we have developed an improved staging system for rectal cancer and also found that two biological processes, angiogenesis and loss of cell adhesion, might be targets for improved treatment strategies.

## **Successful treatment with Pegylated Interferon in HBV non-responders to standard interferon and lamivudine**

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Treatment with antiviral therapy is effective in 20-30% of HBeAg-positive chronic hepatitis B (CHB). Non-response to treatment may result in progression of liver disease and increased risk of hepatocellular carcinoma. As part of a global randomized controlled trial we investigated the efficacy (i.e. loss of HBeAg at end of follow-up) of pegylated interferon (PEG-IFN) in CHB who failed to respond to previous courses of standard interferon or lamivudine.

We analyzed 59 non-responders to previous standard IFN and 39 non-responders to previous lamivudine therapy. All patients received a 52 week course of 100µg PEG-IFN weekly combined with, either 100mg lamivudine or placebo daily. After therapy patients were followed for 26 weeks. Median treatment duration was 24 weeks (range 3–52) for previous standard IFN (median dose 18 MU/week), and 52 weeks (range 2–411) for previous treatment with lamivudine. Median interval until retreatment was 119 weeks (range 26–576) and 43 weeks (range 28–366) for prior IFN and lamivudine, respectively. Eleven patients had a YMDD-mutant at start of Peg-IFN therapy. Seventeen (29%) non-responders to previous IFN and 9 (23%) non-responders to previous lamivudine responded (loss of HBeAg at end of follow-up) to treatment with Peg-IFN. Treatment with the combination of Peg-IFN and lamivudine was not superior to Peg-IFN alone. Non-responders to prior IFN therapy with baseline ALT above 4 x ULN responded better to Peg-IFN than those with ALT levels below 4 x ULN (44% vs. 16 % respectively,  $p = 0.019$ ). A similar trend was found for non-responders to previous lamivudine with ALT above vs. below 4 x ULN (response 37% vs. 13%, respectively;  $p = 0.089$ ).

In conclusion, Peg-IFN is effective in almost one-third of patients who failed previous treatment with standard IFN or lamivudine. High ALT levels at baseline of Peg-IFN therapy was the best predictor for increased response in prior non-responders to either standard IFN or lamivudine therapy.

## **Preoperative estimation of postoperative remnant liver function using hepatobiliary scintigraphy**

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Although mortality has decreased, partial liver resections still have considerable postoperative morbidity, mainly caused by impaired function of the remnant liver, leading to liver failure. Remnant liver function (RLF) can be calculated by hepatobiliary scintigraphy (HBS) using <sup>99m</sup>Tc-mebrofenin. This method is informative for hepatocyte uptake function as well as excretory kinetics into the bile. The aim of this study was to evaluate the prognostic value of HBS on morbidity and mortality after liver resection. HBS was performed in 44 patients selected to undergo hepatic resection.. Future RLF was calculated. In 33 patients HBS was repeated on day 1-3 after resection (2 segments: n=12; 3 segments: n=8; 4 segments or more: n=24). Biochemical values and hospital stay characteristics were recorded. Liver failure was defined as Bilirubin>50 µmol/L, Albumin<35 g/L and Prothrombin time>12 seconds. Mean <sup>99m</sup>Tc-mebrofenin uptake for the total liver was 13.50 ± 3.32 %/min. Future remnant liver (FRL) uptake was 6.98 ± 2.93 %/min. RLF determined preoperatively on HBS correlated well with the actually measured postoperative value (r=0.62;p<0.01;n=33). 17 patients developed liver failure during the first 4 postoperative days. FRL uptake and clearance was significantly decreased in these patients (p=0.02;p=0.04). There was no difference in total liver uptake and clearance in patients with liver failure and patients without liver failure (p=0.67;p=0.16). HBS did not correlate with postoperative in hospital morbidity and mortality. Preoperative HBS of the future remnant liver with <sup>99m</sup>Tc-mebrofenin is able to predict the occurrence of liver failure within the first 4 postoperative days. It can therefore be useful to identify patients who are at risk of developing liver failure after partial hepatectomy.

## **Sustained virological response virtually eliminates liver-related morbidity and mortality of hepatitis C cirrhosis**

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**Introduction:** Patients with chronic hepatitis C and liver cirrhosis have an increased risk for hepatocellular carcinoma, decompensation and liver related death. The effectiveness of antiviral therapy on long-term clinical outcome of cirrhotic patients is expected, but remains unproven. The aim of this study was to assess the long-term clinical outcome of all consecutive cirrhotic patients with chronic hepatitis C, treated with combination therapy in a tertiary referral center for liver disease. **Methods:** Between 1992 and 2003, 41 patients with biopsy-proven cirrhosis finished treatment with combination therapy, consisting of interferon or pegylated interferon plus ribavirin. Sixteen patients (39%) achieved sustained virological response. Patients underwent physical examination, laboratory testing and abdominal ultrasound twice yearly during follow-up. Patients were censored at death or liver transplantation or, in case of non-response, at the time of a second interferon-based treatment.

**Results:** During a mean follow-up of 3.4 years after end-of-treatment, 7 patients developed hepatocellular carcinoma and 4 patients had decompensated cirrhosis. Five patients died of a liver-related cause. Two patients underwent orthotopic liver transplantation and one non-responder was lost to follow-up. Patients with a sustained virological response had a significantly better long-term clinical outcome than non-responders (Log Rank  $p < 0.01$ ). According multivariate Cox regression analysis the only baseline factor associated with an increased chance of clinical events was platelet count below 80,000 per milliliter. The improved long-term clinical outcome of sustained virological responders remained significant after correction for low platelet counts (Log Rank  $p < 0.02$ , see figure).

**Conclusion:** The long-term clinical outcome of patients with hepatitis C related cirrhosis is favourable once sustained virological response is achieved. This effect is independent from baseline platelet counts.



## **HBsAg seroconversion in chronic HBV patients treated with pegylated interferon alpha-2b alone or in combination with lamivudine. The role of HBV genotype**

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HBsAg seroconversion is the hallmark of a complete response during antiviral therapy in chronic hepatitis B (CHB), and is almost absent in patients treated with lamivudine and is 1.6% after one year of adefovir. Since pegylated interferon alpha-2b (PEG-IFN) has an immune modulating, rather than an antiviral effect, we investigated the frequency of HBsAg seroconversion during treatment with PEG-IFN. In a multicenter randomized controlled trial, 266 HBeAg-positive CHB patients were treated for 52 weeks with PEG-IFN 100 µg/week in combination with either lamivudine 100 mg/day or placebo. PEG-IFN dose was halved after 32 weeks of treatment. Post-treatment follow-up was 26 weeks. Ninety-five (36%) of the 266 patients exhibited HBeAg loss, 18 (7%) patients showed loss of HBsAg and 16 (6%) HBsAg seroconversion. HBeAg loss was 47% for genotype A (n=90), 44% for genotype B (n=23), 28% for genotype C (n=39) and 25% for genotype D (n=103) (A vs D and B vs C:  $P < 0.001$ ). Adding lamivudine did not enhance HBeAg loss, HBsAg loss or HBsAg seroconversion. HBsAg seroconversion occurred in 3 patients during treatment and in 13 patients after treatment. All these patients had normal ALT and HBV DNA  $< 10^3$  copies/ml by PCR at the end of follow-up. HBsAg seroconversion rate also differed according to HBV genotype: 13% for genotype A, 9% for genotype B, 0% for genotype C and 2% for genotype D (A vs D:  $P=0.04$ ). Among responders with HBeAg loss the HBsAg seroconversion rate was 29%, 20%, 0%, 8% for genotype A, B, C and D, respectively. In conclusion, one year of PEG-IFN in HBeAg-positive CHB patients leads to an HBsAg seroconversion rate of 6%. Almost one third of the responders with genotype A exhibited HBsAg seroconversion. Our study indicates that future intervention studies for HBeAg-positive CHB may need stratification according to HBV genotype and that PEG-IFN is most beneficial to achieve an HBeAg- and HBsAg response in patients with genotype A and B.

## **Benign recurrent intrahepatic cholestasis type 2 is caused by mutations in *ABCB11*\***

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Progressive familial intrahepatic cholestasis (PFIC) and benign recurrent intrahepatic cholestasis (BRIC) are hereditary liver disorders; PFIC is characterized by severe progressive liver disease, whereas BRIC patients have intermittent attacks of cholestasis without permanent liver damage. Mutations in *ATP8B1* are present in PFIC type 1 and in a subset of BRIC patients. We hypothesized that a genetically distinct form of BRIC is associated with mutations in *ABCB11*. This gene encodes the bile salt export pump (BSEP) and is mutated in PFIC type 2. Patients from 20 families were included; all had normal *ATP8B1* sequence. Sequencing of all 27 coding exons including the splice junctions of *ABCB11* revealed 8 distinct mutations in 11 patients of 8 different families: one homozygous missense mutation (E297G) previously described in PFIC2 patients, 6 novel missense mutations and one putative splice site mutation. In 12 families, no mutations in *ATP8B1* or *ABCB11* were detected. Pancreatitis is a known extrahepatic symptom in BRIC caused by *ATP8B1* mutations, but was not present in BRIC patients with mutations in *ABCB11*. In contrast, cholelithiasis was observed in 7 out of 11 BRIC patients with mutations in *ABCB11*, but has never been described in *ATP8B1*-affected BRIC patients. Conclusions: Mutations in *ABCB11* are associated with BRIC, and consistent with the genetic classification of PFIC into two subtypes, we propose that this disorder be named BRIC type 2.

## Enhanced lentiviral hepatocyte transduction by elimination of Kupffer cells

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Lentiviral vectors can stably transduce dividing and non-dividing cells in vivo. This makes lentiviral vectors attractive to correct dysfunctional hepatocytes in inherited liver diseases. When lentiviral vector containing the GFP gene was injected into the portal vein of mice (n=7), the transduction efficiency of non-parenchymal cells (mainly Kupffer cells and endothelial cells, 2.93±1.46 %) was much higher than that of parenchymal cells (hepatocytes, 0.13±0.046 %).

Endothelial cells form a potential barrier between the sinusoidal lumen and the Space of Disse. Removal of this barrier may improve viral transduction of hepatocytes. Mild damage of the endothelial layer in cyclophosphamide treated animals (n=5) did, however, not significantly increase the percentage of transduced parenchymal cells (0.25±0.22 %) and non-parenchymal cells (3.96±2.99 %).

Kupffer cells may eliminate virus particles from the blood stream, before transduction of hepatocytes. Specific agents, such as gadoliniumchloride (GdCl<sub>3</sub>) can block phagocytosis and transiently deplete Kupffer cells. GdCl<sub>3</sub> treatment of mice (n=7) significantly lowered the non-parenchymal cell transduction to 0.86±1.20 % (p=0.008) and increased the number of hepatocytes that expressed GFP (0.89±1.26 %, p=0.073). The percentage of GFP positive hepatocytes of total GFP positive cells in the liver increased from 8.29±4.64 % to 51.45±13.96 % after treatment with GdCl<sub>3</sub> (p≤0.001).

These studies indicate that the endothelial layer does not interfere significantly in the efficiency of lentiviral vectors to infect hepatocytes. Kupffer cells sequester lentiviral particles and thereby inhibit hepatocyte transduction. Agents that affect the activity of Kupffer cells or that are able to deplete these cells transiently could be important for clinical application of lentiviral vectors in liver disease.

## **MURR1/COMMD1 defines a novel protein family with a possible role in hepatic copper homeostasis and NF- $\kappa$ B signaling**

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Hepatic copper accumulation results in extensive liver damage as seen in Wilson Disease (WD), (Non-) Indian Childhood Cirrhosis ((N)ICC) and Idiopathic Copper Toxicosis (ICT). Copper Toxicosis in Bedlington Terriers, a canine model for WD, (N)ICC and ICT, is caused by a deletion in the Murr1 gene. MURR1 interacts with ATP7B, the copper transporter defective in WD, suggesting that MURR1 and ATP7B cooperate to regulate hepatic copper excretion. No mutations in MURR1 were detected in (N)ICC and ICT patients. To gain further insight into the function of MURR1 and to obtain more candidate genes for (N)ICC and ICT we identified a previously uncharacterized 8-kDa protein as a novel interacting partner of MURR1 using yeast two-hybrid analysis. The validity of this interaction was verified by co-immunoprecipitation on cell lysates and on in vitro synthesized proteins. Mass spectrometry analysis of MURR1-containing protein complexes independently identified the 8-kDa protein and 2 other MURR1 interacting proteins. Interestingly, alignment of the sequences of these proteins revealed that they share a common domain, which is also present in MURR1. We propose to name this the Copper Metabolism MURR1 (COMM) Domain. Database searching revealed a total of 10 human proteins that share this COMM domain. Based on these results we re-annotated MURR1 to COMMD1 and named the 8-kDa protein COMMD6. Further interaction experiments indicated that all COMMD proteins bind COMMD1 and that these interactions are in part mediated by the COMM domain. Nearly all COMMD proteins, including COMMD1, are able to inhibit NF- $\kappa$ B-mediated transcriptional activation, thereby revealing a potential link between copper metabolism and NF- $\kappa$ B signaling. Conclusion: We have identified a novel protein family with a possible role in hepatic copper metabolism. The COMMD family members are regarded as excellent candidate genes for (N)ICC and ICT.

## **Carbon monoxide protects hepatocytes against oxidative stress induced apoptosis**

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Background: Many liver diseases are accompanied by oxidative stress and this may induce apoptosis in hepatocytes. Oxidative stress induces Heme Oxygenase-1 (HO-1) expression. HO-1 is responsible for Heme degradation into carbon monoxide, iron and biliverdin. Carbon monoxide is an important intracellular messenger molecule and modulates crucial signalling pathways. However, whether carbon monoxide is involved in the cytoprotective effect of HO-1 is not known. Aim: To investigate whether HO-1 and its product carbon monoxide protect rat hepatocytes against oxidative stress-induced apoptosis. Methods: Primary cultures of hepatocytes were exposed to the superoxide anion donor menadione (50 $\mu$ M) in a normal and a carbon monoxide containing (250-400ppm) atmosphere. HO-1 expression was determined by PCR and Western blotting. Survival pathways were studied using Western blot and the p38 MAPK inhibitor SB203580 and the ERK MAPK inhibitor U0126. Caspase-6,-9,-3 activation were determined by activity assay and Western blot and necrosis by Sytox Green staining. Results: 1) Superoxide anion induces HO-1 expression in a time- and concentration-dependent manner in hepatocytes. 2) Induction of apoptosis and caspase-6,-9,-3 activation by superoxide anion peaks at 9-12 hrs in a normal atmosphere. Superoxide anion induces JNK phosphorylation at early time points. 3) Carbon monoxide inhibits superoxide anion-induced caspase-6,-9,-3 activation and apoptosis, but does not increase necrosis. Carbon monoxide blocks superoxide anion-induced JNK phosphorylation, which is considered a pro-apoptotic MAPK pathway. Conclusions: Carbon monoxide protects hepatocytes against superoxide anion-induced caspase-6,-9,-3 activation and abolishes apoptosis. The antiapoptotic effect of carbon monoxide is due to inhibition of JNK phosphorylation. Our results explain in part the protection of HO-1 against oxidative stress induced injury. CO could become an important candidate for treatment of liver diseases.

## **Analysis of intrahepatic immune response identifies four stages of chronic HBV infection**

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Based on virologic and biochemical parameters chronic HBV infection (CHB) is divided into 3 phases: 1) immune tolerance (IT) (HBV DNA high, HBeAg+, ALT N), 2) immune clearance (IC) (ALT high, HBeAg +/-), and 3) inactivity (INA) (HBV DNA low, HBeAg-, ALT N). Unclear is whether these phases can be characterised by intrahepatic immune response, as most studies have concentrated on peripheral blood (PB). We aimed to characterise the composition of key populations of immune effector cells in CHB, both in the liver by Fine-Needle-Aspiration-Biopsy and in PB. In 47 patients (IT n=7, IC n=24, INA n=16) the percentage of CD56+ NK cells, CD8+ cytotoxic T cells (CTL) and CD4+ T helper cells (Th) were determined by flow cytometry. In 15 HLA A2+ patients HBc 18-27-tetramers were used to identify HBV-specific CTL. No significant differences were found between the 3 phases in proportion of Th cells and CTL. The proportion NK cells in the liver was higher in IT than in IC and INA-patients (mean % 41.1, 29.8 and 35 resp; IT vs IC and IC vs. INA  $p < 0.05$ ). However, when IC-patients with similar ALT levels were divided according to HBV DNA:  $>10E7$  geq/ml (n=14) vs. HBV DNA  $<10E5$  geq/ml (n=10), Th cells in the liver but not PB were higher in those with low vs. high HBV DNA (mean % 30.4 vs. 22.5 resp.,  $p < 0.05$ ). In contrast, the intrahepatic proportion of CTL was increased in IC-patients with high vs. low HBV DNA (mean % 40.3 vs. 31.7,  $p < 0.05$ ). In 3 of 15 HLA A2+ patients HBV-specific CTL were detected in the liver. All of them were IC-patients and intrahepatic HBV-specific CTL concentration was 10-100 fold higher than in PB. Interestingly, in the only patient with high HBV DNA and intrahepatic HBV-specific CTL, viral load decreased to  $<10E5$  geq/ml soon after sampling. In conclusion, analysis of intrahepatic immunology suggests that CHB can be divided into 4 stages. During the course of infection patients may evolve through these immunological stages to inactive disease.

## Altered disposition of acetaminophen in Mrp3 knockout mice

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MRP3 is an ABC transporter that pumps various anions preferentially glucuronide conjugates. MRP3 is present in the basolateral membrane of several epithelia. The AIM of this study was to investigate the role of Mrp3 in drug disposition. We investigated the metabolism and disposition of acetaminophen (APAP). Metabolism of this drug occurs by sulfation, glucuronidation and glutathione conjugation in the liver. The glutathione conjugate (APAP-GSH) is secreted into bile via Mrp2 while the glucuronide (APAP-GLU) is secreted into the urine. From fasted wild type and Mrp3<sup>-/-</sup> mice the bile duct was cannulated. The animals received APAP after which bile was sampled during 120 min after administration in 10 min intervals. Basolateral and canalicular excretion of APAP and metabolites was assessed in the isolated perfused liver. At 120 min after administration no difference was found in the biliary excretion of APAP-GSH (16% of dose) between Mrp3<sup>-/-</sup> and wild-type mice. However, APAP-GLU excretion in bile of Mrp3<sup>-/-</sup> mice was ten-fold higher in Mrp3<sup>-/-</sup> mice than in wild types (6.1 ± 0.8% vs. 0.6 ± 0.2% of dose respectively). There was 20-fold accumulation of the APAP-GLU in the liver of Mrp3<sup>-/-</sup> compared to wild types (20.5 ± 4.0% vs. 1.0 ± 0.1% of dose respectively). In addition, the plasma APAP-GLU content was more than ten-fold lower in Mrp3<sup>-/-</sup> mice compared to wild types (5.8 ± 3.2% vs. 0.5 ± 0.8%). In line with these data we found a strong decrease of APAP-GLU content in the perfusate of perfused Mrp3<sup>-/-</sup> livers compared to wild types. These data demonstrate that the basolateral excretion of APAP-GLU is nearly completely dependent on the presence of Mrp3. We hypothesize that the affinity of murine Mrp3 for APAP-GLU is much higher than for Mrp2, but that in the absence of Mrp3 there is sufficient accumulation in the liver to redirect the APAP-GLU to bile via low-affinity transport through Mrp2. Conversely, the main metabolite, APAP-GSH is entirely excreted into bile via Mrp2.

## **Selective COX-2 inhibition impairs ileal but not colonic experimental anastomotic healing in the early postoperative period**

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Selective COX-2 inhibitors are considered a safer alternative for NSAIDs and they are increasingly prescribed in the perioperative period, mainly for their analgetic properties. Their supposed anti-neoplastic properties may render them suitable for even more widespread usage in the future. However, the recognition of a possible role for COX-2 in wound healing has raised concern about the safety of their perioperative usage. This study was carried out to investigate the influence of COX-2 inhibition on intestinal anastomotic strength.<sup>48</sup> Male Wistar rats were randomly assigned to a control group (A) receiving saline (n=12) or to experimental groups (n=12 each) receiving the COX-2 inhibitor Celecoxib in a dose of 15(B), 50(C) or 200 mg/kg/day(D) starting one day before both an ileal and a colonic anastomosis were constructed. At the third postoperative day anastomotic strength was assessed by measuring the breaking strength and bursting pressure. Rats from the control group recovered quickly from the operation. In contrast, rats receiving celecoxib suffered from severe complications. Altogether 10 rats died prematurely; 4 in group B and C each and 2 in group D. At necropsy, severe peritonitis resulting from ileal anastomotic dehiscence appeared to be the cause of death. At sacrifice, 8 more ileal anastomoses were dehiscent; 2 in group B, 1 in group C and 5 in group D resulting in a total ileal dehiscence rate of 50% in group B, 42% in group C and 58% in group D. Colonic anastomoses were invariably macroscopically normal. Ileal bursting pressure, but not breaking strength was significantly lower in animals from experimental group D as compared to the control-group (-62%, p=0.004). No significant differences in strength were measured in colonic anastomoses. These results clearly suggest a role for COX-2 in intestinal anastomotic healing warranting further research. In the meanwhile caution should be exerted when prescribing COX-2 inhibitors in the perioperative period.



## **The value of Narrow Band Imaging for the detection of dysplasia in longstanding ulcerative colitis**

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Patients with longstanding UC have an increased risk for the development of colorectal cancer. Narrow Band Imaging (NBI), a new endoscopic technique using special light-filters, allows a more detailed view of the mucosal architecture.

The aim of this study was to assess the feasibility of NBI for UC surveillance, and compare this with conventional colonoscopy for the detection of (pre)neoplastic lesions.

Patients with longstanding pancolitis ( $\geq 8$  years) were screened using both a standard surveillance and NBI-colonoscopy with targeted biopsies of abnormalities only. Both procedures were performed in random order, 6-8 weeks apart and by two different, blinded doctors.

To date, 40 (of scheduled 60) patients have been included in the study. Thirty patients have completed the full protocol and are reported in this abstract. Mean age: 48.4 (20-65) yrs; UC duration 19.2 (8-40) yrs; Disease Activity Index 1.0 (0-6); 12 pts also suffered from PSC.

NBI-colonoscopy was feasible in 29 patients; there was no procedural time difference. The number of biopsies was higher in the conventional colonoscopy-group (41 vs 6). Lesions suspicious for dysplasia were detected in 9 patients during conventional and in 12 patients during NBI-colonoscopy; this was confirmed by pathology in respectively 6 and 9 patients. However, in another 3 patients abnormalities were detected taking random biopsies during conventional colonoscopy. NBI detected a dysplastic lesion in 1 patient not found by conventional colonoscopy; in no cases a lesion was detected during conventional colonoscopy that was not seen during NBI. With NBI, the maximal grade of dysplasia was identical to conventional colonoscopy in 19 patients, upgraded in 5 patients and downgraded in 5 patients.

In conclusion, NBI is feasible for dysplasia screening in patients with longstanding UC. Preliminary results suggest that this technique allows a sensitive diagnosis of dysplasia with a significant reduction of biopsies.

## **EMR improves staging of early neoplastic lesions in Barrett's esophagus**

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Barrett's esophagus is a well-recognised premalignant condition for development of adenocarcinoma of the esophagus. Patients with a known Barrett's esophagus are enrolled in endoscopic surveillance programs with random biopsies to detect high-grade dysplasia (HGD), thereby improving patient survival. Grading of dysplasia, however, is notoriously difficult. Even rigorous random biopsy protocols have a limited aptitude to differentiate HGD from early invasive carcinoma. Reportedly, focal invasive cancer is found in 40-50% of resection specimens in patients operated for presumptive HGD. Endoscopic Mucosal Resection (EMR) is a novel endoscopic technique for removal of mucosal neoplasia. Compared to biopsies, it provides larger tissue samples, wider in-depth margins and better orientation of the specimen, thereby enabling more accurate staging of dysplasia and early invasion. In 36 patients with known Barrett's neoplasia on biopsies, an EMR of the suspected area was performed using a capped technique. No major complications occurred. Two expert pathologists assessed the highest degree of neoplasia. Results of referral biopsies were compared to final assessment after EMR. The diagnosis was altered in 12/36 cases (33%). In 10 patients a diagnosis of HGD was changed to invasive carcinoma: T1m (limited to the mucosa) in 8 and T1sm (submucosal invasion) in 2. Two patients were staged with a less severe dysplasia: 1 suspected carcinoma appeared to be HGD and 1 alleged HGD was assessed indefinite for dysplasia. Of the remaining 24 patients, 15 were diagnosed with HGD and 9 with an invasive cancer. The latter group could be differentiated between T1m infiltration and T1sm using the EMR specimen (4 T1m, 5 T1sm). Conclusion: EMR specimen enabled more accurate staging of a Barrett's neoplasia in 59%. Cancer was found in 40% of patients with suspected HGD in biopsies. We therefore recommend EMR of suspicious focal abnormalities in all patients with early neoplasia in Barrett's.

## **The role of the intestine in reverse cholesterol transport**

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It is generally assumed that hepatobiliary cholesterol excretion is the only way to excrete substantial amounts of cholesterol from the body. Recently, we demonstrated that this statement may require revision. Cholesterol balance studies have shown, that more neutral sterols are present in feces, than can be derived from hepatobiliary excretion. Indeed, in mouse models with abrogated biliary cholesterol secretion, a condition, which should lead to greatly diminished fecal neutral sterol output, no difference in cholesterol excretion was found. It was the aim of this study to investigate the origin of the non-biliary derived cholesterol in the intestinal lumen. To quantify the amount of cholesterol directly derived from the intestine, intestine perfusions were performed. <sup>3</sup>H-cholesterol was injected in the tail vein of male FVB mice. After 30 minutes, gallbladders were cannulated to divert bile and intestinal segments were perfused with, or without, cholesterol acceptor (micelles composed of 10 mM taurocholate and 2 mM phosphatidylcholine). Perfusate was collected every 15 minutes for a period of 90 minutes. After the perfusions, intestinal segments and blood were collected. Specific activity of cholesterol in these tissues and in perfusate was determined. Cholesterol was excreted in duodenum, jejunum, as well as in ileum. Cholesterol flux varied from 0.4 nmol/min/100 g body weight (jejunum) to 1.5 nmol/min/100 g body weight (duodenum). Addition of cholesterol acceptor to perfusate did not change these fluxes appreciably. Specific cholesterol activity in serum was comparable to the specific activity in perfusate. Specific activity in intestinal homogenates was 9 fold lower, indicating that the cholesterol was not derived from shedded intestinal cells. We conclude that there is a substantial transport of cholesterol from blood to the intestinal lumen which may provide an alternative for the hepatobiliary route.

## **A new design esophageal stent (Niti-S stent) for the prevention of migration**

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Self-expanding covered metal stents are effective in the palliation of patients with obstructing esophageal cancer. However, stent migration occurs in 10-20% of patients. The new Niti-S stent (Taewoong Medical/Seoul Korea) has a double layer, consisting of an inner polyurethane layer to prevent tumor ingrowth and an outer uncovered nitinol wire to allow the mesh of the stent to embed itself in the esophageal wall. This should reduce, if not eliminate stent migration. The aim of our study was to evaluate migration of the Niti-S stent in patients with dysphagia due to inoperable esophagogastric carcinoma. Between June 2003 and May 2004, 41 patients with esophagogastric carcinoma were treated with a Niti-S stent. Patients were prospectively followed and were compared with a historical group of 108 prospectively followed patients treated with an Ultraflex stent for the same indication (SIREC study). Differences in frequency of migration and other clinical outcomes were assessed by chi-square tests. Clinical characteristics were similar in the two treatment groups. After 4 weeks, the dysphagia score improved from a median of 3 (liquids only) to 0 (normal diet) in both groups. Recurrent dysphagia was reported in 2/41 (5%) patients treated with a Niti-S stent (Ultraflex stent group: 44/108 (41%) patients;  $p < 0.001$ ). Particularly, stent migration (1 (2%) vs. 18 (17%);  $p = 0.025$ ), (non-)tumoral tissue overgrowth (1 (2%) vs. 16 (15%);  $p = 0.042$ ), and food bolus obstruction (0 (0%) vs. 16 (15%);  $p = 0.006$ ) occurred less frequently with a Niti-S stent compared to an Ultraflex stent. Complications were seen in 9/41 (22%) patients after placement of a Niti-S stent (Ultraflex stent group: 36/108 (33%);  $p = \text{NS}$ ). Conclusions: The Niti-S stent effectively reduces stent migration in patients with inoperable esophagogastric carcinoma. In addition, its completely covered design may be an important factor in preventing the occurrence of (non-)tumoral tissue overgrowth at both ends of the stent.

## **Video Autofluorescence Imaging (AFI) Followed by Narrow Band Imaging (NBI) for Detection of High Grade Dysplasia (HGD) and Early Cancer (EC) in Barrett's Esophagus (BE)**

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**Background** AFI is a novel endoscopic technique that may improve the detection of HGD/EC in BE, but AFI is associated with a high false positive rate. NBI, another new imaging technique, improves the detection of surface patterns in BE, which may correlate with histology. The combination of AFI & NBI may reduce the false positive rate.

**Patients & Methods** 14 patients with BE were investigated with 2 prototypes (Olympus, Tokyo, Japan): 1) AFI, which has a sequential RGB light source and a high-resolution video-endoscope (HRE) with separate CCD's for white light endoscopy (WLE) and AFI; 2) NBI, which has a sequential RGB light source equipped separate rotary filters with narrowed RGB band-pass ranges and increased contribution of blue light illumination. A zoom HRE was used for NBI. The BE was first examined with the WLE of the AFI system and all visible lesions were recorded, followed by AFI for additional lesions. Non-dysplastic BE appeared green on AFI, while suspicious areas were blue/violet. Subsequently, NBI was performed for detection of the mucosal and vascular patterns of all lesions. Irregular patterns as well as abnormal blood vessels were considered suspicious; regular patterns were regarded as not suspicious. Lesions were sampled for histological correlation.

**Results** From a total of 27 lesions with HGD/EC, 16 were identified with WLE (sensitivity 59%) and 26 with AFI (sensitivity 96%). 34 suspicious lesions were detected with AFI: 26 contained HGD/EC and 8 were false positive (23%). After NBI, regular patterns were found in 7 of the 8 false positive lesions and the false positive rate was reduced to 4%.

**Conclusion** This uncontrolled study suggests that AFI can serve as a "red flag" technique to detect suspicious lesions and NBI can be applied to verify the surface patterns. The combination of these 2 novel imaging techniques may improve the detection of HGD/EC.

## **Diagnostic value of videocapsule endoscopy in carcinoid tumours**

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Carcinoid tumours are the most common gastrointestinal neuroendocrine neoplasms. They often originate in the small intestine but many patients are diagnosed by the presence of liver metastases. The primary tumour is difficult to locate. It may cause obstruction, intussusception and ischaemia due to infiltration of mesenteric blood vessels. Therefore resection in an earlier phase would be recommended. The aim of this study was to investigate the diagnostic value of videocapsule endoscopy (VCE) in finding the primary carcinoid tumour in the small intestine. Sixteen consecutive carcinoid patients (M 10/F 6; 63+10 yr) with histologically or cytologically proven metastatic disease of unknown primary were examined. All patients underwent abdominal CT-scanning, enteroclysis, octreotide- or MIBG-scanning and VCE of the small bowel. After an overnight fast, VCE was performed with the Given Imaging M2A swallowable videocapsule. CT-scans demonstrated liver metastases in 10 patients and a mesenteric tumour in 4 patients. One patient was primarily diagnosed with a lesion in the lung. In only one patient CT-scan showed an aspecific lesion in the distal small intestine. One patient had a multifocal carcinoid tumour of the distal ileum at colonoscopy. All patients had a normal enteroclysis. Nuclear imaging demonstrated abnormalities in the abdominal area in 9 patients but was unable to relate this to an intestinal localisation. VCE, which did not visualize the cecum in 6 patients, revealed a small intestinal tumour in 8 patients (50%). These findings led to an ileal resection in 4 patients. In three of these patients a carcinoid tumour was found in the resected intestine. Four patients are scheduled for surgery. Conclusion: VCE has a high diagnostic yield with respect to the intestinal localisation of the primary carcinoid tumour as compared to enteroclysis, abdominal CT-scan and nuclear imaging and may become the diagnostic procedure of first choice in this rare disorder.

### **Third generation autofluorescence endoscopy of the colon**

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Endoscopic screening for colonic cancer relies mainly on the detection of adenomatous polyps. However, non-adenomatous polyps cannot be classified at the time of colonoscopy and non-polypoid (flat) adenomas may go unrecognized. Light-induced fluorescence endoscopy (LIFE) is a technique that has been shown to be of value in discriminating normal from dysplastic mucosa during endoscopy. We report on the pilot experience of a third generation system (ONCO-LIFE, Xillix, Vancouver, Ca) for real-time detection of polypoid and non-polypoid dysplasia in the colon. Ten patients were screened with both standard colonoscopy and LIFE (6 polyp surveillance, 2 HNPCC, 1 longstanding UC, 1 other). All polypoid (9) and non-polypoid (6) abnormalities were inspected and documented. Polypectomy was performed or targeted biopsies were taken of each endoscopic or autofluorescence abnormality. All lesions were immediately judged to be either dysplastic or non-dysplastic using both techniques. Endoscopic findings were correlated to the pathology results. The series included 15 lesions of the colon and 21 random biopsies. Six dysplastic lesions were detected with LIFE that were occult to standard colonoscopy, including 4 lesions in a patient with longstanding quiescent ulcerative colitis. One hyperplastic polyp was judged suspicious on autofluorescence imaging. None of the random biopsies showed dysplasia on histology. **CONCLUSIONS** Fluorescence endoscopy is a clinically useful technique for differentiating dysplastic from non-dysplastic lesions in the colon. Our earliest experience with ONCO-LIFE shows the feasibility to detect adenomatous lesions in the colon that are occult to standard endoscopy, also in longstanding IBD

## **Clinical benefit from video capsule endoscopy - one year after**

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Video capsule endoscopy (VCE) is a new technique used for visualisation of the small intestinal mucosa. The most frequently used indications are intractable blood loss, inflammatory bowel disease and polypoid diseases of the small bowel. The aim of the present study was to define the impact of VCE on patient management. VCE was performed using the Given M2A system according to standard protocol. Follow-up was performed using a standard questionnaire sent to the referring physician one year after the procedure. In the period from September 2001 through April 2003 36 VCEs were performed. One patient was unable to swallow the capsule. The procedure was uneventful in the other cases. There were no cases of non-natural excretion. The coecum was reached in 69% of procedures. A definite diagnosis was made in 53% of patients, a possible diagnosis in 8%, no diagnosis was made in 39%. To date, the follow-up questionnaire was returned by 78% of referring physicians. The result of VCE was helpful in patient management in 36% of patients and not helpful in 61% of patients. In 54% of patients a conservative course was followed, in 14% drug therapy was instituted, 18% underwent a peroperative enteroscopy, 4% underwent surgery and 7% were treated endoscopically. After a median follow up of 23 months (range 12-31) 39% of patients were effectively treated, 39% had unaltered complaints, 14% improved spontaneously. In 4% an alternative diagnosis became apparent. One patient (4%) died postoperatively after a peroperative enteroscopy. In conclusion, VCE is a safe and simple diagnostic tool. In our series, it helped decision making in over 35% of patients and was not helpful in 60%. This number can probably be improved by better patient selection. At the present, VCE is mainly performed when other diagnostic strategies have failed. In this highly selected group of patients, a 35% alteration in management strategy is a significant contribution to patient care.



## **Interobserver variation in the diagnosis of dysplasia in Barrett's Esophagus**

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Histological grading of biopsies from Barrett's esophagus (BE) is the gold standard to determine the risk of progression to adenocarcinoma. Histological interpretation is dependent on the criteria used and the expertise of the pathologist. The aim of this study was to determine interobserver variation in the diagnosis of BE and grading of dysplasia. In this study, 8 general pathologists from different hospitals in the Netherlands graded surveillance biopsies of 183 patients with BE. Subsequently, one of a panel of 5 expert gastrointestinal (GI) pathologists blindly reviewed the biopsies. When there was disagreement on the presence of intestinal metaplasia (IM) or grade of dysplasia, slides were again blindly reviewed by a second panel member. A final conclusion was made if 2 of 3 pathologists agreed on the diagnosis. In 18/183 (10%) patients, no IM was present. In 128/165 (78%) patients, the final diagnosis was no dysplasia (ND), in 32 (19%) low-grade dysplasia (LGD), in 2 (1%) high-grade dysplasia and in 3 (2%) adenocarcinoma. In 73/183 (40%) patients, a third opinion was indicated. In 7/44 (16%) of the third opinions, the first diagnosis was upgraded, mostly from ND to LGD, whereas in 13/44 (30%) it was downgraded. In 24/44 (55%) third opinions, the final diagnosis was not different from that of the general pathologist. The interobserver reproducibility between the first and second pathologist for dysplasia was moderate with a  $\kappa$ -value of 0.43. In contrast to the general pathologist, experts concluded that no IM was present in 5/183 patients, resulting in a  $\kappa$ -value of 0,70, which suggests substantial agreement for IM. Conclusions: There was substantial interobserver variation in the interpretation of dysplasia in BE between general pathologists and expert GI pathologists, but also between expert GI pathologists. The high interobserver variability demonstrates the need for additional markers to determine cancer risk in BE.

## **Characterization of Tissue Autofluorescence (AF) In Non-dysplastic (NDBE) And Dysplastic Barrett Esophagus (BE) By Confocal Fluorescence Microscopy (CFM)**

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BE high grade dysplasia (HGD) and NDBE can be distinguished in vivo by endoscopic AF spectroscopy and imaging. It is unknown whether this is due to differences in epithelial AF or due to changes in tissue architecture and increased hemoglobin content. We used CFM for ex vivo characterization and comparison of epithelial AF in NDBE, low grade dysplasia (LGD) and HGD. Sections cut from 28 snap-frozen biopsies, which were taken from 10 patients with HGD in BE were examined using CFM ( $\lambda_{exc}$ . 458 nm; 2 emission detection channels:  $\lambda_{emiss}$  505-550 nm for green AF, and  $\lambda_{emiss} \geq 560$  nm for red AF; 40x objective). Pseudo-color 8-bit digital CFM micrographs were taken from areas with homogenous histopathology and each image was separated into its respective green and red channels. Image analysis software on the CFM system (LSM510, Carl Zeiss, Jena, Germany) was used to calculate the total detected fluorescence intensities in each channel. These data were used for objective comparison of the total green and red AF in NDBE and HGD. 42 NDBE, 40 LGD and 91 HGD areas were imaged. In all tissues autofluorescence was mainly in the green spectrum and originated from the cytoplasm and lamina propria; nuclear and mucinous vacuole AF was negligible. By visual inspection, there were no significant differences seen in AF intensity and microdistribution between NDBE, LGD and HGD, except for quantitative differences secondary to morphological changes such as increased nuclear/cytoplasmic ratio in HGD. The means and standard deviations for total green and red AF in NDBE and HGD, respectively, were statistically not significant.

Conclusion: Using ex vivo CFM, no significant differences were observed in epithelial AF between NDBE, LGD and HGD. The in vivo detectable differences in AF are therefore not caused by specific changes in epithelial autofluorescence but probably reflect changes in tissue architecture and hemoglobin content that diminish AF from the collagen in the submucosa.

## **Barrett's esophagus is associated with reflux of secondary bile acids into the esophagus**

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Bile reflux has been implicated in the pathogenesis and malignant transformation of Barrett's esophagus (BE), but it is unknown which bile acids contribute to this effect. Ambulatory esophageal measurement of acid and bile reflux was performed with a fiberoptic bilirubin monitoring system (Bilitec 2000) combined with a pH probe in 15 patients with BE, 16 with gastro-esophageal reflux disease (GERD) and 12 patients with reflux-like dyspepsia (RLD). In addition, bile was collected during endoscopy after cholecystokinin administration. There were more males in the BE-group than in the GERD- and RLD-group (60% vs. 35% vs. 33%, resp.;  $p < 0.05$ ). In addition, BE-patients used more often PPI's than GERD- and RLD-patients (94% vs. 50% vs. 58%, resp.  $p < 0.01$ ). Mean age, use of aspirin/NSAID's and H.pylori status were not different. The median (range) percentage total time that the pH was  $< 4$  was 12.7% (6.6-28.1%) in BE, 12.4% (4.3-23.5%) in GERD and 2.1% (0-4.4%) in RLD (BE and GERD vs. RLD:  $p < 0.05$ ). The median (range) percentage total time that bilirubin absorbance was  $\geq 0.14$  was 28.0% (2.4-45%) in BE, 11.8% (0-29.3%) in GERD and 1.1% (0-2.9%) in RLD (BE and GERD vs. RLD:  $p < 0.01$ ; BE vs. GERD:  $p < 0.01$ ). Of all bile acids, glycine-urodeoxycholic (URSO) acid was decreased in bile of PPI-using BE-patients ( $n=14$ ) compared to PPI-using GERD- ( $n=8$ ) and PPI-using RLD-patients ( $n=7$ ) (BE: 133 vs. GERD: 735 ( $p=0.07$ ) vs. RLD: 206 ( $p=0.02$ )  $\mu\text{mol/l}$ ). In addition, taurine-deoxycholic acid (DCA) was increased in bile of PPI-using BE-patients compared to PPI-using GERD- and PPI-using RLD-patients (BE: 1412 vs. GERD: 329 ( $p=0.02$ ) vs. RLD: 476 ( $p=0.20$ )  $\mu\text{mol/l}$ ).

Conclusions: Use of PPI's in BE is associated with increased levels of the toxic secondary bile acid DCA, whereas the 'protecting' bile acid URSO is decreased. It remains therefore to be established whether PPI's sufficiently protect against duodeno-gastro-esophageal reflux in BE, and in that way prevent malignant transformation in BE.

## DNA copy number changes at 8q11-24 in metastasized colorectal cancer

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**Background** C-myc, a well known oncogene located on 8q24.12-q24.23, is often amplified and over-expressed in both primary and metastasizing colorectal cancer. In addition, PTP4A3, a tyrosine phosphatase located on 8q24.3, is amplified in colorectal cancer metastasis. Beside PTP4A3 and c-myc, other oncogenes located on the 8q23-24 region might be involved in this process. Therefore, the present study aims to correlate DNA copy number status of a series of genes at 8q23-24 in colorectal cancer at high resolution in correlation to metastatic disease. **Methods** Thirty-two cases of colorectal cancer, 10 B1, 10 B2 and 12 D (Astler-Coller) with their corresponding liver metastasis and one colorectal cell line were included in this study. A chromosome 8 specific MLPA probe mixture was used to analyze the presence of DNA copy number changes. The probe mixture contained 29 probes covering 25 genes on chromosome 8 and 6 control probes on other chromosomes. **Results** Astler-Coller B1 and B2 colorectal cancers differed significantly in DNA copy number of the genes MOS, MYC, DDEF1, PTK2 and PTP4A3. When comparing these with Astler-Coller D primary tumors, significant differences were seen for several genes as well (MYC, DDEF1, SLA, PTK2, PTP4A3, and RECQL4). When comparing primary Astler-Coller D tumors and their corresponding liver metastases, a similar pattern of gains and losses was observed. Most of the liver metastases showed higher DNA copy number ratios than the corresponding primary tumors, but this difference was only significant for TPD52 and EIF3S6. **Conclusion** In addition to c-myc, multiple genes on chromosome 8 differed significantly between primary colorectal cancers with and without liver metastases. This observation is consistent with the concept that clinical behaviour, like risk of liver metastasis, is determined by the genomic profile that is already present in the primary tumor.

## **Wnt pathway activation in colorectal tumors is associated with an increase in DR4 expression.**

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Activation of the Wnt-APC- $\beta$ -catenin pathway is implicated in the initiation and progression of the majority of colorectal carcinomas. Wnt activation, due to inactivating APC mutations or activating  $\beta$ -catenin mutations, leads to the accumulation of  $\beta$ -catenin in the nucleus where it functions as a cofactor for the T-cell factor (TCF) family of transcription factors. TNF-related apoptosis-inducing ligand (TRAIL) can induce apoptosis by binding to the death receptors DR4 and DR5. We previously found that DR4 and DR5 expression gradually increase during the adenoma-carcinoma sequence. We hypothesize that activation of the Wnt pathway is involved in the upregulation of DR4 and DR5 in colorectal adenomas and carcinomas. Immunohistochemical expression of DR4, DR5 and  $\beta$ -catenin were examined in colorectal adenomas (n=166) and carcinomas (n=57). Immunoreactivity for DR4 and DR5 was evaluated as the percentage of positively staining epithelial cells. The presence and location of membranous, cytoplasmic and nuclear  $\beta$ -catenin staining intensity were evaluated. Nuclear or increased cytoplasmic  $\beta$ -catenin staining were used as markers of Wnt activation. The presence of nuclear  $\beta$ -catenin staining was associated with a higher percentage of DR4 and DR5 expression in comparison with tumors without nuclear  $\beta$ -catenin staining (74.5 % vs. 56.6 %, p = 0.001 and 92.0 % vs. 84.4 %, p = 0.017, respectively) expression. In serial slides, foci of nuclear staining and/or increased intensity of cytoplasmic  $\beta$ -catenin staining co-localize with foci of increased DR4 staining in the majority of adenomas. In carcinomas,  $\beta$ -catenin, DR4 and DR5 staining were homogeneously positive. Increased DR4 expression in colorectal adenomas and carcinomas is associated with activation of the Wnt pathway. This indicates that, in colorectal carcinogenesis, either the expression of DR4 is regulated by Wnt pathway activation or a common factor is involved in Wnt pathway activation and DR4 upregulation.

## **CT colonography in colorectal screening New perspectives by repeating a recent study in replicated samples of autopsy material from an asymptomatic Dutch population**

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Pickhardt (NEJM 2003) showed extremely favourable results of CT colonographic screening in asymptomatic patients, but focussed on patients with polyps  $\geq 6$  mm. To extend the viewing angle we performed a simulation based on autopsy data of 490 asymptomatic subjects. The probability of a positive finding on CT colonography was deduced from the sizes of polypoid lesions in each subject of the autopsy population and polyp size specific sensitivity and false positive rates reported from the Pickhardt study. In the simulation, polyps  $< 6$  mm could only be detected as a result of coexisting lesions measuring  $\geq 6$  mm. Means and confidence intervals of CT findings were calculated from bootstrapped samples of the autopsy material (N=1000, with replacement). Sensitivity rates for patients with polyps  $\geq 6$ ,  $\geq 8$  and  $\geq 10$  mm were 93-95% compared to 89-94% reported by Pickhardt (mean age of the populations 70.1 and 57.8 yrs respectively). However among subjects presenting with lesions  $\geq 10$  mm, the detectable proportion of all subjects with neoplasia was only 30% (95% CI 23-36%) and the sensitivity for all subjects with histologically advanced lesions was 70% (95% CI 56-85%). Comparable data were not provided by Pickhardt et al. although the proposal was made to confine additional optical colonoscopy to subjects presenting polyps  $\geq 10$  mm. Our data showed that if the threshold was lowered to 6 mm the detectable proportion of all neoplasms would be 73% (95% CI 67-79%) and the detectable proportion of all advanced neoplasms 91% (95% CI 84-97%). In view of low sensitivity rates using the  $\geq 10$  mm threshold, repeated CT screening of negative subjects appears inevitable - undoing initial cost savings. For the 6 mm threshold the simulation predicted a positivity rate of 31% (95% CI 28-34%; Pickhardt: 33%). By omitting repeated screening in negative cases the number of CT's and colonoscopies in a life time would be less than in the Pickhardt scenario.

## **Sulindac increases epithelial cell proliferative activity in the proximal colon of HNPCC-patients**

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Sulindac causes reduction in size and number of adenomas in familial adenomatous polyposis. Whether a similar chemopreventive effect can be expected in subjects with the predisposition for hereditary nonpolyposis colorectal cancer (HNPCC) is not sure. We therefore performed a study on the effect of sulindac on epithelial cell proliferative activity as a biomarker of cancer risk in the colon of subjects predisposed to HNPCC. A randomized double-blind cross-over study was performed in 22 subjects (9 female; age 30-66 years, mean 44) with either a pathogenic germline mutation in MLH1 (n=5) or MSH2 (n=8) or with an Amsterdam criteria positive family history and an adenoma before age 40, an advanced adenoma before age 50 or an extracolonic HNPCC-related cancer in the past. Sulindac 150 mg b.i.d. and placebo were given for four weeks each, with an interval of four weeks in between. Colonoscopy was performed at the end of both study periods and biopsies were taken from the ascending and transverse colon, the sigmoid and the rectum. Proliferative activity was determined immunohistochemically using MIB-1 antibodies against Ki-67 and was expressed as labeling index. Labeling index was higher during sulindac than during placebo in both ascending (57.6 vs 52.1%, p=0.048) and transverse colon (53.6 vs 47.7%, p=0.021), but not in the sigmoid (41.6 vs 40.4%, p=0.665) and the rectum (31.2 vs 29.1%, p=0.389). Conclusions: Sulindac induces an increase of epithelial cell proliferative activity in the proximal colon of subjects predisposed to HNPCC. Since colorectal cancer predominantly arises in the proximal colon in HNPCC, these results cast strong doubts about the potential chemopreventive effects of sulindac in HNPCC.

## **Effects of sulindac on apoptotic activity and expression of DR5 and $\beta$ -catenin in normal colon from patients with hereditary non-polyposis colorectal cancer and familial adenomatous polyposis**

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Sulindac is the most studied chemopreventive nonsteroidal anti-inflammatory drug (NSAID) that reduces colorectal cancer risk in genetically susceptible humans and animals. The underlying molecular mechanisms are incompletely understood. Many studies suggest a role for induction of apoptosis, possibly involving the death receptor (DR) pathway. Recent in vitro studies indicated that sulindac induces up-regulation of DR5 in colon cancer cells. Another mechanism may be inhibition of the APC- $\beta$ -catenin-Wnt pathway as sulindac decreased nuclear  $\beta$ -catenin expression in FAP-associated adenomas. The aim of this study was to determine the effects of sulindac in vivo on apoptotic activity and expression of DR5 and  $\beta$ -catenin in normal colon mucosa. Biopsies were obtained during two chemoprevention studies before and after treatment. 18 patients with hereditary non-polyposis colorectal cancer (HNPCC) and 6 patients with FAP received 150 mg sulindac twice daily during 1 and 3 months respectively. Apoptotic activity (assessed by M30 staining) and expression patterns of DR5 and  $\beta$ -catenin were studied by immunohistochemistry. Apoptotic activity was similar before and after sulindac treatment, in both patient groups. At baseline, expression of DR5 was observed in all cases of normal mucosa from HNPCC and FAP patients. No consistent changes in DR5 expression were seen after sulindac. Membranous  $\beta$ -catenin expression was seen in all samples from both patient groups at baseline. No nuclear staining was observed. Following sulindac, intensity of  $\beta$ -catenin staining decreased in 22 out of 42 (52 %) HNPCC and in 3 out of 6 (50 %) FAP samples. Conclusion. Sulindac does not affect apoptotic activity and DR5 expression in normal colorectal mucosa from HNPCC and FAP patients. The decrease in  $\beta$ -catenin staining intensity in approximately half of the cases suggests a possible inhibiting effect of sulindac on APC- $\beta$ -catenin signalling in colon mucosa, before the stage of adenoma development.



## **Abnormal neo-rectal contractility or 'irritable neo-rectum' after preoperative radiotherapy and rectal resection for rectal carcinoma**

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Preoperative radiotherapy followed by rectal resection with total mesorectal excision (TME) and colo-anal anastomosis severely compromises anorectal function. To what extent altered motility of the neo-rectum is involved, remains unknown. The aim of this study was to compare the motor response to rectal filling of the neo-rectum after TME and preoperative radiotherapy with that of healthy volunteers (HV). Ten volunteers (mean age 44.4 years, 7 males) and seven patients (mean age 59.6 years, 4 males) 4 months after surgery for rectal cancer were evaluated. (Neo)rectal function was examined by (neo)rectal barostat. Both a step-wise isovolumetric and isobaric distention protocol were performed to determine thresholds of urge to defecate and discomfort. Subsequently, (neo)rectal motility was recorded during 10 minutes using the barostat with the volume or pressure fixed at the threshold of urge to defecate. An increase in pressure >10 mmHg during isovolumetric distention or a volume decrease of >15% of the baseline volume during isobaric distention were considered as a contraction. The neo-rectal volumes of patients at the thresholds of urge to defecate ( $128 \pm 46$  ml) and discomfort ( $136 \pm 33$ ) were significantly lower compared to the rectal volumes of HV ( $248 \pm 81$  ml and  $292 \pm 8$  ml). No rectal contractions were observed during prolonged rectal distention in HV. In patients prolonged isovolumetric distention induced median 3 (range 1-5) contractions/10 min with a mean increase of  $19.0 \pm 5.3$  mmHg per contraction ( $p=0.003$ ). Similarly, prolonged isobaric distention triggered the occurrence of median 4 (range 3-5) contractions/10 min with a mean volume decrease of  $29.6 \pm 7.3$  % per contraction ( $p=0.006$ ). Conclusions. Prolonged distention of the neo-rectum after preoperative radiotherapy and rectal resection results in reactive contractile activity illustrating abnormal neo-rectal motility representing a new mechanism probably contributing to the compromised anorectal function.

## **Mortality in Families with Hereditary Non Polyposis Colorectal Cancer**

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The prognosis in Hereditary Non Polyposis Colorectal Cancer (HNPCC) has improved over the past decades owing to surveillance programs, which have been introduced in the late Eighties. The aim of the study was to examine the causes of death in members of HNPCC families with a known mismatch repair gene defect. At the Dutch HNPCC database 140 families are registered with a known mutation (55 hMLH1, 67 hMSH2, 18 hMSH6). We selected all mutation carriers, all probable carriers (subjects with colorectal cancer (CRC) or endometrial cancer (EC) diagnosed < age 60) and all their first degree relatives who were alive at or after January 1, 1960 and who were born before April 1, 1984. In the total cohort (n=2716), 791 subjects had died. In 108 subjects the cause of death was unknown (mean age 64.1 yrs). 437 (55.2%) subjects died due to a cancer (mean age 52.5 yrs). In men (n=234) the top three causes of death due to cancer was CRC (56.4%), brain (7.7%), and lung (7.3%). In women (n=203) the top three was CRC (43.8%), EC (11.8%), and brain (6.4%). Before 1990 (n=307) the top five (corrected for gender) was: CRC (53.1%), EC (14.3%), brain (6.5%), stomach (4.9%), and ovary (4.5%). During the period 1990 - 2004 (n=130) the top five was slightly different: CRC (43.8%), EC (12.9), brain (8.5%), female breast (7.1%), and kidney and other urinary organs (not urinary bladder) (5.4%). More than half of the members from HNPCC families die from cancer, in comparison with one third in the Dutch population. CRC and EC are the main causes of death. Since the introduction of surveillance, the rate of mortality due to CRC has been decreased by 10%. These results underscore the importance of lifelong surveillance of at least the colon and endometrium.

## Human liver lymph nodes contain mature dendritic cells with high expression of Programmed Death Ligands

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T-cell responses against dietary, viral and allo-antigens in the liver are relatively weak. Primary T-cell responses are initiated in lymph nodes (LN) by dendritic cells (DC). We investigated whether DC in hepatic LN (h-LN) have properties that may contribute to the T-cell hypo-responsiveness to liver antigens. We compared DC from h-LN with DC in skin/muscle-lymph draining LN. DC were purified by immunomagnetic selection from h-LN obtained from multi-organ donors (MOD, n=8), and from inguinal LN (in-LN) of kidney transplant recipients (n=8). The DC were immunophenotyped by flowcytometry and tested for their capacity to stimulate allogeneic T-cell proliferation. In addition we compared DC in pairs of h-LN and illiacal LN derived from the same MOD (n=5). The isolated DC from h-LN proved to be mature, with significantly higher expressions of HLA-DR (p=0.009), CD80 (p=0.000) and CD86 (p=0.001) as compared to DC from in-LN. Despite the enhanced expression of these co-stimulatory molecules, DC from h-LN had a two times reduced capacity to stimulate allogeneic T-cell proliferation compared to DC from in-LN (p<0.05). The relatively weak T-cell stimulatory capacity of DC from h-LN may be associated with their enhanced expression of the T-cell inhibitory molecules Programmed Death Ligands (PD-L) 1 and 2 compared to DC from in-LN: 59±11% of h-LN DC expressed PD-L1 and 49±14% PD-L2, versus 12±13% and 15±14% respectively of in-LN DC (p<0.002). This difference was confirmed in the comparisons of DC from paired h-LN and illiacal LN from MOD: 58±16% of h-LN DC expressed PD-L1 and 47±12% PD-L2, versus 17±25% and 18±22 respectively of illiacal LN DC. Conclusions: Hepatic LN contain a mature type of DC with high expression of both PD-Ligands and relatively weak T-cell stimulatory capacity. These properties may contribute to the T-cell hypo-responsiveness to liver antigens.

## **Cholestasis associated with acute liver rejection is characterised by selective down-regulation of hepatic bile salt transporters**

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Intrahepatic cholestasis after liver transplantation (OLT) is frequently observed, but the mechanism is not understood. The release of inflammatory cytokines during acute rejection may be involved since cytokines are known to change the expression and activity of hepatobiliary transport systems, which mediate hepatic uptake and biliary excretion of bile salts, organic anions, organic cations, and lipids. Reduced levels of hepatobiliary transport proteins are associated with cholestasis. However, nothing is known about bile salt transporter expression during acute liver rejection in humans. Aim: to study whether changes in bile salt transporter gene expression contribute to cholestasis associated with acute liver rejection. Core needle liver biopsies were studied from: (1) patients with acute liver rejection, and (2) patients who had a routine biopsy post transplantation. Acute rejection was graded according to the Banff protocol. Immunohistochemistry was used to study the expression of the bile salt export pump (BSEP), phosphatidylcholine transporter MDR3, multidrug resistance transporter MDR1, multidrug resistance associated proteins MRP1, MRP2 and MRP3, and sodium-dependent taurocholate co-transporting protein (NTCP). Immunohistochemistry was performed on liver biopsies from 16 patients. Canalicular BSEP staining was reduced in livers from patients with high-grade liver rejection. Basolateral staining of NTCP was inverse related with the grade of rejection. Conversely, MDR1 expression increased with the rejection grade. MRP2 and MDR3 expression did not change. High expression of MRP3 was observed post-OLT, but was not correlated with rejection grade. MRP1 staining was not detectable. Conclusion: Cholestasis during high-grade acute rejection is associated with specific down-regulation of the hepatic bile salt transporters BSEP and NTCP. Furthermore, high MDR1 expression may influence the bioavailability of immunosuppressive drugs in these patients.

## **Mrp-type transporters protect activated hepatic stellate cells against cell death**

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Background: In chronic liver diseases, hepatocytes die, while hepatic stellate cells (HSCs) and hepatic progenitor cells (HPCs) survive and proliferate. Activated HSCs produce excessive extracellular matrix, thereby causing liver fibrosis. ATP-Binding Cassette (ABC) transporters transport toxic compounds out of the cell thereby maintaining cell viability. This may enable HSCs and HPCs to survive in the chronically injured liver. We previously demonstrated that HPCs display high expression levels of Mdr1b, Mrp1 and Mrp3. ABC-transporter expression in HSCs has not been reported so far. Aim: To investigate expression and function of Mrp-type and Mdr-type ABC-transporters in activated HSCs. Methods: HSCs and primary hepatocytes were isolated from male rat livers. Culture-activated HSCs were used. Cytokine mixture (TNF $\alpha$  (20ng/ml), IL-1 $\beta$  (10ng/ml) and IFN $\gamma$  (10ng/ml)) was used to mimic inflammation. mRNA levels were determined by RT-PCR. MK571 (50 $\mu$ M) was used as Mrp-inhibitor and Verapamil (50 $\mu$ M) as Mdr-inhibitor. Apoptosis was determined by activated caspase-3 staining and necrosis by Sytox Green nuclear staining. Results: Activated HSCs express high mRNA levels of Mrp1, Mrp3, Mdr1a and Mdr1b compared to hepatocytes. HSCs do not express Ntcp, Bsep, Mrp2 and Mrp6. Although cytokine mixture induced the NF-kB regulated genes iNOS and A1, expression of Mdr and Mrp family members was not affected in HSCs. Mrp inhibition induced necrosis in >50% of HSCs, while hepatocytes were unaffected. Mdr inhibition did not affect HSC survival. Apoptosis was not detected in any of these conditions. Conclusion: Activated HSCs, unlike hepatocytes, express high mRNA levels of Mrp1, Mrp3, Mdr1a and Mdr1b. HPCs have a similar expression pattern. Mrp inhibition induces necrosis in HSCs, but not in hepatocytes, indicating that Mrp-type transporters are important for HSC survival. Therefore, specific inhibition of Mrp family members represents a potential target for anti-fibrotic therapy.

## **Preferential gene transfer to hepatocytes with baculovirus GP64 pseudotyped lentiviral vectors**

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Lentiviral vectors transduce quiescent cells, exhibit stable genomic integration, and provide long-term expression. These properties make them ideal gene delivery vehicles for the treatment of inherited liver diseases. In vivo studies using lentiviral vectors have a low transduction efficiency in hepatocytes. To improve the transduction efficiency of hepatocytes we investigated targeting of lentiviral vectors to the liver by using an alternative envelope protein. We selected the envelope protein from the baculovirus *Autographa californica* multinuclear polyhedrosis virus (AcMNPV), GP64. AcMNPV is an insect virus that is also capable of infecting mammalian cells, and can give rise to gene expression when a mammalian expression cassette is included. In vivo, AcMNPV preferentially transduces hepatocytes. Lentiviral vectors expressing GFP were produced, pseudotyped with either VSVg or AcGP64. Transductions were performed in parallel on HeLa, HepG2 hepatoma cells, primary human, rat, and porcine hepatocytes and on primary human umbilical vein endothelial cells (HUVEC). Viral titers are expressed as the ratio of GP64 versus VSVg titer on HeLa cells: HeLa: 1.0, HepG2: 0.38, human hepatocytes:  $4.4 \pm 1.6$ , porcine hepatocytes:  $3.4 \pm 2.26$ , rat hepatocytes  $1.9 \pm 0.1$ , and HUVEC 0.88. Ratios that are greater than one suggest that AcGP64 has a higher affinity for the indicated cell type. We conclude that GP64 might be a promising envelope protein for liver directed gene therapy.

## **Reduced incidence of acute rejection in HBV-infected liver transplant recipients may be due to suppression of dendritic cell function by polyclonal anti-HBs immunoglobulin**

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The aims of this study were to establish the effect of intravenous polyclonal immunoglobulins (IVIg) on the incidence of acute rejection after liver transplantation (LTX) and to search for a mechanistical explanation for such an effect. To study the effect of IVIg on acute rejection, a group of 40 LTX-recipients, which were treated with anti-hepatitis B surface Ig (anti-HBs Ig, Hepatect®, Biotest Pharma GmbH) to prevent infection of the liver graft with HBV, was compared to recipients with HCV-infection (n=29), or without viral hepatitis (n=147). To study mechanistical aspects, dendritic cells (DC) were purified from the blood of LTX-recipients, and of healthy volunteers, and their capacity to stimulate allogeneic T-cell proliferation was determined. The cumulative incidence of acute rejection in the anti-HBs Ig-treated LTX-recipients was significantly lower (13%) as compared to recipients without viral hepatitis (34%; p=0.01) or with HCV-infection (31%; p=0.05). Treatment with anti-HBs Ig was, independently of other risk factors, associated with a reduced incidence of acute rejection (Relative Risk compared to recipients without viral hepatitis = 0.36; 95% CI=0.14-0.91; p=0.03). DC isolated from HBV-infected LTX-recipients during anti-HBs Ig treatment had a reduced capacity to stimulate allogeneic T-cell proliferation as compared to DC isolated from HCV-infected LTX-recipients or from healthy individuals. In addition, using DC of healthy volunteers, it was shown that anti-HBs Ig suppresses (p=0.008; n=9) the acquirement of allogeneic T-cell stimulatory capacity during in vitro DC-maturation. Conclusions: Immediate post-transplant treatment with a polyclonal IVIg-preparation is associated with a significantly reduced incidence of acute rejection in liver transplant recipients. This protective effect is at least partially explained by suppression of the allogeneic T-cell stimulatory capacity of DC by anti-HBs Ig.

## **Role of hydrophobic bile salts, phospholipids and cholesterol crystals in a rat model of biliary pancreatitis**

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Biliary reflux into the pancreatic duct is essential in the pathogenesis of biliary pancreatitis. Our aim was to investigate the role of bile composition. We hypothesized that hydrophobic bile salts or cholesterol crystals may enhance, and phospholipids may decrease severity of pancreatitis. Model systems composed with taurodeoxycholate (TDC), mixed bile salts (MBS), or tauroursodeoxycholate (TUDC, all 15mM in PBS), with or without cholesterol crystals or phosphatidylcholine (PC), were infused into bile ducts of male Sprague-Dawley rats. Twenty-four hours later, animals were sacrificed for histopathologic scoring of (peri)pancreatic inflammation. Severity of acute pancreatitis depended on bile salt hydrophobicity (TDC > MBS >> TUDC = PBS; histopathologic scores: 26.3±0.3, 23.3±2.4, 15.7±0.3, 15.5±0.5 respectively; P = 0.002). Phosphatidylcholine protected against the detrimental effect of TDC at high, but not at low concentrations (scores: 17.0±3.1 vs 25.7±3.3 in case of PC/(TDC+PC) ratios 0.25 vs 0.05). Cholesterol crystals increased the severity of pancreatitis in model systems containing TDC or MBS, but not TUDC or PBS (33.0±0.6, 31.3±0.6, 17.5±1.8, 17.5±0.6 respectively; P < 0.001). Conclusions: In the rat model, hydrophobic bile salts and cholesterol crystals aggravate biliary pancreatitis, whereas phospholipids have a protective effect.



## **The interferon-gamma gene in coeliac disease shows expression correlated with tissue transformation but no evidence for genetic susceptibility \***

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Coeliac disease (CD) is a complex genetic disorder characterized by a life-long gluten intolerance mediated by a cytotoxic T cell response. Interferon-gamma (IFN- $\gamma$ ) plays a pivotal role in this Th1 response and is an important determinant of the mucosal remodeling in the small bowel. Our aim was to further ascertain the role of IFN- $\gamma$ , both as key cytokine in the pathology, as well as a possible causative genetic factor in the etiology. Duodenal biopsies were collected from the entire spectrum of disease stages (Marsh 0-IIIc), including those from patients refractory to a gluten-free diet (RCD). RNA was isolated and used to determine IFN- $\gamma$  gene (IFNG) activity by quantitative reverse-transcription PCR. IFNG expression correlated with the extent of tissue transformation and reached 240-fold higher expression in total villous atrophy compared to healthy control tissue. CD and RCD patients with comparable lesions showed similar expression levels. Interestingly, patients on a gluten-free diet who were in complete remission still had 7.6-fold residual over-expression. We further observed remarkable inter-individual expression variability. These observations prompted us to examine IFNG as one of the candidate susceptibility genes. We tested an IFNG microsatellite DNA marker in three large cohorts of Dutch patients for both genetic linkage and association. A sib-pair linkage analysis yielded no significant positive results for IFNG and its flanking markers. Furthermore, the IFNG marker allele frequencies were not differently distributed between cases and controls. Similarly, all alleles were transmitted randomly to affected children in parents-case trios. Hence, genetic association could not be established between CD and IFNG. In conclusion, despite the importance of IFNG in the Th1 response, its mucosal transformation-correlated expression, and its enhanced activity in remission patients, we could not provide evidence for IFNG as one of the causative genes in CD.

## **The role of gene polymorphisms in the bacterial agonist recognizing TLR4 -CD14 system in the susceptibility to and severity of CD and UC in Dutch Caucasian IBD patients**

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**Introduction:** Both genetic and microbial factors seem to play a pivotal role in the aetio-pathogenesis of inflammatory bowel disease (IBD). It is suggested that a genetic predisposition leads to an unregulated intestinal immune response in part due to environmental factors. The recently identified toll-like receptor (TLR) signal transduction route, and specifically TLR4 recognizing among others LPS (via CD14) and host & bacterial heat shock protein is a potential candidate gene involved in mediating essential immune responses in the intestinal tract, predisposing patients to IBD in general or to CD or UC specifically. **Aim:** To assess the frequency of candidate gene polymorphisms in the bacterial agonist recognizing CD14 and TLR4 genes in the susceptibility to and severity of CD and UC in Dutch Caucasian IBD patients. **Methods:** 230 IBD patients (129 Crohn's Disease (CD) patients and 101 Ulcerative Colitis (UC) patients) were recruited from the Outpatient clinic of the Department of Gastroenterology of the VUMC, Amsterdam, The Netherlands. The control group comprised of 170 healthy controls. PCR based RFLPs were used to identify polymorphisms in the CD14-260 and TLR4+896 genes. Genotype and allele frequencies analyses were performed and the Vienna classification (CD patients) was used to assess potential association with disease phenotype. **Results:** The frequency of the 2 allele of TLR4+896 was increased in CD patients compared to controls (18% vs. 10%; p: 0.059). The 2.2 genotype of CD14-260 showed an increased frequency in UC patients compared to healthy controls (34% vs. 24%; p: 0.09). Carriage of TLR4+896\*2 significantly increases the risk for colonic localization of CD compared to non-colonic localization (36% vs. 14%; p: 0.0061; OR: 3.9; 95% CI: 1.5 – 10.2). **Conclusions:** The found trends and associations with the CD14-260 and TLR4+896 polymorphisms suggest a potentially important role for bacteria in the immunopathogenesis of CD and UC.

## **Clinical and functional significance of TNF- $\alpha$ , MMP-2 and MMP-9 gene promoter single nucleotide polymorphisms in inflammatory bowel disease**

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The intestinal levels of TNF- $\alpha$  and matrix metalloproteinases MMP-2 and MMP-9 are markedly increased in patients with inflammatory bowel disease (IBD). Single nucleotide polymorphisms (SNP) in the promoters of the corresponding genes have been shown to affect transcription efficiency in vitro and could also play a role in IBD etiology and/or pathogenesis. Genomic DNA was isolated from patients with Crohn's disease (CD), ulcerative colitis (UC) and controls (colorectal carcinoma (CRC) patients and healthy volunteers) (n=129, 112, 245). Allelic composition at SNP loci was determined by restriction fragment length polymorphism or tetra-primer amplification refractory mutation system (ARMS)-PCR. The MMP genotypes were compared to corresponding protein levels measured in surgically resected CD, UC and CRC intestinal mucosal tissue by ELISA (n=129, 27 and 76). In CD and UC vs controls, genotype distribution at TNF- $\alpha$  -308 G/A was similar (GG;GA;AA: 67.4, 30.2, 2.3 and 70.3, 22.5, 7.2 vs 69.8, 26.5, 3.7%, respectively). Allelic composition at MMP-2 -1306 C/T was not different between these groups (CC; CT; TT: 57.4, 36.4, 6.2 and 57.7, 35.1, 7.2 vs 62.0, 29.8, 8.2%) and also in case of MMP-9 -1362 C/T similar genotype frequencies were observed (CC;CT;TT: 76.0, 23.3, 0.8 and 69.4, 28.8, 1.8 vs 69.8, 28.6, 1.6%). Median MMP-2 and MMP-9 protein levels were 2-3 fold higher in inflamed vs non-inflamed IBD and control tissue but unrelated to MMP genotypes independent from severity of inflammation. We conclude that the SNPs at TNF- $\alpha$  -308, MMP-2 -1306 and MMP-9 -1362 apparently do not relate to IBD susceptibility and that the in vivo tissue levels of the corresponding proteins are regulated by other (genetic) factors.

## **Prevalence of Helicobacter pylori antibiotic resistance in The Netherlands: Trends in time**

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Most patients treated for H. pylori infection receive empirical treatment based on epidemiological data of antibiotic resistance. However, previous European studies indicate that resistance patterns may be changing. Therefore, the aim of this study was to investigate the prevalence of clarithromycin and/or metronidazole-resistant H. pylori over a six-year period. Patients visiting Hospital Slingeland, Doetinchem, The Netherlands between 1997 and 2002 with a positive H. pylori culture were included in this study. Susceptibility to metronidazole and clarithromycin was determined by disk diffusion. The yearly prevalence of primary resistance was calculated and related to baseline characteristics using unadjusted and adjusted logistic regression analyses. Of the 1355 patients with a H. pylori positive culture, 1127 did not have a history of H. pylori eradication, 58 did, and for 170 this information was not available. Mean rates of primary resistance to metronidazole and clarithromycin were 14.4% (162/963) and 1.0% (11/1112), respectively. Primary metronidazole resistance was stable throughout the study period (resistance rates of 15.1%, 13.1%, 20.2%, 14.7%, 9.6%, and 14.3% for the consecutive years). Primary clarithromycin resistance was decreasing (3.2%, 1.2%, 1.6%, 0.5%, 0.5%, and 0% for the consecutive years). Patients of foreign extraction and from secondary care had a higher chance of harboring primary metronidazole resistant H. pylori (adjusted OR(95%CI): 1.69(1.1-2.7), and 1.57(1.1-2.2), respectively). Patients with failed H. pylori eradication had a higher chance of harboring metronidazole resistant H. pylori (43% vs. 14%, p<0.0001) and clarithromycin resistant H. pylori (79% vs. 5%, p=0.004). In conclusion, prevalence of primary clarithromycin and metronidazole resistance is low in the eastern part of The Netherlands and did not increase between 1997 and 2002. Triple therapy with clarithromycin and amoxicillin can remain the empirical treatment of choice.

## **A specific COX-1 polymorphism (A842G/C50T) protects against peptic ulcer disease**

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Background: Cyclooxygenase (COX-1) gene polymorphisms could be important in the pathogenesis of peptic ulcer disease as these influence prostaglandin formation and consequently its protective effect at the level of the gastric mucosa. Objective: The aim of this study was to investigate the association between the functional single-nucleotide polymorphism A842G/C50T in the COX-1 enzyme and peptic ulcer disease. Methods: DNA samples were obtained from 106 patients who underwent an upper gastrointestinal endoscopy and presented with peptic ulcer disease, 133 patients hospitalized for cardiovascular disease and 101 healthy individuals. Genetic polymorphism in A842G/C50T was determined by PCR followed by restriction-fragment-length-polymorphism analyses. Logistic regression analysis was performed to evaluate the association between the polymorphism and peptic ulcer disease. Results: The percentage of patients carrying the A842G/C50T COX-1 allele 7 in the peptic ulcer disease group, 11 in the healthy controls and 18 in the cardiology patients. The adjusted risk for peptic ulcer disease among individuals who were heterozygote or homozygote for the A842G/C50T polymorphism was 0.44 (95% confidence interval: 0.19-1.03) compared with common allele homozygotes. All patients with the A842G/C50T COX-1 polymorphism had a duodenal ulcer. Conclusion: COX-1 A842G/C50T SNP carriers have a lower risk for developing peptic ulcer disease in comparison to those without the polymorphism.

## Octreotide for Therapy-resistant Functional Dyspepsia

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Recent studies indicate that somatostatin affects gastric motor and sensory function. This implicates that the somatostatin-analogue octreotide (OCT) might be of benefit in the treatment of functional dyspepsia (FD). However, a subset of FD-patients is characterized by delayed gastric emptying and OCT is known to further delay gastric emptying. Therefore gastric emptying rate might be a confounder in the response to OCT-therapy. Our aim was to investigate the effect of OCT, both subcutaneous (sc) and intramuscular depot (OCT-LAR), on a) FD-symptoms and quality of life, b) gastric emptying and c) the relation between gastric emptying and clinical response. In an open label study, 12 otherwise therapy-resistant FD-patients (10F, age 27-59 yrs) were treated for 1 month with OCT 50 µg sc tid. and thereafter for 3 months with depot OCT-LAR 20 mg every 4 weeks. Symptoms were scored using symptom diaries (scale 0-5). Quality of life was evaluated using the Gastrointestinal Quality of Life Index (GIQLI-score; 0-144) after each treatment period. Before OCT-treatment gastric emptying was measured twice, (C13-acetate breath test) once with OCT 50 µg sc and once with placebo. Main symptom severity sign. decreased from  $3.8 \pm 0.4$  to  $3.0 \pm 0.5$  ( $P < 0.05$ ) during OCT sc and to  $2.9 \pm 0.5$  ( $P < 0.05$ ) during OCT-LAR. GIQLI-scores increased sign. ( $P < 0.05$ ) from  $91 \pm 6$  to  $101 \pm 4$  during OCT-LAR. (OCT sc versus OCT-LAR n.s.). Gastric emptying was sign. delayed by OCT versus control ( $t_{1/2} = 162 \pm 12$  vs.  $127 \pm 12$  min resp.;  $P < 0.05$ ) Responders to OCT-therapy (>20% symptom reduction; n=6) were characterized by a sign. more rapid control gastric emptying than non-responders ( $t_{1/2} = 115 \pm 4$  min vs.  $139 \pm 7$  min;  $P < 0.05$ ). These data show that in a considerable subset (50%) of FD-patients OCT-treatment decreases symptom severity and improves quality of life. Non-responders were characterized by initial slower gastric emptying. The role of OCT in the treatment of otherwise therapy-resistant FD deserves further evaluation.

## **The effect of a multispecies probiotic on the intestinal flora and bowel habits in healthy volunteers treated with amoxicillin.**

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Studies with monospecies probiotics focussing on clinical outcome have shown a decrease in the duration of antibiotic-associated diarrhoea. However, studies of multispecies probiotics on clinical outcome as well as the intestinal flora are lacking.

The effect of a multispecies probiotic on the composition of the flora and bowel habits was studied in 41 healthy volunteers treated with amoxicillin 500 mg bid for 7 days. In addition, a probiotic (10<sup>10</sup> cfu/day) or placebo was given for 14 days. Faecal samples and questionnaires were collected at 0, 7, 14 and 63 days. Total colony forming units (cfu) per gram faeces of aerobic and anaerobic bacteria were determined by culture. Faecal consistency and frequency were scored daily according to the Bristol scale.

Forty subjects (19 probiotic, 21 placebo) completed the study. After probiotic treatment, compared to placebo, an increase in median faecal enterococci was found at t=7 (5.7 vs 3.9 log cfu/g faeces (p<0.01)) and at t=14 (7.1 vs 4.1 (p<0.01)). In addition, a tendency towards an increase in median total anaerobes was found at t=14 days 8.8 vs 7.9 (p=0.06).

Moreover, in the probiotic group significant increases were observed over time in total aerobes (t=7 vs t=63), enterobacteriaceae (t=7 vs t=14), enterococci (t=7/14 vs t=0/63), total anaerobes (t=14 vs t=7, t=63 vs t=0) and *Bacteroides* spp. (t=7 vs t=14) (p<0.05). Within the placebo group significant increases were found in enterococci (t=14 vs t=0) and total anaerobes (t=63 vs t=0) and significant decreases were found in clostridia (t=7 vs t=0/14/63) (p<0.05).

Bowel movements with a frequency  $\geq 3$  and/or a consistency  $\geq 5$  for at least 2 days, were reported less frequently in the probiotic compared to the placebo group (48% vs 79% (p<0.05)).

The intake of a multispecies probiotic affects the composition of the faecal flora during and after intake of amoxicillin and decreases the number and consistency of bowel movements in healthy volunteers.

## **The effect of the probiotic *L. plantarum* 299v on the faecal and mucosal bacterial flora of patients with inactive ulcerative colitis**

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Beneficial effects of probiotics have been found in ulcerative colitis (UC). Most studies focus on clinical outcome without investigating microbiological parameters. In a placebo-controlled double-blind study, the effect of *L. plantarum* 299v on faecal and mucosal bacterial composition and faecal enzyme activities has been studied in inactive UC patients. Inactive UC patients (CAI<10) consumed a drink with or without *L. plantarum* 299v ( $10^{11}$  cfu/day) during four weeks. Faecal samples were collected before and after consumption. After consumption, rectal biopsies were collected in a subgroup of patients. Colony forming units (CFU) per gram faeces or per biopsy of (total) aerobic and anaerobic bacteria were determined.  $\beta$ -glucosidase (mg/h/g faeces) and  $\beta$ -glucuronidase (mg/1/2h/g faeces) activities were analysed in faecal samples. Twenty-seven patients completed the study (13 probiotic, 14 placebo) of which in 13 (5 probiotic, 8 placebo) biopsies were collected. An increase ( $p<0.05$ ) in the mean number of faecal lactobacilli after probiotic consumption (from  $3.4\pm 1.7$  to  $7.1\pm 0.9$ ) but no significant differences in other faecal aerobic and anaerobic bacterial counts were observed. In rectal biopsies, no significant differences were seen in aerobic and anaerobic bacterial counts and prevalences, with the exception of total anaerobic bacteria, which were lower in the probiotic compared to the placebo group ( $2.3\pm 0.8$  vs  $4.0\pm 1.2$ ) ( $p<0.05$ ). However, prevalences of mucosal lactobacilli, *E. coli*, enterococci, *Bacteroides* spp. and clostridia were low in both groups. Furthermore, after probiotic and placebo consumption,  $\beta$ -glucosidase ( $0.7\pm 0.6$  vs  $0.6\pm 0.4$ ) and  $\beta$ -glucuronidase ( $1.5\pm 1.2$  vs  $1.2\pm 0.8$ ) activities did not change significantly. In UC patients, consumption of *L. plantarum* 299v increased the faecal number of lactobacilli significantly but only small numbers were found in biopsy samples and no effects were observed on other bacterial counts nor on enzyme activities.



## Liver histology in inflammatory bowel disease

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**Introduction:** Liver biopsies specimens of patients undergoing surgery for Crohn's disease (CD) or ulcerative colitis (UC) frequently show histological abnormalities. Hepatotoxicity may be due to the inflammatory bowel disease (IBD) or due to hepatotoxic drugs, in particular immunosuppressive medication (azathioprine, methotrexate or 6-thioguanine). In addition 5-ASA and prednisone have also been associated with liver damage. Though clinical signs of hepatic damage or insufficiency are sparse.

**Aims & Methods:** Assessment of liver histology/liver parameters in a cohort of IBD patients who underwent surgery. Routine blood examinations were performed. Histology was evaluated mainly on inflammatory activity, fibrosis, necrosis and steatosis.

**Results:** Biopsies were performed in 109 UC (F:23/M:23) and CD (F:41/M:22) patients, independent of laboratory results, from 1977 till 1998 during surgical procedures. Mean age at the moment of biopsy was 35 years for CD (SD 13) and 45 years for UC (SD 16). In the group who received 5-ASA alone, 93% (13/14) had liver histology abnormalities, compared to 64% (prednisone (17/28)), 79% (prednisone and 5-ASA (22/28)) and 75% (prednisone and AZA (3/4)). Twenty percent (29/109) of the patients used no medication, 76 percent (22/29) of this group had liver histology abnormalities. The most frequent observed overall histologic abnormalities are binucleation (40%), lymphocyte (34%) and histiocyte (32%) infiltration in the portal area, steatosis (33%) and interstitial fibrosis (22%). Liver test abnormalities (twice UNL) were found in 23.4% (GGT), 7.2% (alkaline phosphatase (AF)) and 5.4% (ALAT) of the total patient population.

**Conclusion:** 75 percent of the patients with CD and UC had abnormal liver histology, laboratory results were shown not be indicative for liver damage in general. The real incidence of induced hepatotoxicity due to medication for IBD might be lower as there is a high background level of liver abnormalities in CD and UC.

## **Inflammatory bowel disease after liver transplantation: a role for cytomegalovirus infection**

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Despite the use of immunosuppressive drugs, recurrent and de novo inflammatory bowel disease (IBD) can develop after orthotopic liver transplantation (OLT). Cytomegalovirus (CMV) infection has been implicated in the pathogenesis of IBD. The aim of this study was to investigate the role of CMV infection on the development of IBD after OLT. All 84 patients (pts) transplanted for primary sclerosing cholangitis (PSC) or autoimmune hepatitis (AIH) between May 1987 and July 2002, without a prior colectomy, who survived the first year after transplantation were studied retrospectively. IBD was diagnosed based on endoscopy and histology. The diagnosis of active CMV-infection was made using the pp65-antigenemia assay. Post-OLT colonic biopsy specimens were tested immunohistochemically for CMV. 31 of 84 pts (37%) had IBD prior to OLT, mostly ulcerative colitis (81%). 18 pts (21%) experienced IBD after OLT, either as flare up (12 pts) or de novo (6 pts), at a median of 1.4 years (range 0.3-6.3) after OLT. 48% of all pts experienced CMV infection after OLT, at a median of 27 days (range 8-193). CMV-infection was primary in half of pts. Active-IBD-free survival after OLT was significantly higher in pts who did not develop CMV infection compared to those who did, at 5 years 88% and 67% respectively (p=0.05). De novo IBD was seen only in pts who had experienced a CMV infection (p=0.02). There was no relationship with duration or severity of CMV-infection. All but one colonic biopsies tested were immunohistochemically negative for CMV. Conclusion: In patients transplanted for end-stage PSC or AIH, active IBD, especially de novo IBD, occurred more often in patients who experienced CMV-infection in the postoperative period. This finding supports a pathogenic role for CMV in the development of IBD.

## **Clinical and endosonographic effect of ciprofloxacin on the treatment of perianal fistulas in Crohn's disease with infliximab: a double-blind placebo-controlled study**

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Ciprofloxacin is moderately effective for treating perianal fistulizing Crohn's disease (CD) but symptoms usually reoccur when treatment is discontinued. Infliximab is effective but often requires maintenance therapy. We aimed to evaluate whether a combination of ciprofloxacin and infliximab is more effective than infliximab alone. A double blind placebo-controlled study in patients with perianal fistulizing CD was performed. Patients were randomly assigned to receive 500-mg ciprofloxacin twice daily or a placebo for 12 weeks. All patients received 5-mg/kg infliximab in week 6, 8 and 12 and were followed for 18 weeks. Primary endpoint was clinical response, defined as a 50% or greater reduction from baseline in the number of draining fistulas. Secondary endpoints were the change in Perianal Disease Activity Index (PDAI) and hydrogen peroxide enhanced 3D-endoanal ultrasonography (3D HPUS) findings between baseline and week 18. Analysis was by intention-to-treat. In total 24 patients were included, two discontinued treatment and 22 completed the study. After six weeks, clinical response was 9% (1/11) in the ciprofloxacin group and 15% (2/13) in the placebo group ( $p=1.0$ ). At week 8 and 12 this increased to 91% (10/11) in the ciprofloxacin group and 62% (8/13) in the placebo group ( $p=0.17$ ). At week 18, response was 73% (8/11) in the ciprofloxacin group and 39% (5/13) in the placebo group ( $p=0.12$ ). Logistic regression analysis showed a tendency for patients treated with ciprofloxacin to respond better (OR= 2.37, CI: 0.94-5.98,  $p=0.07$ ). The PDAI improved ( $p=0.02$ ,  $p=0.008$ ) in the ciprofloxacin group but not in the placebo group. On 3D HPUS, improvement was seen in three patients with a clinical response. Conclusions: A combination of ciprofloxacin and infliximab seems to more effective than infliximab alone in the treatment of perianal fistulas in CD. The PDAI improves significantly. Despite clinical response, persistent fistula tracts remain visible on 3D HPUS.

## **Pharmacokinetic effect of discontinuation of mesalamine on 6-mercaptopurine metabolite levels in IBD patients.**

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**Introduction:** Mesalamine (5ASA) and thiopurines like 6-mercaptopurine (6MP) are widely used in inflammatory bowel disease (IBD). In vitro studies suggest interactions between 5ASA and thiopurines by inhibition of thiopurine methyltransferase (TPMT), which methylates 6MP to 6MMP and influences the balance between hepatotoxic 6MMP and immunosuppressive 6TGN metabolites. Recently the use of 5ASA in M.Crohn (CD) is debated. **Methods:** we performed an interaction study in the Sittard IBD subcohort using 6MP combined with 5ASA for at least 3 months. All patients were in remission at inclusion. 6MMP and 6TGN were measured at t=0, 4 weeks after discontinuation (t=4w) and 4 weeks after reintroduction of 5ASA (t=8w). 6MP was continued during the study interval. Primary outcomes were 6TGN and 6MMP metabolite levels at t=0,4,8w. Secondary outcomes were correlations between 6TGN and 6MMP, 6MP or 5ASA dose in mg/kg, laboratory parameters and disease activity. **Results:** 27 IBD patients used 6MP and 5ASA: 17 were included, 3 had an exacerbation and 7 refused. CD/ulcerative colitis ratio was 12/5. Mean 6MP and 5ASA doses were 0.78 and 43 mg/kg. Mean 6TGN levels were 262 (CI 212-312), 209 (170-247) and 270 (217-322) pmol/10<sup>8</sup> RBC at t=0,4 and 8w and were significantly lower at t=4w than t=0 and 8w (p<0.01). Mean 6MMP levels were 1422 (CI 721-2122), 2149 (730-3568) and 1503 (721-2284) pmol/10<sup>8</sup> RBC at t=0, 4 and 8w, but did not significantly vary. No correlations were found between 6MP/5ASA dose and 6MMP or 6TGN levels or between these levels. Leucocyte counts were stable. Four patients noticed slight worse disease activity at t=4w, recovering spontaneous. **Discussion:** a significant decrease in 6TGN levels was found after interrupting 5ASA, reversible after reintroduction of 5ASA. 5ASA seems to inhibit TPMT, leading to increased 6TGN levels. In theory, 5ASA potentiates 6MP efficacy. **Conclusion:** Because of this synergistic effect on 6MP, 5ASA may still have a place in therapy of CD.

## **Dendritic Cells in Immunotherapy for Esophageal Adenocarcinoma: The Near Future**

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Despite surgical intervention the outcome of these patients with esophageal carcinoma is poor and novel treatments are necessary. Dendritic Cells (DC's) are potent antigen presenting cells, which regulate the immune system and are responsible for the activation of both humoral and cellular immune response, capable of stimulating CD8+, CTLs, CD4+ helper T cells, NK and NK T-cells. Because of this capability, there is a growing interest in using ex-vivo generated DC's loaded with tumor antigen as a tool for cancer immunotherapy. Our aim is to develop immunotherapy for esophageal cancer employing RNA autologous to the cancer cells. Tumor and normal esophageal mucosa biopsies were taken, and 80cc of blood was drawn from patients. Total RNA was extracted from the tumor and normal biopsies. Lymphocytes (T-cells) and monocytes were isolated from blood. Monocytes were matured into DC's and transfected with total tumor RNA or normal RNA. Upon incubation with patient's T-cells, transfected DC's sustained T-cell proliferation and polarization to CD8+ cytotoxic T-cells as compared to cultures incubated with untransfected DCs. Simultaneously, primary cell cultures of the carcinoma and of the patient's normal squamous mucosa were derived. Stimulated lymphocytes were given to the cell cultures and apoptosis was measured by annexin labeling and analysed through flow cytometry. The normal cells exposed to lymphocytes that were stimulated by DC's loaded with tumor and normal RNA, did not show increased apoptosis. The cancer cells showed massive apoptosis, through a cytotoxic lymphocyte response, when incubated with lymphocytes that were stimulated with DC's loaded with tumor RNA, but not when DC's were loaded with normal RNA. This set up will be used as a base to safely treat esophageal cancer patient with immuno-gene therapy. These results represent an important step forward in the development of immuno-gene therapy for esophageal cancer.

## **Fiber modified adenovirus vectors that target to EphrinA2 Receptor and Vascular Endothelial Growth Factor Receptor II reveal enhanced gene transfer to human pancreatic tumor cells**

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Human pancreatic cancer (PC) has a poor prognosis and requires new treatment options such as adenovirus (Ad) gene therapy. Conditionally Replicating Adenoviruses (CRAds) specifically lyse tumors while leaving normal cells unharmed. Low expression of the Coxsackie- and Adenovirus Receptor on PC hampers CRAd efficacy in clinical trials. In this study we test targeting of Ad vectors expressing polypeptides in the fiber knob that bind to human PC cells which express the following membrane antigens: Neurotensin receptor (NTR), Vascular Endothelial Growth Factor receptor II (VEGF-RII), and Ephrin A2 receptor (EphA2). DNA encoding for the peptides neurotensin (NT), K237, YSA/SWL that bind to NTR, VEGF-RII and EphA2, respectively, were cloned into a cytomegalovirus promoter controlled green fluorescent reporter protein (GFP) backbone system. Virus was propagated on 293 cells and titered with the lightcycler. PC and normal human cells were transduced with 1000 viral particles per cell. After 24 h, GFP production was measured. In addition, expression of NTR, VEGF-RII and EphA2 on human PC cells was determined by Western blotting and FACS. Compared to wildtype Ad5, Ad-K237, Ad-YSA, and Ad-SWL showed a 12.1, 8.8, and 8.0-fold increase in transduction, respectively. Ad-NT failed to show increased targeting to human PC cells. Ad-K237 revealed 9.4- and 8.1-fold higher transduction efficiency to fibroblasts and hepatocytes, respectively, than Ad-Wt, indicating non-tumor specific receptor expression of VEGF-RII. In contrast, both Ad-YSA and Ad-Wt reveal limited transduction of fibroblasts, while hepatocytes showed only 3.4-fold increase of Ad gene transfer. Every receptor was detected on the PC cell lines. We conclude that adenoviral gene transfer to PC cells is enhanced by targeting fiber modified Ads to the VEGFR-II and EphA2 receptor. The YSA peptide shows the most specific transduction to PC cells, making this peptide a promising candidate for targeting of CRAds to PC.

## **Glutathione S-transferases and UDP-glucuronosyltransferases in duodenal mucosa of patients with Familial Adenomatous Polyposis and controls**

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The duodenum is the main site for (pre-) malignant extra-colonic manifestations in patients with Familial Adenomatous Polyposis (FAP). The prevalence of the mainly peri-ampullary adenomas varies from 50% to greater than 90%, whereas in 2-5 % of the FAP patients duodenal carcinomas have been detected. Compared to the general population the relative risk of duodenal adenocarcinoma is high. The detoxifying enzymes glutathione S-transferases (GSTs) and UDP- glucuronosyltransferases (UGTs) are involved in the mucosal protection against carcinogenesis. Conjugation with glutathione or glucuronic acid respectively, converts potential carcinogens in the intestine to more water-soluble and less biologically active molecules, which can be excreted in bile or urine. A significant inverse correlation between GST activity in normal mucosa along the gastrointestinal tract and the tumour incidence at these sites has been observed. Furthermore, a decreased GST activity has been observed in the colon of FAP patients, which may also contribute to the high colon cancer risk. The aim of this study is to compare the detoxification capacity in duodenal mucosa of FAP patients with that of healthy controls. Normal appearing duodenal biopsies were obtained from 18 FAP patients (mean age  $49 \pm 15$  years, 10 M / 8 F) and 18 controls ( $50 \pm 13$ , 8 M/ 10 F). The GST isoforms were quantified by SDS PAGE and immunoblotting. GST enzyme activity was measured spectrophotometrically using CNDB as substrate. UGT enzyme activity was assayed spectrofluorometrically with 4-MUB as substrate. No differences were observed in the GST isoform levels, as well as in the GST and UGT enzyme activities, in the duodenal mucosa of FAP patients compared to healthy duodenal mucosa. In conclusion GST isoform levels, GST and UGT enzyme activities are not lower in the duodenum of FAP patients as compared to controls. Therefore, these detoxification enzymes seem not to be involved in the duodenal adenomatosis in FAP.

## **Specific kinase profiling in colon cancer predicts stage of disease and identifies potential molecular targets for therapy.**

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The main prognostic marker in colon carcinoma (CC) is nodal status at time of primary resection. However, staging of CC fails to accurately predict outcome of disease. The number of patients needed to treat with chemotherapy to save one life is 10 for stage III, and even 30 for stage II CC. Rapid technological advances in molecular biology has resulted in array based techniques for the detection of kinase activity. Kinomic profiling is a promising new approach for cancer diagnostics, since the biological function and cellular metabolism is resembles the phenotype of cancer. We hypothesized that kinomic profiling differentiates normal epithelial from CC and tumor from metastasis of 10 patients. A newly developed protein microarray containing 1200 peptides in duplo with specific phosphorylation sites (PepChip) was used for in vitro phosphorylation analysis with <sup>33</sup>p-gamma-ATP. Histological analysis of cryosections was performed to ensure the quality of the tissue before frozen tissue specimens were lysed. We optimized statistical correlation coefficient dissimilarity measurements to extract kinases that were significantly up- or downregulated. Specific kinomic profiles of normal colon (10) versus colon cancer (10) and metastasis (10) were detected by the kinase array. Significantly enhanced activity of the kinases IGFr, PI3k, Akt, and PKC was observed in tumour compared to normal tissue. Distinct kinomic profiles marked metastatic lesions from primary tumour. The results were reproducible with correlation coefficients of at least 0.80 for the duplo. All findings were validated using western blot analysis and in vitro kinase assays. We conclude that the kinase peptide substrate array is a powerful method to detect cellular metabolism profiles that distinguish normal colon, colon tumour tissue and colon metastasis. In addition important kinases responsible for oncogenic behaviour are elucidated which potentially provide targets for therapy.



## **Kinome Analysis Reveals Lck and Fyn As Novel Targets Of Glucocorticoid Action In Activated T cells**

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Glucocorticoids (GCs) are effective in inhibiting inflammatory and immune responses. Apart from their classic genomic effects, it is known that GCs have rapid, nongenomically mediated effects. However, the molecular mechanisms being responsible for these effects are still largely unknown. The aim of this study was to evaluate the rapid effects of GCs on signal transduction in activated T cells, as these immune cells are important target cells for GC action. Therefore, human CD4 lymphocytes were treated with dexamethasone (10<sup>-6</sup>M), a synthetic GC, for 10 minutes and subsequently activated with anti-CD3 and anti-CD28 antibodies for 15 minutes. Total cell lysates were used for arrays, containing up to 1.200 kinase substrate peptides on a single chip, in order to study the effects of GCs on kinase activity. Additionally, outcomes were validated by in vitro kinase assay and western blot. Our kinase arrays revealed significant alterations of kinomic profiles due to dexamethasone treatment in activated CD4 lymphocytes. Interestingly, Lck and Fyn activity turned out to be down regulated in dexamethasone present conditions, and this was reconfirmed by in vitro kinase assays. Lck and Fyn, members of the Src family, are key players in T-cell receptor (TCR) signaling, since they are responsible for the initial phosphorylation of the TCR, leading to recruitment and subsequent activation of various signaling molecules downstream of the TCR. Accordingly, western blot showed decreased phosphorylation of various signaling molecules downstream of the TCR due to dexamethasone treatment, pointing out the rapid effects of GCs on early signaling events initiated upon TCR activation. Conclusion: Kinome analysis revealed rapid effects of GCs on various kinases in immune cells. Lck and Fyn were identified as novel targets for GC treatment in activated T cells.

## **Internalization of *Campylobacter jejuni* into Caco-2 cells is lipo-oligosaccharide class A/B dependent**

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*C. jejuni* infection, the most frequent cause of bacterial gastro-enteritis, may result in Guillain-Barré syndrome (GBS), an immune-mediated disorder affecting peripheral nerves. Pathology of GBS involves cross-reactive anti-ganglioside antibodies that arise from molecular mimicry between *Campylobacter* lipo-oligosaccharides (LOS) and neuronal gangliosides, both containing sialic acid. The LOS biosynthesis gene clusters class A/B are found more often in GBS-associated *C. jejuni* strains than in enteritis-associated strains. It is known from the literature that LOS composition may affect adhesion and internalization of *C. jejuni* into host intestinal epithelial cells. We hypothesise that internalization of *C. jejuni* may result in escape from immediate elimination by the innate immune system and thereby aid adaptive immune responses, including production of anti-ganglioside antibodies. We studied 7 GBS- and 8 enteritis-associated *C. jejuni* strains from Dutch patients for their capacity to internalize into Caco-2 intestinal epithelial cells. On average GBS strains and enteritis strains internalized equally well. However, *C. jejuni* with LOS class A/B internalized significantly better than *C. jejuni* with LOS class C/D/E. In three *C. jejuni* strains with LOS class A/B, knockout mutants of a putative acetyltransferase (*putA*) and a *cstII* gene involved in transfer of sialic acid respectively, were constructed. *CstII* and *putA* mutants did not differ in motility compared to their wild type. However, knock out mutagenesis resulted in a significant reduction of internalization of Caco-2 cells by one strain, whereas the internalization capacity of the two other strains was not affected significantly. Conclusion: Strains from *Campylobacter* LOS classes show strongly differing internalization capacity in Caco-2 cells, but bacterial factors other than LOS ganglioside epitopes are involved in the internalization of *C. jejuni* into intestinal epithelial cells.

## **Bacterial DNA exacerbates the inflammatory response and gut barrier failure caused by systemic hypotension.**

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Bacterial DNA is characterized by unmethylated CpG motifs that induce Th1 cytokines such as TNF- $\alpha$  and IFN- $\gamma$ . Recently was discovered that oligodeoxynucleotides (ODN) containing unmethylated CpG motifs aggravate local intestinal inflammation in experimental colitis. Here, we investigate the role of CpG-ODN, as common denominator of bacterial infection, on intestinal barrier function in sepsis. The effects of CpG-ODN on the inflammatory response and gut barrier failure were studied in a model of sepsis (hemorrhagic shock) in rats. Rats were exposed to CpG-ODN or not exposed (control) before hemorrhagic shock (n=7 per group). At 4 hours after hemorrhagic shock TNF- $\alpha$ , IL-6 (proinflammatory), IL-10 (anti-inflammatory) and NO<sub>2</sub><sup>-</sup> were determined in plasma. Gut barrier function was assessed by measurement of horseradish peroxidase (HRP) leakage in ileum segments and bacterial translocation to distant organs by culture. Exposure to CpG-ODN (without shock) impaired gut barrier function and caused a mild inflammatory response. Interestingly, administration of CpG-ODN together with hemorrhagic shock significantly augmented inflammatory mediators: TNF- $\alpha$  (68 $\pm$ 13 pg/ml vs. control (c): not detectable, p<0.001), IL-6 (171 $\pm$ 33 pg/ml, vs. c: 12 $\pm$ 6 p<0.005) and NO<sub>2</sub><sup>-</sup> (169 $\pm$ 38  $\mu$ M vs. c: 97 $\pm$ 7  $\mu$ M, p<0.05) and caused a defective IL-10 response (62 $\pm$ 4 pg/ml, vs. c: 148 $\pm$ 13 pg/ml, p<0.01). Intestinal barrier function, measured as permeability for HRP was increased (60 $\pm$ 11  $\mu$ g/ml, vs. c: 36 $\pm$ 3  $\mu$ g/ml p<0.05) and bacterial translocation to distant organs was elevated (total 819 cfu/g vs. c: total 330 cfu/g, p<0.05). In conclusion, it is shown that immunostimulatory CpG-ODN causes gut barrier failure and induces a "two-hit" phenomenon, exacerbating the host response to systemic hypotension. These data reveal potential harmful effects of CpG-ODN on gut barrier function in compromised and non-compromised animals.

## **Regulation of the murine Muc2 mucin gene by GATA factors in intestinal cells \***

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Mucin production and secretion by specialized epithelial cells is a common mechanism used by mammals to protect the underlying mucosae. MUC2 is the major mucin of the mucus layer covering both the human and rodent colonic epithelium. Previous studies have shown that the expression of MUC2 is altered in inflammatory bowel disease. However the molecular mechanisms responsible for MUC2 specific expression in the intestine are still largely unknown. Aim: Having found putative GATA binding sites by computer analysis both in the human and murine genes, and because of the restricted pattern of expression of MUC2 in the intestine, we undertook to study the regulation of the mouse promoter by GATA-4/-5/-6 factors. Methods: GATA binding sites were identified by electrophoretic mobility shift assays. A panel of deletion constructs up to 2.2 kb of the mouse Muc2 promoter made in pGL3 basic vector were used to transfect murine goblet cell-like (CMT-93) and rat intestinal crypt-like (IEC6) cell lines, respectively. The role of the GATA factors on the Muc2 gene regulation was investigated by the means of RT-PCR and co-transfections in the presence of expression vectors encoding either wild-type or mutated GATA factors or by mutating the GATA site within the promoter of Muc2. Results: GATA-4 expression was found in the nuclei of goblet cells in the small intestine. Four GATA-4 cis-elements were identified in the promoter and Muc2 promoter was efficiently activated when GATA-4 was overexpressed in the cells with a loss of transactivation when those sites were mutated or a mutated form of GATA-4 was used. Overexpression of GATA-5 and GATA-6 also induced activity of Muc2 promoter. Conclusion: Altogether these results point out an important role for GATA factors as a potent activator of Muc2 expression in intestinal cells during embryonic development of the intestine and in maintaining intestinal homeostasis in adults.

## **The NikR Protein Mediates Nickel-Responsive Induction of *Helicobacter pylori* Urease via Binding to the ureA Promoter**

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To survive in its acidic habitat, *Helicobacter pylori* requires high-level production of the nickel-containing metalloenzyme urease. The nickel-regulatory protein NikR was previously shown to be involved in acid- and nickel-responsive induction of urease expression and activity, but the molecular mechanism behind this regulation is so far unknown. Therefore the aim of this study was to further investigate the role of the NikR protein in regulation of the urease virulence factor. *H. pylori* reference strain 26695 and its isogenic *nikR* mutant were grown in Brucella media supplemented with 20 and 200  $\mu\text{M}$   $\text{NiCl}_2$ , and with 20  $\mu\text{g/ml}$  chloramphenicol when appropriate. Urease expression was determined by urease activity measurement and SDS-PAGE. Transcriptional regulation of urease genes was monitored by Northern hybridization, while gel mobility shift assays and DNase footprint assays were used to characterize the interaction of recombinant *H. pylori* NikR with the *ureA* promoter. The transcription of the urease genes and urease activity was nickel-induced in wild-type *H. pylori*, whereas this nickel-induction was absent in the *nikR* mutant. Supplementation of cultures with the translation inhibitor chloramphenicol also abolished most of the nickel-responsive induction of urease activity, demonstrating that not altered mRNA stability, but increased transcription is responsible for nickel-responsive induction of urease expression. Recombinant NikR protein was able to bind to the *ureA* promoter only in the presence of nickel. Removal of a palindromic sequence from the *ureA* promoter also abolished binding of NikR. Conclusion: The NikR protein directly binds the *ureA* promoter of *H. pylori* in a nickel- and sequence-dependent manner, resulting in nickel-responsive activation of urease expression. This indicates that NikR can function as activator of gene transcription, which contrasts with the repressor function attributed to this class of regulatory proteins.