LISTA CELOR MAI RELEVANTE 12 LUCRĂRI

ANCA TRIFAN

1. **Anca Trifan,** Carol Stanciu, Cristina Muzica (Editors). **ESSENTIALS OF NON-ALCOHOLIC FATTY LIVER DISEASE. COMPLICATIONS AND EXTRAHEPATIC MANIFESTATIONS. Springer 2023,** ISBN 978-3-031-33547-1. ISBN 978-3-031-33548-8 (eBook).
https://doi.org/10.1007/978-3-031-33548-8
Volumul, publicat în cadrul unei Edituri Internaționale prestigioase, prezintă metodele științifice, atât cele standard, cât și cele de ultimă generație, utilizate în diagnosticul și stadializarea NAFLD - Boala ficatului gras non-alcoolic, și în evaluarea manifestărilor extrahepatice ale acesteia. Sunt sintetizate cele mai recente trialuri care evaluează eficacitatea noilor agenți terapeutici și rolul intervențiilor nutriționale pentru NAFLD.
Până în prezent, volumul a fost accesat de 6610 de ori pe portalul Editurii Springer.
2. **Anca Trifan,** C. Gheorghe, D. Dumitrașcu, M. Diculescu, Liana Gheorghe, I. Sporea, M. Tanțău, T. Ciurea. **GASTROENTEROLOGIE ȘI HEPATOLOGIE CLINICĂ** (Ediție revizuită și completată), Editura Medicală, București, 2023. 2 volume. ISBN 978-973-39-0942-2.
Ediție revizuită și completată a Tratatului de specialitate din 2018, elaborat la inițiativa Președintei Societății Române de Gastroenterologie și Hepatologie, profesor Anca Trifan. Tratat de referință pentru pregătirea medicilor în specialitatea Gastroenterologie și Hepatologie.
3. **Trifan A. MANUAL DE ENDOSCOPIE. VOL. 2. COLONOSCOPIA**. Iași: Ed Junimea, 2003, ISBN 973-37-0871-2. 224 pag.
Volumul reprezintă primul și singurul manual de colonoscopie din România, elaborat concis și sintetic, dar în același timp cu o bogăție de informații și imagini, într-o manieră ideală atât pentru începători, cât și pentru cei avansați în această tehnică.
4. **Trifan A**, J Ren, R Arndorfer, C Hofmann, E Bardan, R Shaker. Inhibition of progressing primary esophageal peristalsis by pharyngeal water stimulation in humans. *Gastroenterology*. 1996; 110(2): 419–423. IF 9.329; 24 de citări conform Web of Science.
https://www.gastrojournal.org/article/S0016-5085(96)00072-8/pdf
Gastroenterology este prima revistă din domeniul gastroenterologiei, pe plan mondial. Trifan A., singurul prim autor român, cu apartenență la o Universitatea de Medicină din România.
5. **Trifan A**, Reza Shaker, Junlong Ren, Ravinder K. Mittal, Kia Saeian, Kulwinder Dua, Motoyasu Kusan. Inhibition of resting lower esophageal sphincter pressure by pharyngeal water stimulation in humans. Gastroenterology. 1995; 108(2): 441–446. IF 8.203; 47 de citări conform Web of Science.
https://www.gastrojournal.org/article/0016-5085(95)90072-1/pdf
Gastroenterology este cea mai importantă revistă din domeniul patologiei digestive, pe plan mondial. Trifan A., singurul prim autor român, cu apartenență la o Universitate de Medicină din România.
6. Rondonotti E, Pennazio M, Toth E, Menchen P, Riccioni ME, De Palma GD, Scotto F, De Looze D, Pachofsky T, Tacheci I, Havelund T, Couto G, **Trifan A,** Kofokotsios A, Cannizzaro R, Perez-Quadrado E, de Franchis R; European Capsule Endoscopy Group; Italian Club for Capsule Endoscopy (CICE); Iberian Group for Capsule Endoscopy. **Small-bowel neoplasms in patients undergoing video capsule endoscopy: a multicenter European study.** ***Endoscopy.*** 2008; 40(6): 488-495. **IF 6.091;**159 citări conform Web of Science.
https://www.thieme-connect.com/products/ejournals/abstract/10.1055/s-2007-995783
Includerea ca singurul centru din România în acest studiu european multicentric de videocapsulă reprezintă recunoașterea valorii și a experienței în activitatea cu videocapsula endoscopică, introdusă în premieră națională la Iași în 2003, datorită profesorilor Carol Stanciu și Anca Trifan.

7. **Trifan A,** Stanciu C, Girleanu I, Stoica OC, Singeap AM, Maxim R, Chiriac SA, Ciobica A, Boiculese L. Proton pump inhibitors therapy and risk of Clostridium difficile infection: Systematic review and meta-analysis. World J Gastroenterol. 2017; 23(35): 6500-6515 IF 3.365
https://www.wjgnet.com/1007-9327/full/v23/i35/6500.htm
Lucrare citată de 264 de ori conform Google Academic, si de 156 de ori conform Web of Science, Thomson Reuters.

8. Singeap AM, Stanciu C, **Trifan A.** Capsule endoscopy: The road ahead. World J Gastroenterol. 2016; 22(1): 369-378. IF 2.787.
https://www.wjgnet.com/1007-9327/full/v22/i1/369.htm
Lucrare de tip review, care sintetizează rolul actual și prezintă perspectivele de viitor ale videocapsulei endoscopice, reflectând expertiza în domeniul acestei investigații introduse de către profesorii Carol Stanciu și Anca Trifan în premieră națională la Iași.
Articol citat de 91 de ori conform Google Academic, și de 56 de ori conform Web of Science, Thomson Reuters.

9. **Trifan A**, Stanciu C. Checkmate to liver biopsy in chronic hepatitis C? World J Gastroenterol. 2012; 18(39): 5514-5520. IF 2.547; 25 de citări conform Web of Science.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3482637/
Lucrarea reflectă viziunea inovativă în domeniul patologiei hepatice, contribuind la redefinirea abordării bolilor hepatice în manieră non-invazivă. Biopsia hepatică a fost înlocuită cu metode non-invazive (elastometrie), atât în practică, dar și în cadrul protocoalelor diagnostice, naționale și internationale.
Articolul a fost imediat remarcat și comentat în ”Chronic hepatitis: New insights for the Healthcare Professional” 2013.

10. **Trifan A**, Chiriac S, Stanciu C Update on adrenal insufficiency in patients with liver cirrhosis. World J Gastroenterol. 2013; 19(4): 445-456. IF 2.433
https://www.wjgnet.com/1007-9327/full/v19/i4/445.htm
Lucrarea dezbate un domeniu al hepatologiei extrem de puțin explorat până în acel moment. 54 de citări conform Web of Science.

11. **Trifan A**, Stanciu C, Gheorghe L, Iacob S, Curescu M, Cijevschi Prelipcean C, et al. Efficacy and safety of paritaprevir/ritonavir, ombitasvir, and dasabuvir with ribavirin for the treatment of HCV genotype 1b compensated cirrhosis in patients aged 70 years or older. Medicine (Baltimore). 2017 Dec;96(50): e9271; IF = 2.02; 21 de citări conform Web of Science.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5815789/
Prim autor al unui studiu multicentric de mare anvergură (1008 pacienți cu vârsta peste 70 de ani, tratați cu antivirale directe).

12. Poynard T, de Ledinghen V, Zarski JP, Stanciu C, Munteanu M, Vergniol J,France J, **Trifan A**, Le Naour G, Vaillant JC, Ratziu V, Charlotte F; Fibrosis-TAGS group. Relative performances of FibroTest, Fibroscan, and biopsy for the assessment of the stage of liver fibrosis in patients with chronic hepatitis C: a step toward the truth in the absence of a gold standard. J Hepatol. 2012; 56(3): 541-548 IF: 9.858;
https://www.journal-of-hepatology.eu/article/S0168-8278(11)00663-5/fulltext
Am fost cooptată în acest studiu internațional multicentric, ca promotoare a tehnicii noi, non-invazive, de evaluare a fibrozei hepatice prin elastometrie – primul aparat de tip FibroScan adus în Iași. Articol citat de 105 ori conform Google Academic, 64 de citări conform Web of Science.

Essentials of Non-Alcoholic Fatty Liver Disease

Complications and Extrahepatic Manifestations

Anca Trifan
Carol Stanciu
Cristina Muzica
Editors

Springer

Editors
Anca Trifan
Department of Gastroenterology and Hepatology
Grigore T. Popa University of Medicine and Pharmacy
Iasi, Romania

Carol Stanciu
Department of Gastroenterology and Hepatology
Grigore T. Popa University of Medicine and Pharmacy
Iasi, Romania

Cristina Muzica
Department of Gastroenterology and Hepatology
Grigore T. Popa University of Medicine and Pharmacy
Iasi, Romania

ISBN 978-3-031-33547-1 ISBN 978-3-031-33548-8 (eBook)
https://doi.org/10.1007/978-3-031-33548-8

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Contents

Endocrinopathies in Nonalcoholic Fatty Liver Disease

16

Ana Maria Singeap and Laura Huiban

16.1 Introduction

Nonalcoholic fatty liver disease (NAFLD) represents one of the main causes of chronic liver disease, which includes a wide spectrum from simple steatosis to advanced fibrosis, cirrhosis, and, eventually, even hepatocellular carcinoma. Evidence so far sustains a clear relationship between various endocrine dysfunctions and nonalcoholic fatty liver disease (Fig. 16.1). Endocrinopathies may be involved in the development and progression of NAFLD. Awareness, early diagnosis, appropriate surveillance, and treatment are mandatory for optimal patients' approach.

A. M. Singeap (✉) · L. Huiban
Gastroenterology Department, Grigore T. Popa University of Medicine and Pharmacy, Iasi, Romania

Institute of Gastroenterology and Hepatology, St. Spiridon Emergency Hospital, Iasi, Romania

A. Trifan et al. (eds.), *Essentials of Non-Alcoholic Fatty Liver Disease*,
https://doi.org/10.1007/978-3-031-33548-8_16

srgh
1958

Societatea Română de Gastroenterologie şi Hepatologie

GASTROENTEROLOGIE ŞI HEPATOLOGIE CLINICĂ

EDIŢIE REVIZUITĂ ŞI COMPLETATĂ

VOL. 1

Anca TRIFAN • Cristian GHEORGHE • Dan DUMITRAŞCU • Mircea DICULESCU

Liana GHEORGHE • Ioan SPOREA • Marcel TANŢĂU • Tudorel CIUREA

EDITURA MEDICALĂ

srgh
1958

Societatea Română de Gastroenterologie şi Hepatologie

GASTROENTEROLOGIE ŞI HEPATOLOGIE CLINICĂ

EDIŢIE REVIZUITĂ ŞI COMPLETATĂ

VOL. 2

Anca TRIFAN • Cristian GHEORGHE • Dan DUMITRAŞCU • Mircea DICULESCU

Liana GHEORGHE • Ioan SPOREA • Marcel TANŢĂU • Tudorel CIUREA

EDITURA MEDICALĂ

EDITURA MEDICALĂ ESTE MARCĂ ÎNREGISTRATĂ A S.C. EDITURA MEDICALĂ S.A.

Descrierea CIP a Bibliotecii Naţionale a României
Gastroenterologie şi hepatologie clinică / Anca Trifan, Cristian Gheorghe, Dan Dumitraşcu, ... - Ediţie revizuită şi completată - Bucureşti : Editura Medicală, 2023

2 vol.

ISBN 978-973-39-0942-2
Vol. 1. - 2023. - Conţine bibliografie. - ISBN 978-973-39-0943-9

I. Trifan, Anca
II. Gheorghe, Cristian
III. Dumitraşcu, Dan

616

Această carte a fost tipărită cu sprijinul financiar al Fundației „Educația și sănătate.”

Redactor carte: **Ana-Maria SÎNGEAP**
Tehnoredactor: **Sorin POPESCU**
Ilustrațiile de la capitolul „Transplantul hepatic”: **Marius ATANASIU**

srgh
1958

Societatea Română de Gastroenterologie și Hepatologie

GASTROENTEROLOGIE
ȘI
HEPATOLOGIE CLINICĂ

EDIŢIE REVIZUITĂ ŞI COMPLETATĂ

VOL. 1

Anca TRIFAN • Cristian GHEORGHE • Dan DUMITRAȘCU • Mircea DICULESCU

Liana GHEORGHE • Ioan SPOREA • Marcel TANȚĂU • Tudorel CIUREA

Editura Medicală
București – 2023

EDITURA MEDICALĂ ESTE MARCĂ ÎNREGISTRATĂ A S.C. EDITURA MEDICALĂ S.A.

Descrierea CIP a Bibliotecii Naţionale a României
Gastroenterologie şi hepatologie clinică / Anca Trifan, Cristian Gheorghe, Dan Dumitraşcu, ... - Ediţie revizuită şi completată - Bucureşti : Editura Medicală, 2023

2 vol.

ISBN 978-973-39-0942-2
Vol. 2. - 2023. - Conţine bibliografie. - ISBN 978-973-39-0944-6

I. Trifan, Anca
II. Gheorghe, Cristian
III. Dumitraşcu, Dan

616

Această carte a fost tipărită cu sprijinul financiar al Fundației „Educația și sănătate.”

Redactor carte: **Ana-Maria SÎNGEAP**
Tehnoredactor: **Sorin POPESCU**
Ilustrațiile de la capitolul „Transplantul hepatic”: **Marius ATANASIU**

srgh
1958

Societatea Română de Gastroenterologie și Hepatologie

GASTROENTEROLOGIE
ȘI
HEPATOLOGIE CLINICĂ

EDIŢIE REVIZUITĂ ŞI COMPLETATĂ

VOL. 2

Anca TRIFAN • Cristian GHEORGHE • Dan DUMITRAȘCU • Mircea DICULESCU

Liana GHEORGHE • Ioan SPOREA • Marcel TANȚĂU • Tudorel CIUREA

Editura Medicală
București – 2023

C U P R I N S

VOLUMUL 1

INTESTIN SUBȚIRE ȘI COLON

VOLUMUL 2

FICAT ȘI CĂI BILIARE

ASPECTE ETICE ÎN GASTROENTEROLOGIE

LISTĂ DE AUTORI

Monica ACALOVSCHI	profesor emerit, Universitatea de Medicină și Farmacie „Iuliu Haţieganu” Cluj-Napoca, Clinica Medicală III
Luana ALEXANDRESCU	șef de lucrări, Facultatea de Medicină, Universitatea „Ovidius” din Constanța, Spitalul Clinic Județean de Urgență Constanța, Clinica de Gastroenterologie
Teodora ALEXESCU	șef de lucrări, Universitatea de Medicină și Farmacie „Iuliu Haţieganu” Cluj-Napoca, Spitalul Universitar CFR, Cluj-Napoca
Florina ALMARII	asistent universitar, Universitatea de Medicină și Farmacie “Carol Davila” București, Institutul Clinic Fundeni București
Radu Ion BADEA	profesor, Universitatea de Medicină și Farmacie „Iuliu Haţieganu” Cluj-Napoca, Institutul Regional de Gastroenterologie și Hepatologie „Octavian Fodor”
Vasile Daniel BALABAN	șef de lucrări, Universitatea de Medicină și Farmacie „Carol Davila” București, Spitalul Universitar de Urgență Militar Central „Dr. Carol Davila”
Christian BANCIU	conferențiar, Universitatea de Medicină și Farmacie „Victor Babeș” Timișoara, Spitalul Clinic Căi Ferate Timișoara
Ligia Ariana BANCU	conferențiar, Universitatea de Medicină și Farmacie Târgu Mureș, Spitalul Clinic Județean de Urgență Târgu-Mureș, Clinica Medicină Internă 2
Gheorghe G. BĂLAN	șef de lucrări, Universitatea de Medicină și Farmacie „Grigore T. Popa” Iași, Spitalul Clinic Județean de Urgențe „Sfântul Spiridon” Iași, Institutul de Gastroenterologie și Hepatologie
Ioan BĂNCILĂ	cercetător științific, medic primar, Institutul Clinic Fundeni, Centrul de Gastroenterologie și Hepatologie
Oana-Bogdana BĂRBOI	șef de lucrări, Universitatea de Medicină și Farmacie „Grigore T. Popa” Iași, Spitalul Clinic Județean de Urgențe „Sfântul Spiridon” Iași, Institutul de Gastroenterologie și Hepatologie
Simona Maria BĂȚAGĂ	profesor, Universitatea de Medicină și Farmacie Târgu Mureș, Spitalul Clinic Județean de Urgență Târgu-Mureș, Clinica Medicină Internă 1
Gabriel BECHEANU	conferențiar, Universitatea de Medicină și Farmacie „Carol Davila” București, Institutul Clinic Fundeni
Mihaela BLAJ	șef de lucrări, Universitatea de Medicină și Farmacie „Grigore T. Popa” Iași, Spitalul Clinic Județean de Urgențe „Sfântul Spiridon” Iași, Secția A.T.I.
Ciprian BRISC	profesor, Universitatea din Oradea, Facultatea de Medicină și Farmacie, Clinica de Gastroenterologie
Sergiu CAZACU	conferențiar, Universitatea de Medicină și Farmacie din Craiova, Spitalul Clinic Judeţean de Urgență Craiova, Clinica Medicală I - Gastroenterologie
Romeo CHIRA	profesor, Universitatea de Medicină și Farmacie „Iuliu Haţieganu” Cluj-Napoca, Spitalul Clinic de Urgență Cluj-Napoca, Clinica de Gastroenterologie
Ștefan CHIRIAC	șef de lucrări, Universitatea de Medicină și Farmacie „Grigore T. Popa” Iași, Spitalul Clinic Județean de Urgențe „Sfântul Spiridon” Iași, Institutul de Gastroenterologie și Hepatologie
Cristina CIJEVSCHI-PRELIPCEAN	profesor, Universitatea de Medicină și Farmacie „Grigore T. Popa” Iași, Spitalul Clinic Județean de Urgențe „Sfântul Spiridon” Iași, Institutul de Gastroenterologie și Hepatologie

Irina CIORTESCU	șef de lucrări, Universitatea de Medicină și Farmacie „Grigore T. Popa” Iași, Spitalul Clinic Județean de Urgențe „Sfântul Spiridon” Iași, Institutul de Gastroenterologie și Hepatologie
Lorena CIUMĂRNEAN	șef de lucrări, Universitatea de Medicină și Farmacie „Iuliu Hațieganu” Cluj-Napoca, Spitalul Universitar CFR, Cluj-Napoca
Tudorel CIUREA	profesor, Universitatea de Medicină şi Farmacie din Craiova, Spitalul Clinic Judeţean de Urgenţă Craiova, Clinica Medicală I - Gastroenterologie
Camelia COJOCARIU	profesor, Universitatea de Medicină și Farmacie „Grigore T. Popa” Iași, Spitalul Clinic Județean de Urgențe „Sfântul Spiridon” Iași, Institutul de Gastroenterologie și Hepatologie
Rareș CRĂCIUN	medic specialist gastroenterologie, Institutul Regional de Gastroenterologie şi Hepatologie „Octavian Fodor“ Cluj-Napoca
Dana CRIȘAN	conferențiar, Universitatea de Medicină și Farmacie „Iuliu Hațieganu” Cluj-Napoca, Spitalul Clinic Municipal Cluj-Napoca, Compartimentul de Gastroenterologie
Tudor CUCIUREANU	asistent universitar, Universitatea de Medicină și Farmacie „Grigore T. Popa” Iași, Spitalul Clinic Județean de Urgențe „Sfântul Spiridon” Iași, Institutul de Gastroenterologie și Hepatologie
Sorina DIACONU	șef de lucrări, Universitatea de Medicină și Farmacie „Carol Davila” București, Spitalul Universitar de Urgență București, Clinica de Medicină Internă și Gastroenterologie
Mihai Mircea DICULESCU	profesor, Universitatea de Medicină și Farmacie „Carol Davila” București, Institutul Clinic Fundeni, Centrul de Gastroenterologie şi Hepatologie, Centrul de Boli digestive și Transplant hepatic
Rucsandra Ilinca DICULESCU	doctorand, Universitatea de Medicină și Farmacie „Carol Davila” București, Institutul Clinic Fundeni, Centrul de Gastroenterologie şi Hepatologie
Mihaela DIMACHE	șef de lucrări, Universitatea de Medicină și Farmacie „Grigore T. Popa” Iași, Spitalul Clinic Județean de Urgențe „Sfântul Spiridon” Iași, Institutul de Gastroenterologie și Hepatologie
Ion DINA	profesor, Universitatea de Medicină și Farmacie „Carol Davila” București, Spitalul Clinic „Sf. Ioan” București, Clinica de Gastroenterologie
Daniela DOBRU	profesor, Universitatea de Medicină și Farmacie Târgu Mureș, Spitalul Clinic Județean Mureș, Clinica de Gastroenterologie
Mihaela DRANGA	șef de lucrări, Universitatea de Medicină și Farmacie „Grigore T. Popa” Iași, Spitalul Clinic Județean de Urgențe „Sfântul Spiridon” Iași, Institutul de Gastroenterologie și Hepatologie
Vasile Liviu DRUG	profesor, Universitatea de Medicină și Farmacie „Grigore T. Popa” Iași, Spitalul Clinic Județean de Urgențe „Sfântul Spiridon” Iași, Institutul de Gastroenterologie și Hepatologie
Dan L. DUMITRAȘCU	profesor, Universitatea de Medicină și Farmacie „Iuliu Hațieganu” Cluj-Napoca, Spitalul Clinic Județean de Urgență Cluj-Napoca, Clinica Medicală II
Andrei DUMITRU	asistent universitar, Facultatea de Medicină, Universitatea „Ovidius” din Constanța, Spitalul Clinic Județean de Urgență Constanța, Clinica de Gastroenterologie
Eugen DUMITRU	profesor, Facultatea de Medicină, Universitatea „Ovidius” din Constanța, Spitalul Clinic Județean de Urgență Constanța, Clinica de Gastroenterologie

Valentin ENACHE	medic primar anatomie patologică, Spitalul Clinic de Urgență Floreasca, București
Carmen FIERBINȚEANU-BRATICEVICI	profesor, Universitatea de Medicină şi Farmacie „Carol Davila” Bucureşti, Spitalul Universitar de Urgenţă Bucureşti, Secția de Gastroenterologie
Petruța FILIP	asistent universitar, Universitatea de Medicină şi Farmacie „Carol Davila” Bucureşti, Spitalul Universitar de Urgență București, Clinica de Medicină Internă și Gastroenterologie
Petra FISCHER	cercetător ştiinţific, Universitatea de Medicină şi Farmacie „Iuliu Haţieganu” Cluj-Napoca, Institutul Regional de Gastroenterologie şi Hepatologie „Octavian Fodor“
Dan Nicolae FLORESCU	asistent universitar, Universitatea de Medicină şi Farmacie din Craiova, Spitalul Clinic Judeţean de Urgenţă Craiova, Clinica Medicală I - Gastroenterologie
Dan Ionuț GHEONEA	profesor, Universitatea de Medicină şi Farmacie din Craiova, Spitalul Clinic Judeţean de Urgenţă Craiova, Clinica Medicală I - Gastroenterologie
Cristian GHEORGHE	profesor, Universitatea de Medicină şi Farmacie „Carol Davila” Bucureşti, Institutul Clinic Fundeni, Clinica de Gastroenterologie I
Liana GHEORGHE	profesor, Universitatea de Medicină şi Farmacie „Carol Davila” Bucureşti, Institutul Clinic Fundeni, Centrul de Gastroenterologie şi Hepatologie
Georgiana GÎLCĂ-BLANARIU	asistent universitar, Universitatea de Medicină şi Farmacie „Grigore T. Popa” Iaşi, Spitalul Clinic Judeţean de Urgenţe „Sfântul Spiridon” Iaşi, Institutul de Gastroenterologie şi Hepatologie
Irina GÎRLEANU	conferenţiar, Universitatea de Medicină şi Farmacie „Grigore T. Popa” Iaşi, Spitalul Clinic Judeţean de Urgenţe „Sfântul Spiridon” Iaşi, Institutul de Gastroenterologie şi Hepatologie
Adrian GOLDIŞ	profesor, Universitatea de Medicină şi Farmacie Timişoara, Spitalul Judeţean Timişoara, Clinica de Gastroenterologie şi Hepatologie
Simona GRAD	şef de lucrări, Universitatea de Medicină şi Farmacie „Iuliu Haţieganu” Cluj-Napoca, Spitalul Clinic de Urgenţă Cluj-Napoca, Clinica Medicală II
Mircea GRIGORESCU	profesor, Universitatea de Medicină şi Farmacie „Iuliu Haţieganu” Cluj-Napoca, Institutul Regional de Gastroenterologie şi Hepatologie „Octavian Fodor“
Claudia HAGIU	şef de lucrări, Universitatea de Medicină şi Farmacie „Iuliu Haţieganu” Cluj-Napoca, Institutul Regional de Gastroenterologie şi Hepatologie „Octavian Fodor“
Laura HUIBAN	asistent universitar, Universitatea de Medicină şi Farmacie „Grigore T. Popa” Iaşi, Spitalul Clinic Judeţean de Urgenţe „Sfântul Spiridon” Iaşi, Institutul de Gastroenterologie şi Hepatologie
Răzvan IACOB	şef de lucrări, Universitatea de Medicină şi Farmacie „Carol Davila” Bucureşti, Institutul Clinic Fundeni, Centrul de Gastroenterologie şi Hepatologie
Speranţa IACOB	şef de lucrări, Universitatea de Medicină şi Farmacie „Carol Davila” Bucureşti, Institutul Clinic Fundeni, Centrul de Gastroenterologie şi Hepatologie
Mirela IONESCU	conferenţiar, Universitatea de Medicină şi Farmacie „Carol Davila” Bucureşti, Spitalul Clinic de Urgenţă „Elias” Bucureşti, Clinica de Gastroenterologie
Elena Tatiana IVAN	asistent universitar, Universitatea de Medicină şi Farmacie din Craiova, Spitalul Clinic Judeţean de Urgenţă Craiova, Clinica Medicală I - Gastroenterologie
Mariana JINGA	profesor, Universitatea de Medicină şi Farmacie „Carol Davila” Bucureşti, Spitalul Universitar de Urgenţă Militar Central „Dr. Carol Davila”, Clinica de Medicină Internă şi Gastroenterologie

Melania MACARIE	asistent universitar, Universitatea de Medicină și Farmacie Târgu Mureș, Spitalul Clinic Județean de Urgență Târgu-Mureș, Clinica Medicină Internă 1
Radu Bogdan MATEESCU	profesor, Universitatea de Medicină și Farmacie „Carol Davila” București, Spitalul Clinic Colentina București, Secția Gastroenterologie
Daniela MATEI	șef de lucrări, Universitatea de Medicină și Farmacie „Iuliu Haţieganu” Cluj-Napoca, Institutul Regional de Gastroenterologie şi Hepatologie „Octavian Fodor“
Laura MAZILU	profesor, Disciplina de Oncologie, Facultatea de Medicină, Universitatea „Ovidius” din Constanța
Mircea MĂNUC	conferențiar, Universitatea de Medicină și Farmacie „Carol Davila” București, Institutul Clinic Fundeni, Clinica de Gastroenterologie II
Teodora MĂNUC	asistent universitar, Universitatea de Medicină și Farmacie „Carol Davila” București, Institutul Clinic Fundeni, Clinica de Gastroenterologie II
Sergiu Ioan MICU	asistent universitar, Universitatea „Ovidius” din Constanța, Spitalul Clinic Județean de Urgență Constanța, Clinica de Gastroenterologie
Egidia MIFTODE	profesor, Universitatea de Medicină și Farmacie „Grigore T. Popa” Iași, Spitalul Clinic de Boli Infecțioase “Sf. Parascheva” Iași
Cătălina MIHAI	conferențiar, Universitatea de Medicină și Farmacie „Grigore T. Popa” Iași, Spitalul Clinic Județean de Urgențe „Sfântul Spiridon” Iași, Institutul de Gastroenterologie și Hepatologie
Florin MIHAI	șef de lucrări, Universitatea de Medicină și Farmacie „Grigore T. Popa” Iași
Petru-Adrian MIRCEA	profesor emerit, Universitatea de Medicină și Farmacie „Iuliu Haţieganu” Cluj-Napoca, Clinica Medicală I, Secția Gastroenterologie
Cecil Sorin MIREA	conferențiar, Universitatea de Medicină şi Farmacie din Craiova, Spitalul Județean de Urgențe din Craiova, Clinica Chirurgie II
Tudor MOCAN	medic specialist gastroenterologie, Institutul Regional de Gastroenterologie şi Hepatologie „Octavian Fodor“, Cluj-Napoca
Ofelia MOȘTEANU	șef de lucrări, Universitatea de Medicină și Farmacie „Iuliu Haţieganu” Cluj-Napoca, Institutul Regional de Gastroenterologie şi Hepatologie „Octavian Fodor”
Cristina MUZICA	asistent universitar, Universitatea de Medicină și Farmacie „Grigore T. Popa” Iași, Spitalul Clinic Județean de Urgențe „Sfântul Spiridon” Iași, Institutul de Gastroenterologie și Hepatologie
Georgiana NAGY	șef de lucrări, Universitatea de Medicină și Farmacie „Iuliu Haţieganu” Cluj-Napoca, Spitalul Clinic de Urgență Cluj-Napoca, Clinica de Gastroenterologie
Lucian NEGREANU	profesor, Universitatea de Medicină și Farmacie „Carol Davila” București, Spitalul Universitar de Urgență București, Clinica Medicină Internă II
Dragoș NEGRU	profesor, Universitatea de Medicină și Farmacie „Grigore T. Popa” Iași, Spitalul Clinic Județean de Urgențe „Sfântul Spiridon”, Laboratorul de Radiologie și Imagistică Medicală
Oana NICOARĂ-FARCĂU	asistent universitar, Universitatea de Medicină și Farmacie „Iuliu Haţieganu” Cluj-Napoca, Institutul Regional de Gastroenterologie şi Hepatologie „Octavian Fodor“
Alexandru OPROIU	profesor, Universitatea de Medicină și Farmacie „Carol Davila” București, Spitalul „Agrippa Ionescu”
Oliviu PASCU	profesor emerit, Universitatea de Medicină și Farmacie „Iuliu Haţieganu” Cluj-Napoca

Oana PETREA	asistent universitar, Universitatea de Medicină și Farmacie „Grigore T. Popa” Iași, Spitalul Clinic Județean de Urgențe „Sfântul Spiridon” Iași, Institutul de Gastroenterologie și Hepatologie
Cristina POJOGA	medic primar gastroenterolog, Institutul Regional de Gastroenterologie şi Hepatologie „Octavian Fodor“, Cluj-Napoca
Corina POP	profesor, Universitatea de Medicină și Farmacie „Carol Davila” București, Spitalul Universitar de Urgență București, Clinica de Medicină Internă și Gastroenterologie
Ștefan-Lucian POPA	șef de lucrări, Universitatea de Medicină și Farmacie „Iuliu Haţieganu” Cluj-Napoca, Spitalul Clinic Județean de Urgență Cluj-Napoca
Alina POPESCU	profesor, Universitatea de Medicină și Farmacie „Victor Babeș” Timișoara, Spitalul Clinic Județean de Urgență "Pius Brînzeu" Timișoara, Clinica de Gastroenterologie și Hepatologie
Irinel POPESCU	profesor, Universitatea de Medicină și Farmacie „Carol Davila” București, Institutul Clinic Fundeni Bucuresti, Centrul de Boli Digestive si Transplant Hepatic
Carmen PREDA	șef de lucrări, Universitatea de Medicină și Farmacie „Carol Davila” București, Institutul Clinic Fundeni, Clinica de Gastroenterologie II
Bogdan PROCOPEȚ	conferențiar, Universitatea de Medicină și Farmacie „Iuliu Haţieganu” Cluj-Napoca, Institutul Regional de Gastroenterologie şi Hepatologie „Octavian Fodor“
Ion ROGOVEANU	profesor, Universitatea de Medicină şi Farmacie din Craiova, Spitalul Clinic Judeţean de Urgenţă Craiova, Clinica Medicală I - Gastroenterologie
Adrian SĂFTOIU	profesor, Universitatea de Medicină și Farmacie Carol Davila București, Spitalul Universitar de Urgență Elias București, Clinica Gastroenterologie și Hepatologie
Viorel SCRIPCARIU	profesor, Universitatea de Medicină și Farmacie „Grigore T. Popa” Iași, Institutul Regional de Oncologie Iași, Secția Chirurgie Oncologică I
Dragoș-Viorel SCRIPCARIU	șef de lucrări, Universitatea de Medicină și Farmacie „Grigore T. Popa” Iași, Institutul Regional de Oncologie Iași, Secția Chirurgie Oncologică I
Andrada SEICEAN	profesor, Universitatea de Medicină și Farmacie „Iuliu Haţieganu” Cluj-Napoca, Institutul Regional de Gastroenterologie şi Hepatologie „Octavian Fodor“
Cătălin SFARTI	conferențiar, Universitatea de Medicină și Farmacie „Grigore T. Popa” Iași, Spitalul Clinic Județean de Urgențe „Sfântul Spiridon” Iași, Institutul de Gastroenterologie și Hepatologie
Ana-Maria SÎNGEAP	conferențiar, Universitatea de Medicină și Farmacie „Grigore T. Popa” Iași, Spitalul Clinic Județean de Urgențe „Sfântul Spiridon” Iași, Institutul de Gastroenterologie și Hepatologie
Zeno SPÂRCHEZ	profesor, Universitatea de Medicină și Farmacie „Iuliu Haţieganu” Cluj-Napoca, Institutul Regional de Gastroenterologie şi Hepatologie „Octavian Fodor“
Ioan SPOREA	profesor, Universitatea de Medicină și Farmacie „Victor Babeș” Timișoara, Clinica de Gastroenterologie și Hepatologie
Carol STANCIU	profesor, Universitatea de Medicină și Farmacie „Grigore T. Popa” Iași, Spitalul Clinic Județean de Urgențe „Sfântul Spiridon” Iași, Institutul de Gastroenterologie și Hepatologie
Andra-Iulia SUCEVEANU	profesor, Facultatea de Medicină, Universitatea „Ovidius” din Constanța, Spitalul Clinic Județean de Urgență Constanța, Clinica de Gastroenterologie

Adrian-Paul SUCEVEANU	conferențiar, Facultatea de Medicină, Universitatea „Ovidius” din Constanța, Spitalul Clinic Județean de Urgență Constanța, Clinica de Gastroenterologie
Teodora SURDEA-BLAGA	conferențiar, Universitatea de Medicină și Farmacie „Iuliu Hațieganu” Cluj-Napoca, Clinica Medicală II
Roxana ȘIRLI	profesor, Universitatea de Medicină și Farmacie „Victor Babeș” Timișoara, Spitalul Clinic Județean de Urgență "Pius Brînzeu" Timișoara, Clinica de Gastroenterologie și Hepatologie
Gabriela ȘTEFĂNESCU	conferențiar, Universitatea de Medicină și Farmacie „Grigore T. Popa” Iași, Spitalul Clinic Județean de Urgențe „Sfântul Spiridon” Iași, Institutul de Gastroenterologie și Hepatologie
Horia ȘTEFĂNESCU	medic primar gastroenterolog, Universitatea de Medicină și Farmacie „Iuliu Hațieganu” Cluj-Napoca, Institutul Regional de Gastroenterologie și Hepatologie „Octavian Fodor“
Alina TANȚĂU	profesor, Universitatea de Medicină și Farmacie „Iuliu Hațieganu” Cluj-Napoca, Clinica Medicală IV
Marcel TANȚĂU	profesor, Universitatea de Medicină și Farmacie „Iuliu Hațieganu” Cluj-Napoca, Institutul Regional de Gastroenterologie și Hepatologie „Octavian Fodor”
Cristian TIERANU	șef de lucrări, Universitatea de Medicină și Farmacie „Carol Davila” București
Elena TOADER	profesor, Universitatea de Medicină și Farmacie „Grigore T. Popa” Iași, Spitalul Clinic Județean de Urgențe „Sfântul Spiridon” Iași, Institutul de Gastroenterologie și Hepatologie
Cristina TOCIA	șef de lucrări, Facultatea de Medicină, Universitatea „Ovidius” Constanța, Spitalul Clinic Județean de Urgență Constanța, Clinica de Gastroenterologie
Alina TOMESCU	șef de lucrări, Universitatea Bioterra București, Spitalul Universitar de Urgență București, Clinica de Medicină Internă și Gastroenterologie
Anca TRIFAN	profesor, Universitatea de Medicină și Farmacie „Grigore T. Popa” Iași, Spitalul Clinic Județean de Urgențe „Sfântul Spiridon” Iași, Institutul de Gastroenterologie și Hepatologie
Anamaria TRANDAFIR	asistent universitar, Facultatea de Medicină, Universitatea „Ovidius” Constanța
Roxana VĂDAN	cercetător științific, Institutul Clinic Fundeni, Centrul de Gastroenterologie și Hepatologie
Simona VĂLEAN	conferențiar, Universitatea de Medicină și Farmacie „Iuliu Hațieganu” Cluj-Napoca, Clinica Medicală I, Secția Gastroenterologie
Cristin-Constantin VERE	profesor, Universitatea de Medicină și Farmacie din Craiova, Spitalul Județean de Urgențe din Craiova, Clinica Medicală I - Gastroenterologie
Andrei VOIOSU	asistent universitar, Universitatea de Medicină și Farmacie „Carol Davila” București, Spitalul Clinic Colentina București, Secția de Gastroenterologie
Bianca Diana VOIOSU	medic specialist gastroenterologie, Compartimentul de Gastroenterologie, Spitalul Clinic „Ioan Cantacuzino” București
Radu VOIOSU	profesor, Universitatea de Medicină și Farmacie „Carol Davila” București, Spitalul Clinic „N. Gh. Lupu” București, Secția de Gastroenterologie
Theodor VOIOSU	șef de lucrări, Universitatea de Medicină și Farmacie „Carol Davila” București, Spitalul Clinic Colentina București, Secția de Gastroenterologie

Anca TRIFAN, conferențiar la Disciplina de Semiologie Medicală, Universitatea de Medicină şi Farmacie "Gr. T. Popa" Iaşi.
Doctor în ştiințe medicale
Medic primar Medicină internă şi Gastroenterologie
Şef de Laborator Endoscopie, Institutul de Gastroenterologie şi Hepatologie Iaşi

Descrierea CIP a Bibliotecii Naționale a României
TRIFAN ANCA
Manual de endoscopie / Anca Trifan; Iaşi: Junimea, 2002
2 vol.
Vol. 2 – Colonoscopia – 2003 – ISBN 973-37-0871-2

616-072.1(075.8)

Desenator: MariusAtanasiu
Tehnoredactor: Valentin Cozmescu

ISBN: 973-37-0871-2

ANCA TRIFAN

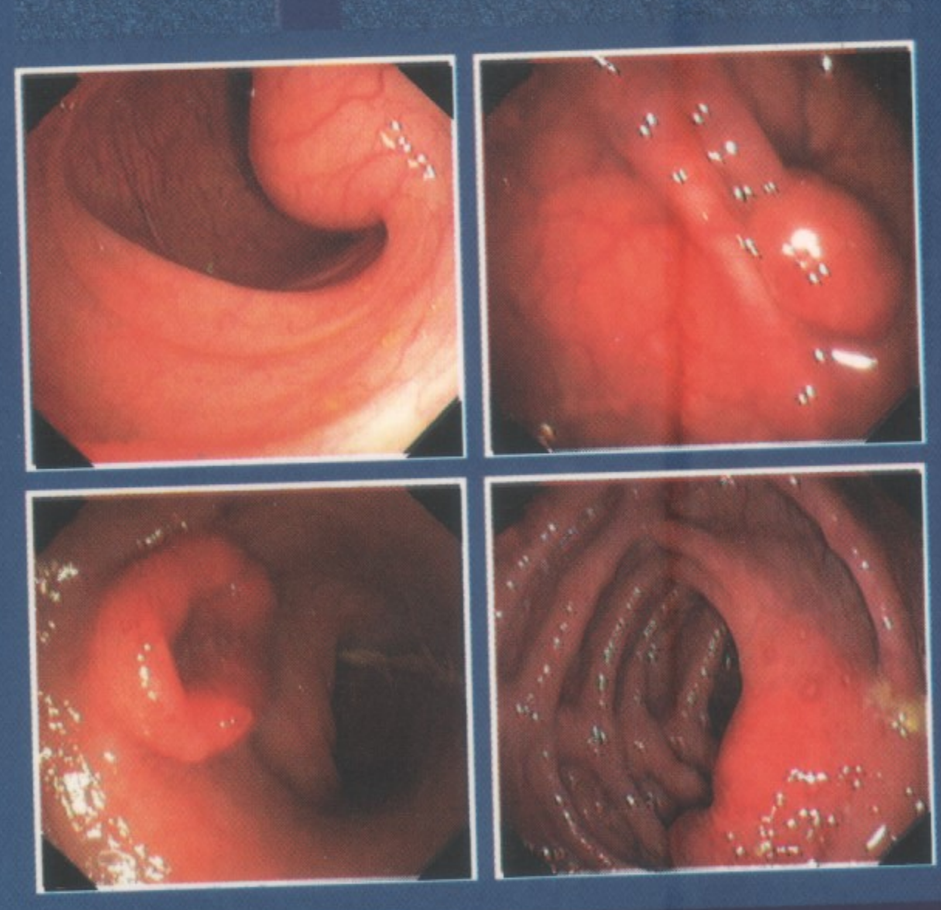
MANUAL DE ENDOSCOPIE

– volumul II –

Colonoscopia

ANCA TRIFAN

MANUAL DE ENDOSCOPIE



– volumul 2 –

COLONOSCOPIA

Editura Junimea

Scrisă cu talent şi cu multă trudă, cartea de faţă are calităţi care o fac să fie una ideală atât pentru începători cât şi pentru cei avansaţi în tehnica colonoscopiei. Maniera concisă şi sintetică, dar care conferă o „mină" de informaţii, dublată de calitatea imaginilor endoscopice şi claritatea figurilor recomandă COLONOSCOPIA ca o excelentă apariţie editorială cel puţin egală cu unele „rivale" publicate în străinătate.
Într-un cuvânt, acest al doilea volum al *Manualului de endoscopie* oferă un spectru unic de experienţă al unui endoscopist desăvârşit: Dr. Anca Trifan.

Prof. dr. Carol Stanciu

ISBN: 973-37-0871-2

Inhibition of Progressing Primary Esophageal Peristalsis by Pharyngeal Water Stimulation in Humans

ANCA TRIFAN,* JUNLONG REN,† RONALD ARNDORFER,† CANDY HOFMANN,† EYTAN BARDAN,† and REZA SHAKER†

†Medical College of Wisconsin Dysphagia Institute, Departments of Medicine, Radiology, and Otolaryngology & Human Communication, Medical College of Wisconsin, and VA Medical Center, Milwaukee, Wisconsin; and *University of Medicine, Iasi, Romania

Background & Aims: **Sensory impulses initiated from the pharynx exert differing effects on the deglutitive apparatus. They have an inhibitory effect on the lower esophageal sphincter but an excitatory effect on the upper esophageal sphincter. The aim of this study was to systematically investigate the effect of pharyngeal sensory impulses evoked by water stimulation on the progressing esophageal peristalsis.** ***Methods:*** **Sixteen healthy young volunteers were studied in the supine position. The presence of normal peristalsis was verified. Esophageal peristalsis was recorded 3, 6, 9, 12, 15, and 18 cm above the lower esophageal sphincter. Pharyngeal stimulation was performed by injecting a predetermined threshold volume into the pharynx 2 cm above the upper esophageal sphincter, directed posteriorly. The injections were timed to coincide with the arrival of the peristaltic wave induced by dry swallows at respective recording sites.** ***Results:*** **Injection of the threshold volume (0.5 ± 0.1 mL) stopped the progression of peristalsis at both the striated and smooth muscle esophagus. Topical pharyngeal anesthesia blocked this inhibitory effect ($P < 0.01$).** ***Conclusions:*** **Sensory impulses initiated from the pharynx evoked by water injection inhibit the progression of primary esophageal peristalsis. Although the clinical significance of these findings is not determined, they may explain the mechanism of some of the failed esophageal peristalsis.**

Sensory impulses initiated from the pharynx exert differing effects on the deglutitive apparatus. They have an inhibitory effect on the lower esophageal sphincter (LES), resulting in its complete or, less commonly, partial relaxation.[1] On the upper esophageal sphincter (UES), they exert an excitatory effect, resulting in an increase in its resting tone.[1–3] However, the effect of these sensory impulses on the esophageal body motor function is not known. A preliminary study in our laboratory suggested an inhibitory effect on esophageal peristalsis. The aim of the present study was to systematically investigate the effect of pharyngeal sensory impulses evoked by water stimulation on the progressing esophageal peristalsis.

Materials and Methods

We studied 16 healthy young volunteers (5 female and 11 male; age, 32 ± 2 years; age range, 19–44 years). The studies were performed with the subjects in the supine position. The study protocols were approved by the Human Research Review Committee of The Medical College of Wisconsin, and the subjects gave informed written consent before their studies.

The UES, esophageal body, LES, and gastric pressure phenomena were recorded concurrently using two sleeve assemblies, which were passed through each nostril and positioned so that the LES sleeve device (6 × 0.5 × 0.4 cm; Dentsleeve, Adelaide, Australia) straddled the LES and the UES sleeve device (6 × 0.5 × 0.3 cm; Dentsleeve) straddled the UES. With this arrangement, the esophageal body pressure phenomena were recorded at the top of the LES sleeve 3, 6, 9, 12, 15, and 18 cm proximal to the LES. The upper sleeve assembly also incorporated an injection port located 2 cm proximal to the sleeve device. This manometric assembly was positioned so that the injection port faced posteriorly. The subjects were monitored for 10 minutes after the positioning of the two manometric assemblies.

Subsequently, the presence of normal peristalsis was confirmed for each subject by monitoring 10 dry swallows before pharyngeal stimulation; only subjects with normally progressing esophageal peristalsis during dry swallows were studied.

To study the effect of pharyngeal water stimulation on the progression of esophageal primary peristalsis, subjects were asked to swallow on command, and their pharynx was stimulated by injections of minute amounts of water. Water injections were timed to coincide with complete UES relaxation or arrival of the peristaltic pressure wave at each recording site.

Pharyngeal water stimulation was initiated by a rapid pulse injection of 0.1 mL of water directed toward the posterior pharyngeal wall. The volume of injected water was increased by 0.1-mL increments until either the progression of the peristaltic wave was halted or an irrepressible swallow occurred.

Abbreviations used in this paper: UES, upper esophageal sphincter.

0016-5085/96/$3.00

Table 1. Effect of Pharyngeal Water Stimulation on the Amplitude of the Peristaltic Pressure Wave

	Sites of pressure wave above LES coincident with pharyngeal water injection				
	18 cm	15 cm	12 cm	9 cm	6 cm
Amplitude (*mm Hg*)					
Before injection	79 ± 4	63 ± 4	72 ± 6	72 ± 6	72 ± 6
After injection	56 ± 6[a]	50 ± 5[a]	47 ± 7[a]	59 ± 8[a]	36 ± 6[a]
Percent decrease	29 ± 6	18 ± 8	33 ± 10	13 ± 5	49 ± 9

NOTE. Pharyngeal water injection significantly reduced the amplitude of the developing pressure wave in both the striated and smooth muscle portions of the esophagus.
[a]$P < 0.05$.

Each volume was repeated three times for each recording site, and the subjects withheld swallowing after water injection for as long as they could. Occurrence of the swallow was judged by typical deglutitive UES and LES relaxation, by subject's signal using a handheld marker, and by observer's marks on the polygraph paper. Each swallow tested by pharyngeal stimulation was performed 25–30 seconds after a control swallow and was followed 25–30 seconds later by a second control swallow. Subsequently, the pharyngeal mucosa of each subject was anesthetized by the application of 4% topical lidocaine spray (Roxan Laboratories Inc., Columbus, OH), and the test was repeated 5 and 20 minutes afterward. Inhibition of progressing peristalsis after each pharyngeal water injection was accepted when the pressure wave was completely eliminated after the injection. Frequency of inhibition after each water injection was determined as a percentage of the trials for each site.

To correlate the effect of pharyngeal water stimulation on progressing esophageal peristalsis with its effect on respiration, the above protocol was repeated in 5 additional subjects while the respiration was monitored by a pneumobelt wrapped around the subject's chest.[4] The output signal induced by the respiratory chest wall movement was recorded on the same polygraph paper used for recording esophageal peristalsis.

We measured the threshold volume for inhibition of peristalsis in each subject and determined the presence or absence of development of a new peristaltic pressure wave after each inhibited peristalsis. We also determined the effect of pharyngeal water injection on the amplitude of the pressure wave at whose onset the water was injected into the pharynx. In subjects in whom respiration was monitored, duration of deglutitive apnea, the presence or absence of apnea induced by water injection, and the respiratory rate for the 10-second period immediately after pharyngeal water injection were determined and compared with the period before water injection. Statistical analysis was performed using analysis of variance with repeated measures and χ^2 tests, when appropriate. Data are presented as mean ± SE unless otherwise stated.

Results

At a threshold volume of 0.5 ± 0.1 mL, progression of primary esophageal peristalsis induced by dry swallows was inhibited in all volunteers except in 2 subjects, in whom the mere injection of 0.1 mL of water resulted in a pharyngeal swallow. Therefore, the inhibitory effect of pharyngeal water stimulation on the progression of primary peristalsis could not be evaluated in these 2 subjects. The level of peristaltic inhibition was dependent on the extent to which peristalsis had progressed before the development of pharyngeal water injection; specifically, water injected before the development of complete UES relaxation did not induce inhibition. Likewise, when the peristaltic wave reached the most distal site, it did not inhibit the development of the pressure wave even if the water was injected coincidently with the onset of the pressure-wave upstroke. However, it frequently resulted in the reduction of the amplitude of the pressure wave at this site compared with dry swallows before and after water injection. As a rule, at all recording levels, the pressure wave coincident with the water injection was not inhibited, but the peristaltic wave inhibition occurred at the next recording site.

The uninhibited pressure wave had a significantly lower amplitude than its counterparts induced by swallows before and after pharyngeal water injection ($P < 0.05$). The attenuating effect was observed in both the striated and the smooth muscle portion of the esophagus. Although there was a trend for a larger reduction in the amplitude of the pressure wave in the distal esophagus compared with the proximal esophagus, the difference did not reach statistical significance ($P = 0.06$) (Table 1).

Figure 1 shows the inhibition of progressing primary peristalsis at various segments of the esophagus that were induced by pharyngeal water injection. This inhibition occurred in both the proximal striated muscle portion and the distal smooth muscle portion of the esophagus. This inhibitory effect was significantly reduced by the application of topical pharyngeal anesthesia (Figure 2). However, the effect of topical anesthesia was reversible (Figure 3).

Analysis of concurrent recordings of esophageal peri-

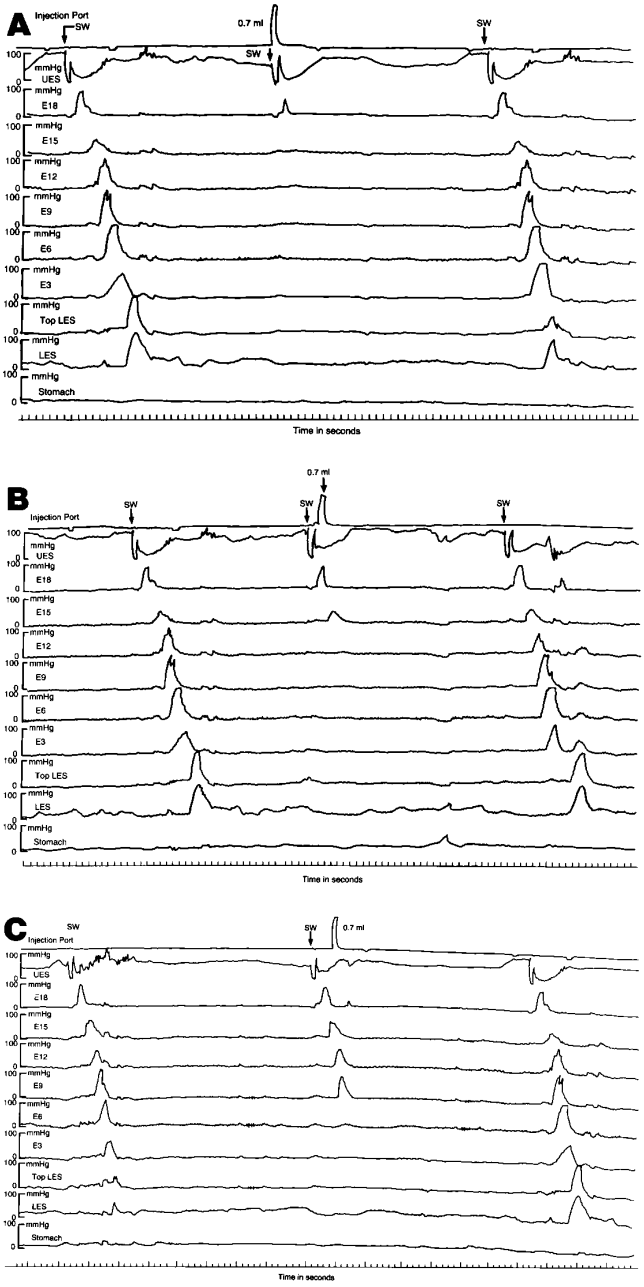


Figure 1. Examples of inhibition of progressing primary esophageal peristalsis in the (*A* and *B*) proximal striated and (*C*) distal smooth muscle esophagus by pharyngeal water stimulation. (*A*) Rapid injection of 0.7 mL room-temperature water into the pharynx immediately after UES relaxation and arrival of peristaltic wave at the site 18 cm above LES inhibited the progression of peristalsis to the sites below. (*B*) Similar injection when the peristaltic wave had reached the site 15 cm above the LES inhibited its progression to the more distal sites. (*C*) Rapid pulse injection of 0.7 mL room-temperature water into the pharynx when the peristaltic wave was 9 cm above the LES resulted in its inhibition in the smooth muscle portion in the distal 6 cm of the esophagus. Note that these inhibitions were not followed by another peristaltic pressure wave. Each inhibition trial is preceded and followed by a normal peristaltic pressure wave induced by a dry swallow. SW, swallow.

stalsis and respiration showed that inhibition of esophageal peristalsis by pharyngeal water injection was associated with a small but significant decrease in the respiratory rate (18 ± 0.1 vs. 15 ± 0.2/min; $P < 0.05$) at all levels. This decrease in respiratory rate lasted for an average of 11 ± 3 seconds and was frequently reset to the preinjection rate after a swallow. Comparison of the respiratory rate in the periods before and after swallows that were not challenged by pharyngeal water stimulation did not show any significant difference. The duration of deglutitive apnea for swallows that were followed by pharyngeal water injection was similar to that of spontaneous swallows. There was no detectable apnea besides the deglutitive apnea identified after pharyngeal water stimulation.

In 5 subjects, we also determined the threshold volume for inducing isolated LES relaxation. The threshold volumes for induction of LES relaxation and inhibition of progressing primary esophageal peristalsis were similar.

Discussion

In this study, we determined the effect of pharyngeal sensory impulses induced by water stimulation on the progressing esophageal peristalsis. Our study findings show that abrupt injection of minute amounts of water toward the posterior pharyngeal wall results in the inhibition of a swallow-induced progressing peristaltic wave

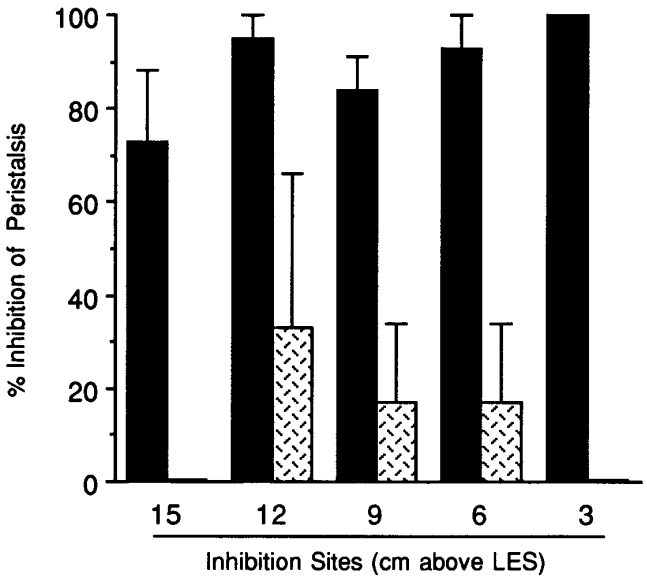


Figure 2. Effect of topical pharyngeal anesthesia on the inhibition of progressing primary esophageal peristalsis by pharyngeal water stimulation. Pharyngeal water stimulation at a threshold volume inhibited the progression of the peristalsis in both the striated and smooth muscle portions of the esophagus. Topical pharyngeal anesthesia significantly reduced this inhibitory effect ($P < 0.01$). ■, Before anesthesia; ⊠, after anesthesia.

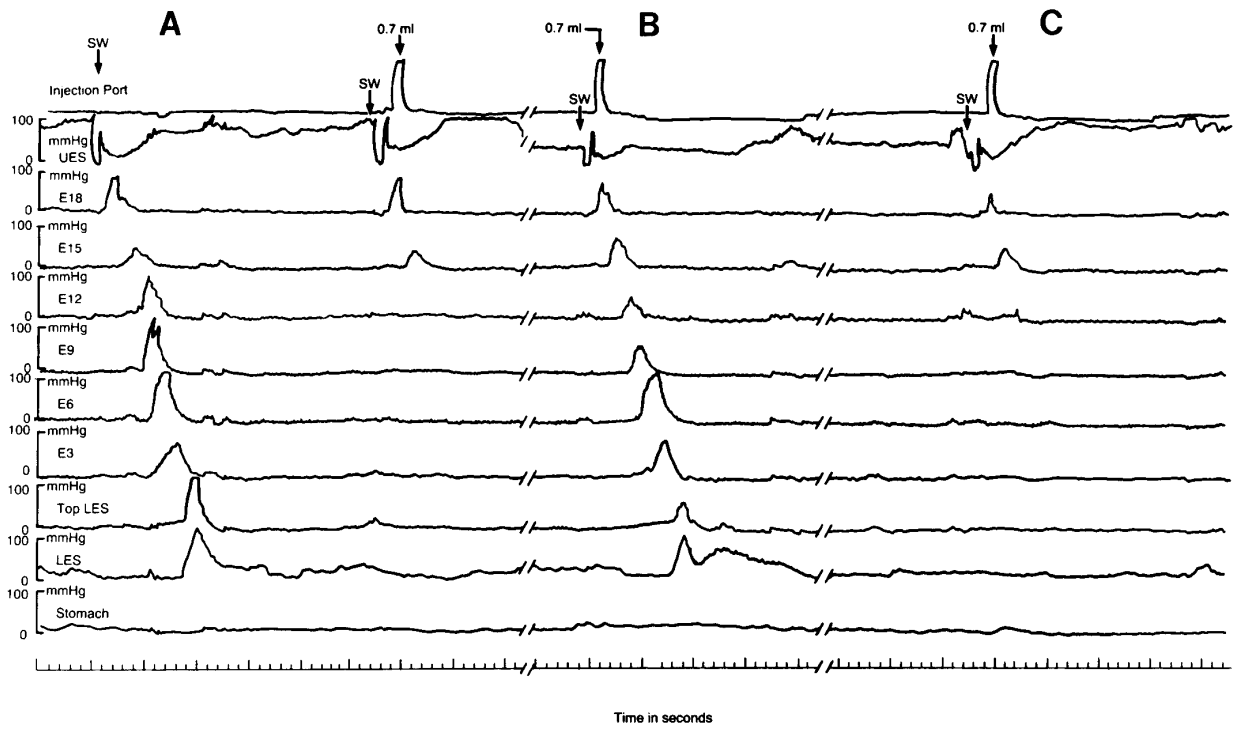


Figure 3. An example of the effect of pharyngeal topical anesthesia on the inhibition of progressing esophageal peristalsis by pharyngeal water stimulation. (*A*) Inhibition of progressing esophageal peristalsis by pharyngeal water stimulation before application of topical pharyngeal anesthesia. (*B*) Five minutes after topical anesthesia, injection of the same volume of water into the pharynx did not result in inhibition of progressing esophageal peristalsis. (*C*) Twenty minutes after topical pharyngeal anesthesia, the inhibitory effect of pharyngeal water injection has returned. SW, swallow.

in both the striated and smooth muscle portions of the esophagus.

Previous studies have shown that minute amounts of water injected into the pharynx induce isolated LES relaxation in humans.[1] Findings of the present study support the notion that pharyngeal water stimulation results in a generalized inhibition of the contractile activity of the esophageal body and the LES. Mechanisms of this inhibitory effect are not currently known. However, it may be postulated that it is mediated centrally through the brain stem swallowing center.

Our study findings concur with previous reports that the inhibitory effect of the swallowing centers on the deglutitive apparatus could be uncoupled from its excitatory effect by pharyngeal water stimulation.[1] This technique may be useful in further delineating the complex mechanism of deglutitive peristalsis.

Various factors are known to affect esophageal peristalsis. Sensory feedback, such as that originating from the presence of a bolus, is known to increase the amplitude of the peristaltic pressure wave[5,6] and reduce the rate of failed peristalsis.[5,6] A swallow occurring in close temporal proximity to a previous swallow tends to either inhibit or attenuate the preceding peristaltic pressure wave.[7–9] This inhibitory effect may occur in both the striated and smooth muscle portions of the esophagus, as shown by Vanek and Diamant.[10] However, the peristaltic wave generated by the second swallow progresses uninterrupted, although it may be attenuated. The latter study confirms the presence of a central inhibition that precedes the stimulation of the deglutitive esophageal peristalsis. The inhibitory effect of pharyngeal stimulation on progressing esophageal peristalsis described in the current study is different from that of the above mentioned studies by not inducing a second peristaltic wave after the inhibition of the original peristalsis. Whether this finding is another manifestation of deglutitive inhibition, or simply shows the isolated stimulation of inhibitory function of the brainstem swallowing center through an unrelated pathway, or yet suggests the presence of a different inhibitory pathway is not clear at this time.

Considering the fact that pharyngeal stimulation induces a centrally mediated contraction of the cricopharyngeus striated muscle[1–3] while inhibiting the proximal esophageal striated muscle layer, these findings suggest that pharyngeal water stimulation seems to have a dual effect on the brain stem neurons: an inhibitory effect on one group of neurons and an excitatory effect on the other group. The findings also suggest the possibility that the excitatory effect of the pharyngeal stimulation

on the cricopharyngeus muscle may not be mediated through the deglutitive pathways.

Previous studies have shown the existence of a close coordination between deglutition and respiration.[4,11–13] Earlier studies have documented the effect of alterations of the respiratory function on the coordination of deglutition with the phases of respiration.[4] On the other hand, pharyngeal water stimulation in a feline model has been shown to inhibit the activities of the inspiratory neurons while increasing the activities of expiratory neurons.[14] Our finding of the association of inhibition of esophageal peristalsis by pharyngeal water stimulation with a reduction in the respiratory rate is another example of the close central coordination of the deglutitive and respiratory functions.

In conclusion, sensory impulses initiated from the pharynx by water injection inhibit the progression of primary esophageal peristalsis. Although the clinical significance of these findings is not currently determined, it is conceivable that they explain the mechanism of some of the failed esophageal peristalsis.

References

1. Trifan A, Shaker R, Ren J, Mittal RK, Saeian K, Dua K, Kusano M. Inhibition of resting lower esophageal sphincter pressure by pharyngeal water stimulation in humans. Gastroenterology 1995;108:441–446.
2. Medda BK, Lang IM, Layman R, Hogan W, Dodds WJ, Shaker R. Characterization and quantification of a pharyngo-UES reflex in the cat. Am J Physiol 1994;267:G972–G983.
3. Shaker R, Ren J, Zamir Z, Sarna A, Liu J, Sui Z. Effect of aging, position, and temperature on the threshold volume triggering pharyngeal swallows. Gastroenterology 1994;107:396–402.
4. Shaker R, Li Q, Ren J, Townsend WF, Dodds WJ, Martin BJ, Kern MK, Rynders A. Coordination of deglutition and phases of respiration: effect of aging, tachypnea, bolus volume, and chronic obstructive pulmonary disease. Am J Physiol 1992;263:G750–G755.
5. Dodds WJ, Hogan WJ, Reid DP, Stewart ET, Arndorfer RC. A comparison between primary esophageal peristalsis following wet and dry swallows. J Appl Physiol 1973;35:851–857.
6. Hollis JB, Castell DO. Effect of dry swallows and wet swallows of different volumes on esophageal peristalsis. J Appl Physiol 1975;38:1161–1164.
7. Ask P, Tibbling L. Effect of time interval between swallows on esophageal peristalsis. Am J Physiol 1980;338:G485–490.
8. Meyer Gw, Gerhardt DC, Castell DO. Human esophageal response to rapid swallowing: muscle refractory period or neutral inhibition? Am J Physiol 1981;G129–136.
9. Hellman J, Vantrappen G, Janssens J. Electromyography of the esophagus. The deglutitive inhibition. In: Vantrappen G, Hellmans J, eds. Diseases of esophagus. New York: Springer–Verlag, 1974:280–284.
10. Vanek AW, Diamant NE. Responses of the human esophagus to paired swallows. Gastroenterology 1987;92:643–650.
11. Martin BJW, Logemann JA, Shaker R, Dodds WJ. Coordination between respiration and swallowing: respiratory phase relationships and temporal integration. J Appl Physiol 1994;76:714–723.
12. Sherman DJ, Ross MG, Day L, Humme J, Ervin MG. Fetal swallowing: response to graded maternal hypoxemia. J Appl Physiol 1991;71:1856–1861.
13. Nishino T, Yonezawa T, Honda Y. Effects of swallowing on the pattern of continuous respiration in human adults. Am Rev Respir Dis 1985;132:1219–1222.
14. Hukuhara T, Okada H. Effects of deglutition upon the spike discharges of neurones in the respiratory center. Jpn J Physiol 1956;6:162–166.

Received July 19, 1995. Accepted September 25, 1995.

Address requests for reprints to: Reza Shaker, M.D., Gastrointestinal Section/111C, VA Medical Center, 5000 West National Avenue, Milwaukee, Wisconsin 53295. Fax: (414) 384-8480.

Supported in part by National Institutes of Health grants RO1-DC00669 and RO1-DK25731 and by a Merit Review Grant from the Department of Veterans Affairs. Dr. Trifan's research fellowship was supported in part by an Astra Merck research fellowship grant.

GASTROENTEROLOGY 1995;108:441–446

Inhibition of Resting Lower Esophageal Sphincter Pressure by Pharyngeal Water Stimulation in Humans

ANCA TRIFAN,* REZA SHAKER,† JUNLONG REN,† RAVINDER K. MITTAL,§ KIA SAEIAN,† KULWINDER DUA,† and MOTOYASU KUSANO‖

†MCW Dysphagia Institute, Medical College of Wisconsin, Department of Veterans Affairs Medical Center, Milwaukee, Wisconsin; *University of Medicine, Iasi, Romania; §University of Virginia, Charlottesville, Virginia; and ‖Gunma University, Maebashi, Japan

Background/Aims: **Normal inhibition of lower esophageal sphincter (LES) tone occurs during swallowing and belching. However, it is known that it may occur independently of these functions. The aim of this study was to characterize the effect of pharyngeal water stimulation on resting LES pressure.** ***Methods:*** **The effect of rapid-pulse and slow continuous intrapharyngeal injection of minute increments of water on the resting tone of the upper and LES of 14 healthy young volunteers was evaluated by concurrent manometry, submental electromyography, and respirography.** ***Results:*** **At a threshold volume, pharyngeal water injection induced an isolated LES relaxation in all volunteers. The threshold volume inducing LES relaxation by rapid-pulse injection, 0.16 ± 0.01 mL, was significantly lower than that with slow continuous injection (0.5 ± 0.05 mL) ($P < 0.05$). The duration and magnitude of LES relaxation were not volume dependent. The duration of LES relaxation induced by rapid-pulse injection was significantly longer than that of swallows.** ***Conclusions:*** **Minute amounts of liquid injected into the pharynx induce LES relaxation different from that of the normal swallow. Neither the duration nor the magnitude of this relaxation is volume dependent. Whereas the contribution of this finding to the mechanism of transient LES relaxation remains to be ascertained, it may partially explain the variability of the basal LES pressure.**

Normal inhibition of lower esophageal sphincter (LES) tone occurs during swallowing and belching. However, it is known that sphincter relaxation may occur independently of these functions.[1–6] During our studies to characterize swallows triggered by pharyngeal water stimulation, it was noted that subthreshold volumes for pharyngeal swallow resulted in LES relaxation without stimulation of swallowing.

Because the effect of direct stimulation of the pharynx on the resting LES tone in humans has not been systematically investigated, this study was undertaken to characterize the effect of pharyngeal water stimulation on the resting LES pressure.

Materials and Methods

We studied 14 healthy young volunteers (6 women and 8 men; age range, 20–32 years). The studies were performed with the subjects in the supine position after overnight fasting. The study protocols were approved by the Human Research Review Committee of the Medical College of Wisconsin, and the subjects gave informed written consent.

LES pressure and esophageal-body and gastric pressure phenomena were monitored by a catheter assembly that incorporated a sleeve device (6 × 0.5 × 0.4 cm) (Dentsleeve, Adelaide, Australia); four esophageal recording sites located 3, 6, 12, and 15 cm above the sleeve; and one gastric port located at the distal end of the sleeve device. A recording site at the proximal end of the sleeve was used for manometric positioning.

The upper esophageal sphincter was monitored by a second catheter assembly that incorporated an upper esophageal sphincter sleeve device (6 × 0.5 × 0.3 cm) (Dentsleeve). The sleeve assembly had recording ports at the proximal and distal ends of the sleeve for manometric positioning. It also incorporated an injection port located 2 cm proximal to the sleeve device. This manometric assembly was positioned such that the injection port faced posteriorly. The subjects were monitored for 10 minutes after positioning of the two manometric assemblies, after which pharyngeal water injections were performed by two methods: rapid-pulse and slow continuous injections. Rapid-pulse injections were started with 0.1 mL water, and the volume was increased by 0.1-mL increments until an irrepressible swallow occurred. Slow continuous infusion was performed in 10 subjects at a rate of 5.5 mL/min with a Harvard infusion pump (model N0975; Harvard Apparatus Co., Inc., Dover, MA) until an irrepressible swallow occurred. Each injection was started 5–10 seconds after the LES pressure stabilized at baseline after a swallow, and subjects withheld swallowing as long as they could. Each volume was tested three times.

Respiration was monitored with a Respitrace system (Ambulatory Monitoring, Inc., Ardsley, NY), which recorded respiration-induced rib cage movement through a coiled insulated electric wire. Swallowing was monitored by submental surface

0016-5085/95/$3.00

electromyography with a surface electrode taped beneath the chin over the geniohyoid-mylohyoid muscle complex. The electromyographic signals and the respirographic tracings were recorded on the same polygraph paper on which the manometric pressure phenomena were recorded. In addition, subjects signaled swallowing with a handheld event marker. During the actual test period, the polygraph paper was run at a speed of 25 mm/s. Between the water injections, the paper speed was kept at 10 mm/s. We evaluated the nature of the LES pressure response to pharyngeal water stimulation during rapid-pulse and slow continuous injection, the onset and duration of the LES response to pharyngeal water stimulation, the magnitude of the LES pressure response to pharyngeal water stimulation, the upper esophageal sphincter pressure response to pharyngeal water stimulation, and esophageal-body activity in response to pharyngeal water stimulation.

LES relaxation induced by pharyngeal water stimulation was compared with LES relaxation induced by the preceding volitional swallow. The nadir of the relaxation was used for comparison. The LES pressure response to pharyngeal water stimulation at each volume was considered positive when it occurred during all three trials. Occurrence of swallow was determined by the presence of submental electromyographic activity, deglutitive apnea, the volunteer's signal, and the observer's marking. Statistical analysis was performed with analysis of variance with repeated measures and χ^2 when appropriate. Data in the text are presented as mean ± SEM unless stated otherwise.

Results

Rapid-Pulse Injection

A total of 212 rapid water injections were performed. In all volunteers, rapid water injections into the pharynx resulted in a decline in the resting LES pressure, which occurred in response to 188 of 212 injections. The LES pressure decline began 2.4 ± 0.17 seconds after the completion of water injection. The smallest volume that induced a decline in resting LES pressure in all three trials, the threshold volume, averaged 0.16 ± 0.01 mL. Injection of the threshold volume resulted in complete LES relaxation in half of the trials. In the rest, the pressure decline ranged between 30% and 85% of the resting LES pressure (Figure 1).

In each subject, with each incremental increase in the injected volume, the LES relaxation continued to occur until the injected volume reached the threshold for the stimulation of pharyngeal swallows (0.8 ± 0.1 mL). These swallows occurred immediately after the completion of water injection.

In 6 subjects, injection of the threshold volume induced complete LES relaxation in all three trials. In this group, complete relaxation continued with increasing increments of injected volume until a pharyngeal swallow occurred. Among the remaining 8 subjects, 3 developed complete LES relaxation in response to volumes larger than the threshold volume. Among the other 5 subjects, the LES relaxation in response to all volumes of pharyngeal injection remained partial in 4 but was variable in 1 subject.

In most instances, the LES relaxations were ended by spontaneous swallows. However, in a total of 70 instances (38%), LES pressure recovered spontaneously before a swallow occurred (Figure 1*B* and *C*). These trials were used to measure the duration of LES relaxation. Analysis of these instances showed that there was no direct relation between the volume of injected water and the duration of LES relaxation. However, the duration of LES relaxation induced by threshold volume (6.0 ± 1.0 seconds) was significantly longer than that induced by a primary (3.0 ± 0.3 seconds) or pharyngeal swallow (3.2 ± 0.4 seconds).

When the LES relaxation recovered spontaneously, in 33% of instances it was followed by a postrelaxation contraction (Figure 2) that resulted in an LES pressure higher than the preinjection value. In the remaining instances, the LES pressure returned to the preinjection level without a postrelaxation contraction (Figure 1*B* and *C*).

In 35 of 188 trials (19%), pharyngeal water injection was associated with minimal submental electromyographic activity. Twenty-two of these activities (12%) occurred 7.5 ± 0.7 seconds (range, 3–15 seconds) after the onset of LES relaxation. The rest (7%) occurred either simultaneously with the onset of LES relaxation or within 1 second of its occurrence (Figure 3).

In nine instances, pharyngeal water injection was associated with distal esophageal motor activity. In eight of them, simultaneous contractions extended caudally from 6 cm above the LES, and in one, the simultaneous contraction involved the entire esophagus. These contractions occurred concurrently with the onset of the recovery of LES pressure (Figure 3*A*).

In 90% of the trials, rapid pharyngeal water injection resulted in an increase in upper esophageal sphincter pressure by 170% ± 10% over the preinjection values (Figures 1–3). In the remaining instances, the upper esophageal sphincter pressure did not change.

Slow Continuous Injection

Similar to rapid-pulse injection, slow continuous injection of water into the pharynx invariably resulted in LES relaxation (Figure 4). The smallest slowly injected volume that caused LES relaxation, the threshold volume, averaged 0.5 ± 0.05 mL. This volume was significantly larger than that of the rapid-pulse injection ($P < 0.05$). A comparison of the threshold volumes required to induce

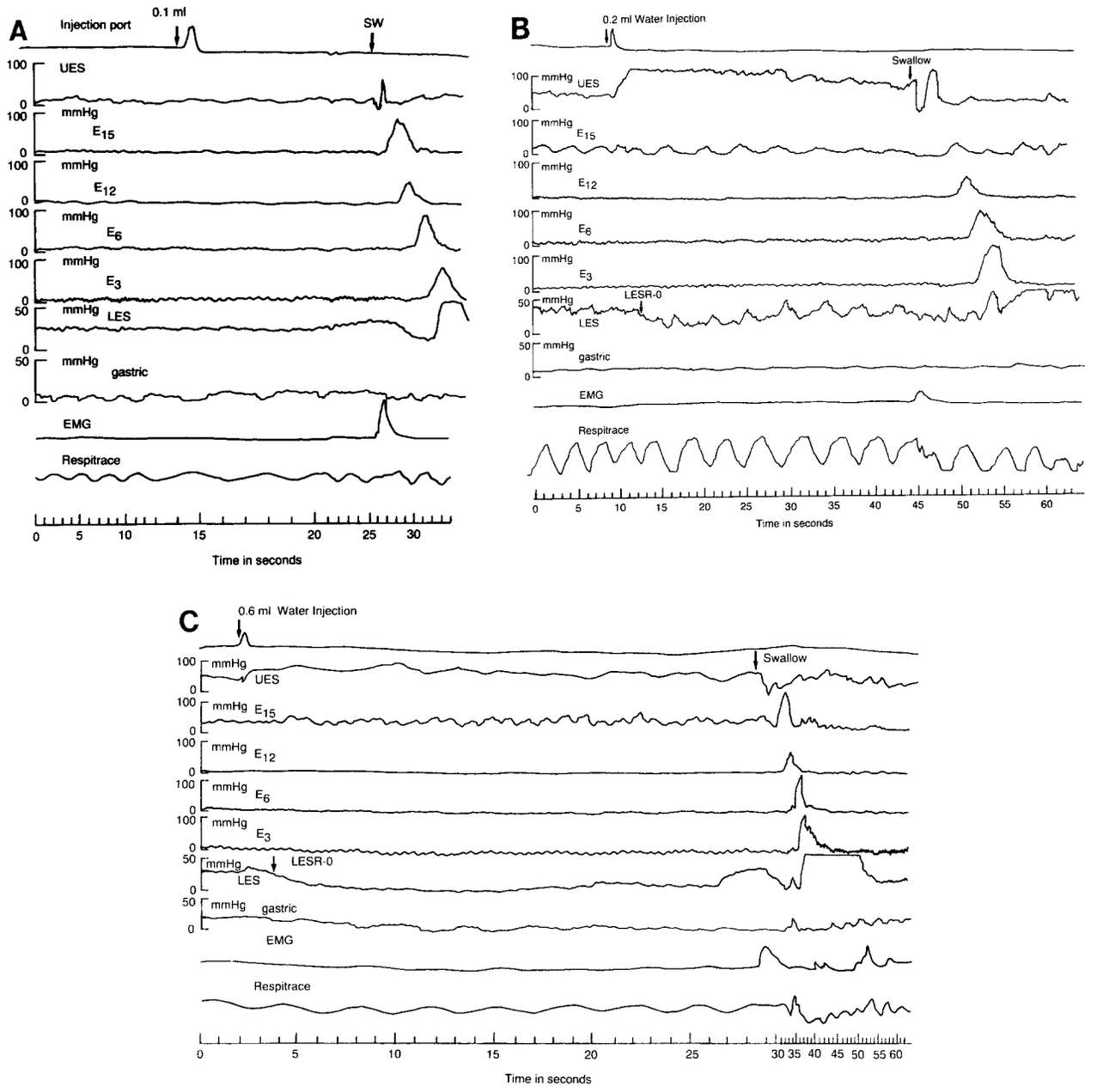


Figure 1. The effect of incremental amounts of water injected rapidly into the pharynx on LES resting pressure. (*A*) Subthreshold volume. Injection of 0.1 mL water directed to the posterior wall of the pharynx did not induce any change in the LES pressure. Water injection is followed by a spontaneous swallow 10 seconds later. (*B*) Partial LES relaxation induced by 0.2 mL water injected into the pharynx. (*C*) Complete LES relaxation induced by injection of 0.6 mL water into the pharynx. In these examples, the LES recovers from relaxation spontaneously. In addition, this recovery is not accompanied by a postrelaxation contraction. In *B* and *C*, there is an increase in the upper esophageal sphincter resting pressure after the injections.

LES relaxation and to induce a pharyngeal swallow is shown in Figure 5. In both modes of water injection, a significantly larger volume of water was required to induce a pharyngeal swallow than to induce isolated LES relaxation.

In 5 of 10 subjects, the threshold volume for slow continuous injection induced complete relaxation of the LES. Four of these subjects were among those with complete LES relaxation at the threshold volume for rapid-pulse injections. In the remaining subjects, the LES relaxation was partial, ranging between 40% and 80% of the preinjection level. The LES relaxation occurred 5.6 ± 0.5 seconds after the initiation of slow water injection. In cases of partial relaxation, the LES pressure did not decrease further between the onset of LES relaxation and the occurrence of pharyngeal swallow. Contrary to rapid-pulse injection, none of the volunteers with partial LES relaxation at the threshold volume developed complete LES relaxation with continued water infusion.

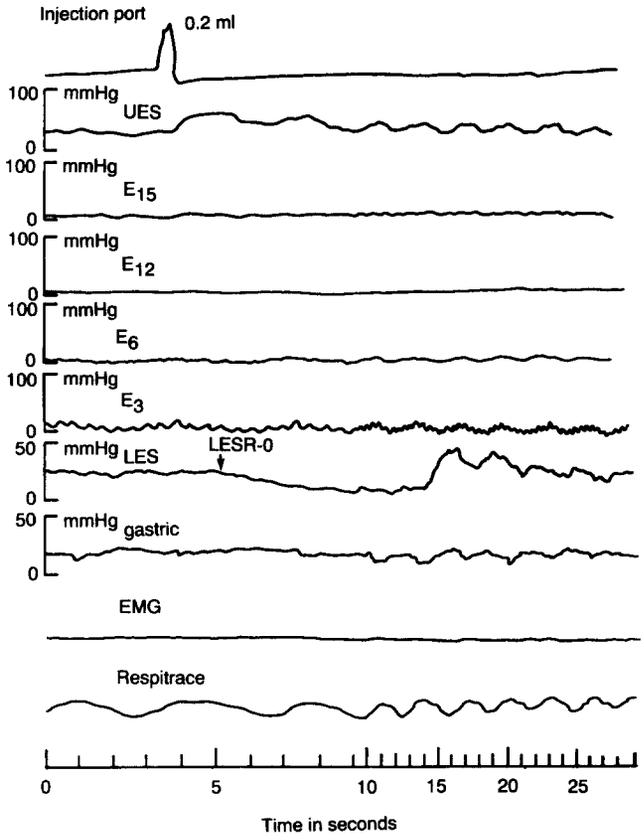


Figure 2. Postrelaxation contraction after the recovery of LES from relaxation induced by pharyngeal water injection. A 0.2-mL water injection into the pharynx induced an 80% LES pressure decline from the pre-ejection value. The total duration of this relaxation was 9 seconds. Recovery of the LES from this relaxation was accompanied by a postrelaxation contraction. This type of recovery was found in 33% of the trials.

Similar to rapid-pulse injection, upper esophageal sphincter pressure increased during slow continuous injection (Figure 4). This finding occurred in 90% of the trials. The increase averaged 138% ± 12% over the preinjection values. In the rest, the upper esophageal sphincter pressure did not change during slow pharyngeal water injection.

Discussion

In this study, we characterized the effect of pharyngeal water stimulation on the resting LES pressure. Our study findings indicate that rapid or slow injection of minute amounts of water into the pharynx induces relaxation of the LES. This relaxation was complete in about half of the subjects. An increase in the volume of injected water resulted in complete LES relaxation in an additional 4 subjects, whereas in the remaining subjects, LES relaxation remained partial regardless of the volume of injected water. Our study findings also indicate that if the water is injected slowly, a significantly larger vol-

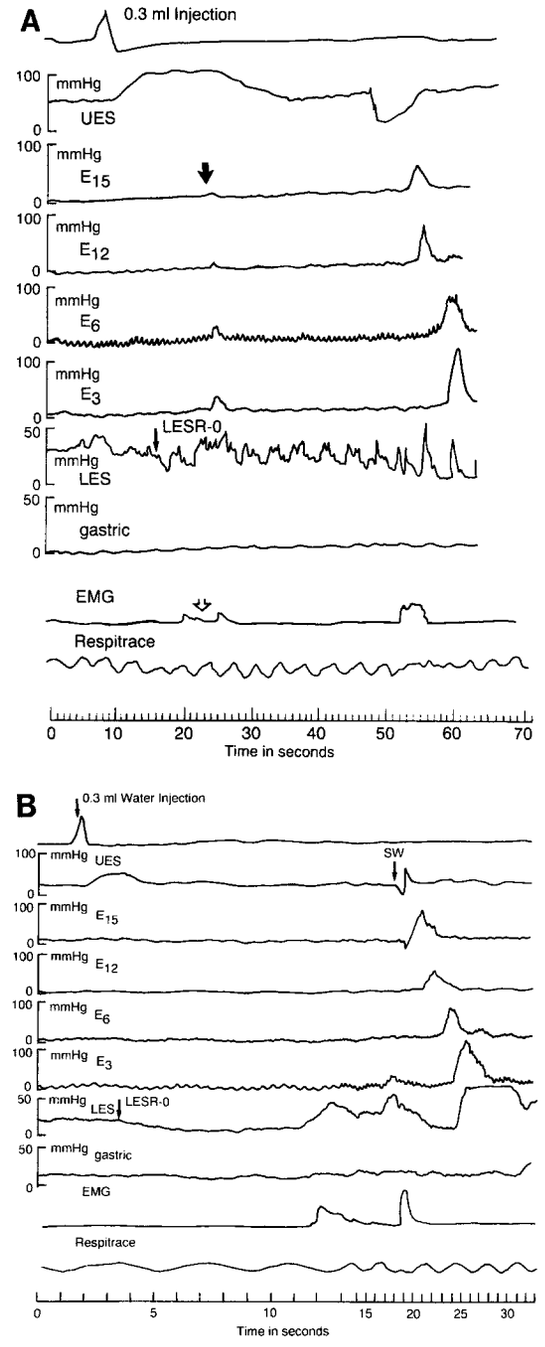


Figure 3. LES relaxations induced by pharyngeal water injection that were associated with submental electromyographic activities. (*A*) Injection of 0.3 mL water into the pharynx induced a partial LES relaxation. Four seconds after the onset of LES relaxation, electromyographic activity of submental muscles occurred (*open arrow*). This activity was accompanied by a small simultaneous contraction of the body of the esophagus (*closed arrow*). These events were associated with the return of LES pressure to the preinjection value. (*B*) Injection of 0.3 mL water into the pharynx in a different volunteer resulted in complete LES relaxation. Eight seconds after the onset of LES relaxation, a submental electromyographic signal that was temporally related to the termination of the LES relaxation developed. Contrary to the example in Figure 1*A*, this signal was not accompanied by an esophageal contraction. In both examples, there was a short duration of increased upper esophageal sphincter pressure after water injection.

ume is required to induce LES relaxation than during rapid-pulse injection.

Earlier studies have shown that pharyngeal water injection at a threshold volume during both rapid-pulse and slow continuous injection results in an irrepressible swallow (pharyngeal, or secondary, swallow).[7] The findings of this study indicate that a significantly smaller volume of water is required to induce LES relaxation than to induce a pharyngeal swallow.

The mechanism of LES relaxation induced by pharyngeal water stimulation remains to be elucidated. However, previous studies of pharyngeal mechanical stimulation in opossums[2] suggest that isolated LES relaxation may occur as part of the stimulation of the swallowing pathway. The fact that our subjects were instructed to resist swallowing may have eliminated swallows that would have occurred otherwise and may have isolated LES relaxation from the rest of the swallowing complex.

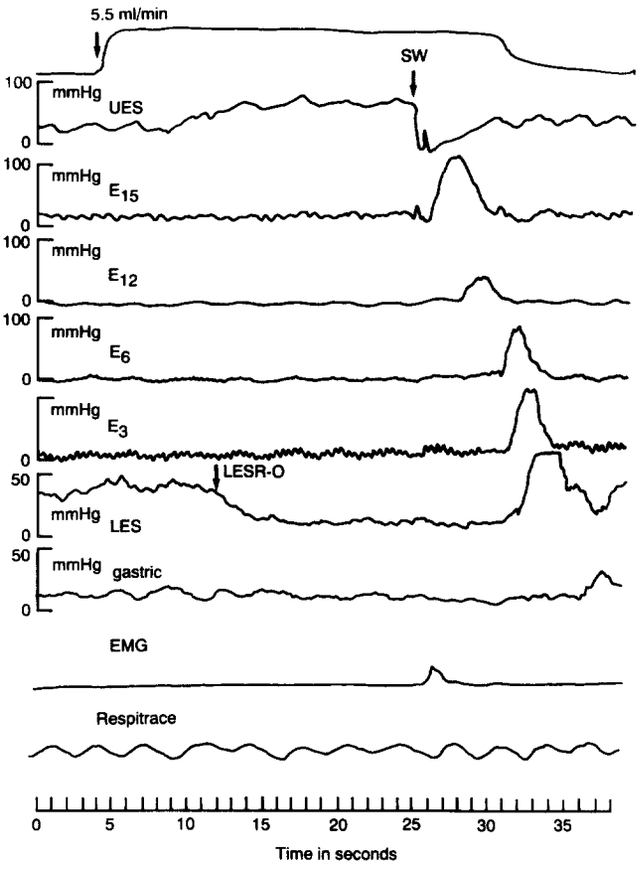


Figure 4. LES relaxation induced by intrapharyngeal slow continuous water injection. Injection of water at the rate of 5.5 mL/min resulted in complete LES relaxation (*arrow*) after 8 seconds. This relaxation was not accompanied by either electromyographic or esophageal contractile activities. Similar to rapid-pulse injection, this mode of injection also resulted in an increase in the upper esophageal sphincter resting pressure. Twelve seconds later, pharyngeal water infusion reached the threshold for a pharyngeal swallow, marked by SW. This threshold is heralded by a submental electromyographic signal.

Swallowing results in deglutitive inhibition in the esophagus and LES, followed by excitation. Previous studies have shown that the threshold for activation of the deglutitive inhibition is lower than that of the deglutitive excitation.[8] Our finding that a significantly smaller volume is needed to induce LES relaxation than to initiate an irrepressible swallow corroborates these earlier findings.

LES tone is modulated by the effect of excitatory and inhibitory vagal[9] impulses to the LES muscle. In the current study, injection of the threshold volume resulted in complete LES relaxation in approximately half of the subjects. An additional 3 subjects' response could be converted from partial to complete relaxation with an increase in the volume of injected water. However, in 4 individuals, the LES relaxation was consistently incomplete. These findings suggest that pharyngeal mechanoreceptor stimulation results in various combinations of activation of inhibitory and inhibition of excitatory pathways that control the LES tone. It also suggests the existence of intersubject variation in the threshold of activation of these pathways.

Inhibition of LES tone independent of swallowing and belching is known to occur; it is called transient LES relaxation. Although the exact mechanism of these relaxations is not known, it has been proposed that they may occur as part of subthreshold swallows,[1,2] incomplete belch events, or both.[3] Presence of a manometric catheter

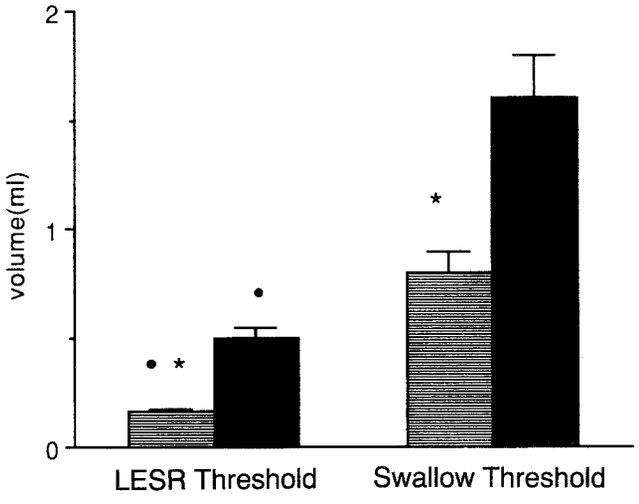


Figure 5. Comparison of the threshold volumes that induce LES relaxation (LESR Threshold) with that of pharyngeal swallow (Swallow Threshold) induced by rapid (≡) and slow (■) injection. For both modes of water injection, the threshold volume required to induce LES relaxation was significantly smaller than that for the induction of pharyngeal swallow (*$P < 0.05$). In addition, for both events to occur, a significantly smaller volume was required when the water was injected rapidly than when it was injected slowly (*$P < 0.05$).

in the pharynx[4] and gastric fundal distention[5,6] have been shown to increase the frequency of these relaxations of the LES. The present study identifies yet another factor that influences LES resting tone. Whereas the possible contribution of this finding to the mechanism of transient LES relaxation remains to be ascertained, it may explain the fluctuations of LES resting pressure observed during long-term measurements.

It is conceivable that minute amounts of fluid introduced into the pharynx through salivary production, postnasal drip, or other aerodigestive tract discharges may induce complete or partial relaxation of the LES and facilitate gastroesophageal reflux. This may also explain why elimination of a transnasal catheter did not completely abolish the occurrence of transient LES relaxation.[4] Further studies are needed to investigate these issues.

Our findings also corroborate earlier studies, which showed that pharyngeal mechanical stimulation in cats[10] and water stimulation in humans[7] results in an increase in the resting tone of the upper esophageal sphincter, the pharyngo–upper esophageal sphincter contractile reflex. Although the physiological role of this reflex remains to be elucidated, it might be speculated that it functions as an airway-protective mechanism whereby retrograde entry of small volumes of liquid into the pharynx from the stomach results in augmentation of upper esophageal sphincter tone, reducing the chance of further regurgitation into the pharynx.

In conclusion, minute amounts of liquid injected either abruptly or slowly into the pharynx induce LES relaxation. The duration and magnitude of LES relaxation induced by pharyngeal water injection are different from those of the normal swallow. Neither the duration nor the magnitude of this relaxation is volume dependent. The physiological contributions of this finding remain to be elucidated.

References

1. Mittal RK, McCallum RW. Characteristics of transient lower esophageal sphincter relaxation in humans. Am J Physiol 1987;252:G636–641.
2. Paterson WG, Rattan S, Goyal RK. Experimental induction of isolated lower esophageal sphincter relaxation in anesthetized opossums. J Clin Invest 1986;77:1187–1193.
3. Dodds WJ, Hogan WJ, Helm JF, Dent J. Pathogenesis of reflux esophagitis. Gastroenterology 1981;81:386–390.
4. Mittal RK, Stewart WR, Schirmer BD. Effect of a catheter in the pharynx on the frequency of transient lower esophageal sphincter relaxations. Gastroenterology 1992;103:1236–1240.
5. Holloway RH, Hongo M, Berger K, McCallum RW. Gastric distention: a mechanism for postprandial gastroesophageal reflux. Gastroenterology 1985;89:779–784.
6. Martin CJ, Patrikios MJ, Dent J. Abolition of gas reflux and transient lower esophageal sphincter relaxation by vagal blockade in the dog. Gastroenterology 1986;91:890–896.
7. Shaker R, Ren J, Zamir Z, Sarna A, Liu J, Sui Z. Effect of aging, position and temperature on the threshold volume triggering pharyngeal swallowing. Gastroenterology 1994;107:396–402.
8. Gidda JS, Goyal RK. Swallow-evoked action potentials in vagal preganglionic efferents. J Neurophysiol 1984;52:1169–1180.
9. Roman C, Gonella J. Extrinsic control of digestive tract motility. In: Johnson LR, ed. Physiology of the gastrointestinal tract. Volume 1. 2nd ed. New York: Raven, 1987:507–522.
10. Medda B, Lang IM, Layman RD, Hogan WJ, Dodds WJ, Shaker R. Characterization and quantification of a pharyngo-UES contractile reflex in the cat. Am J Physiol (in press).

Received June 23, 1994. Accepted October 18, 1994.

Address requests for reprints to: Reza Shaker, M.D., GI Section/111C, VA Medical Center, 5000 West National Avenue, Milwaukee, Wisconsin 53295. Fax: (414) 384-8480.

Supported in part by grant RO1DC00669-5, a merit review grant from the Department of Veterans Affairs. Dr. Trifan was supported in part by an Astra–Merck Research Fellowship grant.

Presented in part at the annual meeting of the American Gastroenterological Association in May 1994 in New Orleans, Louisiana, and published in abstract form (Gastroenterology 1994;106:A580).

Small-bowel neoplasms in patients undergoing video capsule endoscopy: a multicenter European study

Authors

E. Rondonotti[1], M. Pennazio[2], E. Toth[3], P. Menchen[4], M. E. Riccioni[5], G. D. De Palma[6], F. Scotto[7], D. De Looze[8], T. Pachofsky[9], I. Tacheci[10], T. Havelund[11], G. Couto[12], A. Trifan[13], A. Kofokotsios[14], R. Cannizzaro[15], E. Perez-Quadrado[16], R. de Franchis[1] on behalf of the European Capsule Endoscopy Group (ECEG), the Italian Club for Capsule Endoscopy (CICE) and the Iberian Group for Capsule Endoscopy (IGCE). Also see Appendix for additional contributors.

Institutions

Institutions are listed at the end of article.

submitted
4 September 2007
accepted after revision
16 January 2008

Bibliography
DOI 10.1055/s-2007-995783
Published online
8 May 2008
Endoscopy 2008; 40:
488–495 © Georg Thieme
Verlag KG Stuttgart · New York
ISSN 0013-726X

Corresponding author
E. Rondonotti, MD
Università degli Studi di Milano
IRCCS Fondazione Policlinico,
Mangiagalli, Regina Elena
Dipartimento di Scienze
Mediche
Unità Operativa
Gastroenterologia 3
Via Pace 9
20122 Milano
Italy
Fax: +39-02-50320747
emanuele.rondonotti@unimi.it

Background and study aim: Small-bowel tumors account for 1%–3% of all gastrointestinal neoplasms. Recent studies with video capsule endoscopy (VCE) suggest that the frequency of these tumors may be substantially higher than previously reported. The aim of the study was to evaluate the frequency, clinical presentation, diagnostic/therapeutic work-up, and endoscopic appearance of small-bowel tumors in a large population of patients undergoing VCE.

Patients and methods: Identification by a questionnaire of patients with VCE findings suggesting small-bowel tumors and histological confirmation of the neoplasm seen in 29 centers of 10 European Countries.

Results: Of 5129 patients undergoing VCE, 124 (2.4%) had small-bowel tumors (112 primary, 12 metastatic). Among these patients, indications for VCE were: obscure gastrointestinal bleeding (108 patients), abdominal pain (9), search for primary neoplasm (6), diarrhea with malabsorption (1). The main primary small-bowel tumor type was gastrointestinal stromal tumor (GIST) (32%) followed by adenocarcinoma (20%) and carcinoid (15%); 66% of secondary small-bowel tumors were melanomas. Of the tumors, 80.6% were identified solely on the basis of VCE findings. 55 patients underwent VCE as the third procedure after negative bidirectional endoscopy. The lesions were single in 89.5% of cases, and multiple in 10.5%. Retention of the capsule occurred in 9.8% of patients with small-bowel tumors. After VCE, 54/124 patients underwent 57 other examinations before treatment; in these patients enteroscopy, when performed, showed a high diagnostic yield. Treatment was surgery in 95% of cases.

Conclusions: Our data suggest that VCE detects small-bowel tumors in a small proportion of patients undergoing this examination, but the early use of this tool can shorten the diagnostic work-up and influence the subsequent management of these patients.

Introduction

Although the small bowel represents 75% of the length and 90% of the overall mucosal surface of the alimentary tract, it is considered a rare location for the development of neoplasms, accounting for only 1%–3% of all primary gastrointestinal tumors [1,2]. A review of the Utah Cancer Registry from 1966 through 1990 showed that the overall age-adjusted yearly incidence of small-bowel cancers was 1.4 per 100 000. Over a 30-year period [3], Barclay [4] reported an incidence of 0.7/0.6 (male/female) malignant small-bowel tumors per 100 000, which accounted for 1.6% of all gastrointestinal tumors [5].

Approximately 40 different histological types of small-intestinal tumors have been identified [6]. Among malignant tumors, about 30%–50% are adenocarcinomas, 25%–30% are carcinoids, and 15%–20% are lymphomas. The majority of benign small-bowel tumors originate from the stromal layer [7] accounting for about 15%–20% of all small-bowel primary neoplasms [8,9].

Secondary neoplasia has been reported to be more frequent than primary small-intestinal neoplasms. Primary tumors of the colon, ovary, uterus, and stomach can metastasize to the small bowel by direct invasion or by intraperitoneal spread, whereas primaries from breast, lung, and melanoma metastasize by the hematogenous route [7]. In patients with skin melanoma, small-bowel metastases have been described in 1.5%–4.4% of cases in in-vivo studies [10,11] and in 58% of post-mortem specimens [10].

Small-bowel tumors grow slowly, extraluminally, remaining asymptomatic for years or presenting insidiously in patients with nonspecific complaints such as abdominal pain, diarrhea, iron de-

ficiency anemia, bleeding, extraintestinal symptoms (flushing, paraneoplastic syndromes), or acute obstruction [12]. In these patients, the results of routine diagnostic laboratory and other diagnostic tests, such as push enteroscopy, small-bowel series (SBS) or enteroclysis, computed tomography, and magnetic resonance imaging may frequently be inconclusive. For these reasons the diagnosis is often delayed [6,12], thus failing to prevent the development of locally advanced lesions or metastatic disease.

The development and clinical implementation of video capsule endoscopy (VCE), an accurate, safe, and painless method of endoscopically evaluating all of the small bowel, has opened a new frontier in the field of small-bowel investigation. Since the introduction of this device into clinical practice, a few small series have been published showing an frequency of small-bowel neoplasms higher than previously expected, ranging between 2% and 9% [13–17], and some authors have speculated that routine use of wireless capsule endoscopy in the diagnostic algorithm for obscure gastrointestinal bleeding, iron deficiency anemia, and abdominal pain would lead to earlier diagnosis, and therefore improve the overall prognosis associated with malignant small-bowel tumors [18].

The aim of the present study was to describe the frequency, clinical presentation, endoscopic appearance, and diagnostic work-up related to small-bowel tumors in a large population of patients undergoing VCE.

Patients and methods

This study was carried out in 29 centers from 10 European countries. Each participating center reviewed its own series of consecutive patients undergoing VCE, from the beginning of the use of this device in clinical practice until October 2006.

For each patient in whom VCE showed one or more lesions suggesting small-bowel neoplasia, and a subsequent diagnostic/therapeutic work-up led to histological confirmation, a specific structured questionnaire was completed.

We decided to exclude from the study all patients with a known condition that increases the risk of small-bowel neoplasms (e.g. patients with refractory celiac disease or patients with familial adenomatous polyposis [FAP] or Peutz–Jeghers syndrome with alarm symptoms or under surveillance).

The questionnaire collected data on:

- the center where VCE was performed (name of referring physician, number of VCE procedures performed at the time of data submission),
- the patient (age, sex, and length of clinical history),
- indication for VCE (for patients with obscure gastrointestinal bleeding [OGIB], their hemoglobin level at the time of VCE),
- diagnostic work-up before VCE,
- results of VCE (endoscopic appearance of the lesion, and location, estimated by the physician reviewing the video),
- complications related to VCE (e.g. capsule retention),
- diagnostic-therapeutic work-up after VCE (particularly a brief description of surgical intervention, if done, with appearance and location of the lesion),
- final histological diagnosis.

Statistical analysis

This was done using SPSS software (SPSS 14.0 for Windows, SPSS Inc. Chicago, Illinois, USA). To describe the population, we used mean and SD for data with a Gaussian distribution (e.g. age) and median and range for data with a non-Gaussian distribution (e.g. length of clinical history). To compare unpaired groups, we used the two-tailed *t* test for unpaired groups or the Mann–Whitney test, respectively, for data with Gaussian or non-Gaussian distributions.

The Spearman correlation test was used to quantify the association between variables. We calculated the value of *r*, which ranges between + 1 and – 1; a value of 0 means that the two variables do not vary together at all, while + 1 or – 1 indicate perfect correlation (respectively, direct or inverse).

To analyze contingency tables we used the X^2 test or Fisher's exact test as appropriate. As usual, for all these tests, a *P* value of less than 0.05 was considered to be statistically significant.

Results

Frequency of small-bowel tumors

In 29 centers from 10 European countries, 5129 VCE examinations were performed, for any indication. Unfortunately we did not know when each center started to use VCE in clinical practice; thus we were not able to calculate the mean rate of examinations performed per center per year but only the total number of VCEs done at each center. From the 5129 examinations (**Table 1**), we collected data on 160 patients. A total of 36 questionnaires were excluded from further evaluation: 13 described small-bowel neoplasms in patients with refractory celiac disease (n = 8) or Peutz–Jeghers syndrome (n = 5), while 23 were incomplete. Thus, data from a total of 124 patients (mean age ± SD 60.3 ± 14.3 years) with histologically proven small-bowel neoplasms were evaluated.

The overall frequency of small-bowel tumors identified at VCE was 2.4%, ranging between 0.75% (3/400) and 9.3% (7/75) for the 29 centers. We found an inverse correlation (Spearman *r* = – 0.56, 95% confidence interval [CI] – 0.77 to – 0.23; *P* < 0.002; **Fig. 1**) between the frequency of identification of small-bowel neoplasms and the number of VCE examinations performed at each center.

Indication for VCE

The indication for VCE was obscure gastrointestinal bleeding (OGIB) in 108 patients (108/124, 87.1%), which was obscure-occult in 52 (52/108, 48.2%), ongoing overt in 36 (36/108, 33.3%) and previous overt in 20 (20/108, 18.5%). In the remaining 16 patients (16/124, 12.9%), the indication for VCE was abdominal pain in 9, investigation for primary neoplasms in patients with liver metastases or carcinoid syndrome in 6, and diarrhea with severe malabsorption in 1.

Diagnostic work-up before VCE

Of the 124 patients, 55 (44.4%) underwent VCE as the third examination after negative bidirectional endoscopy, while the remaining 69 (55.6%) had undergone at least one further examination aimed to evaluate the small-bowel before VCE. These 69 patients underwent 102 examinations specifically addressed to evaluate the small bowel (not including repeated gastroscopies and colonoscopies) before capsule endoscopy (mean number of diagnostic procedures per patient, 1.47); in 45 out of these 69 pa-

Table 1 Centers participating the study. For each center, the table shows the number of video capsule endoscopy (VCE) examinations performed, the number of small-bowel tumors identified at VCE, and the resulting percentage frequency of small-bowel tumors identification at VCE

Center	Country	VCE procedures performed, n	Small-bowel tumors identified at VCE, n	Frequency of identification of small-bowel tumors at VCE, %
Ghent	Belgium	70	4	5.7
Aviano	Italy	121	4	3.3
Bari	Italy	75	7	9.3
Bologna	Italy	41	1	2.4
Busto Arsizio	Italy	264	6	2.3
Ferrara	Italy	120	2	1.7
Genoa	Italy	110	3	2.7
Milan	Italy	400	9	2.2
Naples 1	Italy	86	1	1.2
Naples 2	Italy	265	7	2.6
Polla	Italy	226	3	1.3
Rome 1	Italy	366	8	2.1
Rome 2	Italy	58	1	1.7
Venafro	Italy	15	1	6.7
Turin	Italy	280	10	3.6
Barcelona	Spain	156	2	1.3
Murcia	Spain	67	2	3.0
Toledo	Spain	141	4	2.8
Madrid	Spain	400	3	0.7
Madrid 2	Spain	375	10	2.6
Bilbao	Spain	50	2	4.0
Pamplona	Spain	132	3	2.3
Thessalonika	Greece	35	1	2.8
Iasi	Romania	26	2	7.7
Hradec Kralove	Czech Republic	61	3	4.9
Malmø	Sweden	864	16	1.8
Vienna	Austria	149	4	2.7
Lisbon	Portugal	48	2	4.1
Odense	Denmark	128	3	2.3
Total		**5129**	**124**	**2.4**

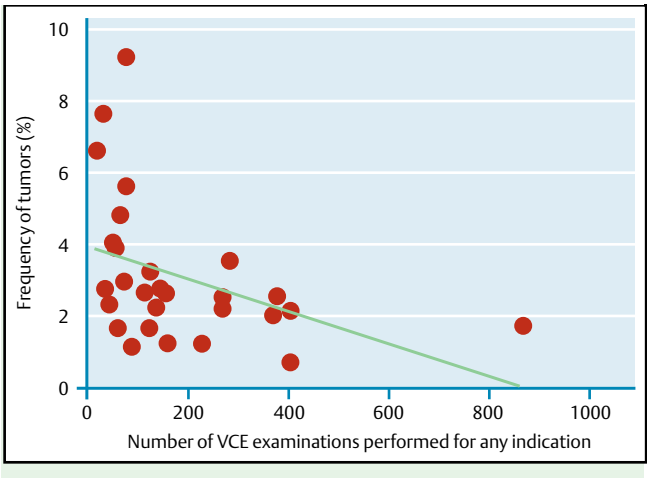


Fig. 1 Correlation between number of video capsule endoscopy (VCE) examinations done for any indication and frequency of identification of small-bowel tumors, at 29 centers participating in the study (Spearman correlation test, $r = -0.56$, 95% confidence interval [CI] – 0.77 to – 0.23; $P < 0.002$).

tients the diagnostic work-up was completely negative while 24 patients had at least one examination with positive results. The examinations performed before VCE and their diagnostic yield are shown in **Table 2**.

Taking together the patients who underwent VCE immediately after negative bidirectional endoscopy (n = 55) and the patients with a negative diagnostic work-up despite further examinations (n = 45), capsule endoscopy had a direct impact on diagnosis, identifying an unexpected small-bowel tumor, in 80.6% of patients (100/124).

Endoscopic appearance and histological classification

The endoscopic appearance of lesions identified by VCE was: polyp or mass in 94 patients (75.8%); ulcers in 10 (8.1%); fresh blood in 8 (6.5%); stenoses in 8 (6.5%); and cobblestone in 4 (3.2%). **Fig. 2** shows a polypoid, ulcerated lesion that was confirmed at surgical intervention (**Fig. 3**).

These lesions were single in 111 cases (89.5%) and multiple in 13 (10.5%)

Of 124 lesions identified, 112 were primary neoplasms (112/5129 or 2.2%) while the other 12 cases were small-bowel metastases (12/5129 or 0.2%). The most frequent histological type of primary tumors was gastrointestinal stromal tumor (GIST) (32.1%) followed by adenocarcinoma (20.5%) and carcinoid (15.6%). In 8 out of 12 cases with metastatic small-bowel tumors, the metastases were from a previously removed skin melanoma. The his-

Table 2 Diagnostic yield of examinations performed before video capsule endoscopy (VCE) in 69 patients, and after VCE in 112 patients without capsule retention

	Examinations performed before VCE Total patients, n = 69		Examinations performed after VCE Total patients, n = 112	
	Number of examinations, n	Diagnostic yield, %	Number of examinations, n	Diagnostic yield, %
Small-bowel series/small-bowel enteroclysis	49	10.2	2	100
Abdominal CT scan	19	47.3	2	50
Bleeding nuclear scan	13	53.8	1	0
Push enteroscopy	10	10.0	30	77
Octreoscan	4	50.0	None	
Angiography	2	0.0	4	75
SPECT	2	0.0	None	
Meckel's scan	1	0.0	None	
Double-balloon enteroscopy	1	0.0	5	80
Surgical intervention	1	0.0	None	
Gastroscopy	None		5	100
Colonoscopy	None		3	0
MRI enteroclysis	None		3	66
CT enteroclysis	None		2	50
Total	**102**	**24.3**	**57**	**72**

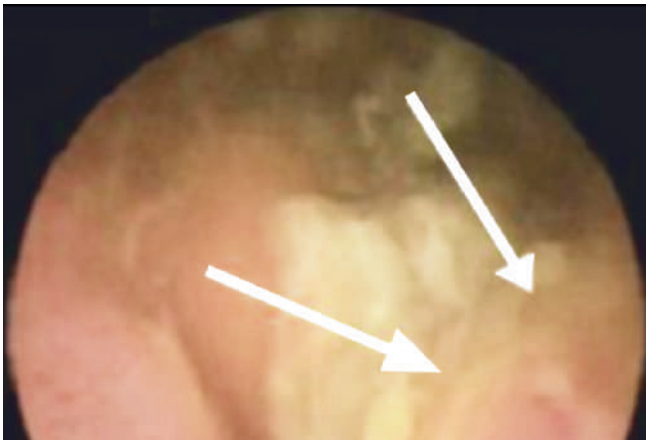


Fig. 2 Capsule endoscopy image showing a polypoid ulcerated lesion (white arrows).

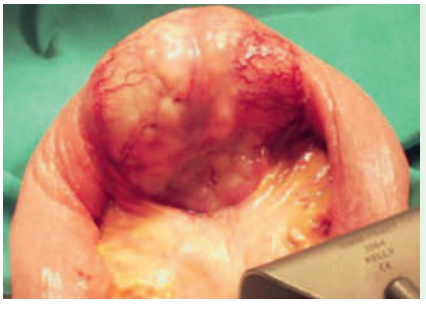


Fig. 3 Same patient as in **Fig. 2**. Small-bowel tumor, 3 cm in size, involving the wall of the small bowel circumferentially.

Table 3 Histological classification of small-bowel neoplasms

	n	%
Primary small-bowel neoplasms		
GIST	36	32.1
Adenocarcinoma	23	20.5
Carcinoid	17	15.2
Lymphoma	12	10.7
Lipoma	10	8.9
Angioma	4	3.6
Neuroendocrine tumor	4	3.6
Sarcoma	3	2.7
Juvenile hamartoma	2	1.8
Kaposi's sarcoma	1	0.8
Total	**112**	**100%**
Small-bowel metastases		
Melanoma	8	66.4
Colonic carcinoma	2	16.0
Seminoma	1	8.3
Hepatocellular carcinoma	1	8.3
Total	**12**	**100%**

GIST, gastrointestinal stromal tumor.

tological classification of the small-bowel primary neoplasms and metastases is shown in **Table 3**.

Location of tumors

On the basis of further diagnostic and/or therapeutic work up carried out after VCE, the definitive location of single lesions was established as the jejunum in 70.3% of cases, the ileum in 22.5%, and the duodenum in 7.2%. There was 92.8% agreement (103/111 cases) between the location of single lesions as assessed by VCE and that established on the basis of further diagnostic and/or therapeutic work-up.

Complications of capsule endoscopy

All patients enrolled in this study (except one in whom the capsule was placed in the stomach using the endoscope), swallowed the capsule easily. Among the 38 patients (30.9%) in whom the

Table 4 Cases of capsule retention: demographic data of patients, indication for video capsule endoscopy (VCE) and clinical data, endoscopic appearance and location of tumor, and histological findings, and method used to retrieve capsule

Age, sex	Indication for VCE	Length of clinical history, months	Hb at time of VCE, g/dl	Tumor			Capsule retrieval
				Endoscopic appearance	Location	Histology	
61, M	OGIB (obscure occult)	6	8.3	Polyp/mass	Jejunum	Adenocarcinoma	Push enteroscopy
68, M	OGIB (obscure occult)	12	11.8	Stenosis	Multiple	Adenocarcinoma	Surgery
78, M	OGIB (ongoing overt)	8	10.0	Stenosis	Jejunum	Adenocarcinoma	Surgery
52, M	OGIB (ongoing overt)	4	6.0	Polyp/mass	Duodenum	Adenocarcinoma	Push enteroscopy
56, M	OGIB (ongoing overt)	1	8.3	Polyp/mass	Ileum	Colonic carcinoma (metastasis)	Surgery
50, M	OGIB (ongoing overt)	0.5	7.5	Polyp/mass	Ileum	Adenocarcinoma	Surgery
35, M	OGIB (ongoing overt)	7	8.0	Polyp/mass	Jejunum	Angiosarcoma	Surgery
66, M	OGIB (ongoing overt)	3	9.0	Polyp/mass	Jejunum	GIST	Surgery
63, M	OGIB (ongoing overt)	2	8.6	Stenosis	Jejunum	Lymphoma	Surgery
66, M	OGIB (previous overt)	24	6.8	Stenosis	Jejunum	Lymphoma	Surgery
53, M	Investigation for primary neoplasm	6	NA	Stenosis	Ileum	Lymphoma	Surgery
61, M	Investigation for primary neoplasm	10	NA	Stenosis	Jejunum	Carcinoid	Surgery

OGIB, obscure gastrointestinal bleeding; Hb, hemoglobin; GIST, gastrointestinal stromal tumor; NA, not applicable.

capsule did not reach the ileocecal valve during the examination period, in 12 (12/124, 9.7%) the capsule was stuck at the site of the tumor. Five of these 12 patients (41.6%) had undergone an SBS or small-bowel enteroclysis before VCE, with negative results. In 2/12 patients (16%) the capsule was retrieved with the push enteroscope and in 10 (84%) by surgical intervention. The relevant data for these 12 patients is presented in **Table 4**. Capsule retention occurred only in patients with stenoses (n = 6) or polyps/masses (n = 6). As expected, stenoses led to capsule retention more frequently than polyps/masses (6 cases of retention in 8 patients with stenoses vs. 6 in 94 with polyps/masses; Fisher test, $P = 0.002$).

There was no difference in the occurrence of retention according to type of OGIB, location, or histological type of tumor.

None of these 12 patients had acute obstruction due to capsule retention. In the remaining 26 patients, in whom the capsule did not reach the ileocecal valve during the examination period but was not retained, the capsule was egested naturally in the stool within 7 – 15 days.

Diagnostic and therapeutic work up after capsule endoscopy

A total of 110 patients were treated by surgery alone, 8 received a combination of surgery and chemotherapy, 1 underwent endoscopic polypectomy, while 1 was left untreated because of poor general condition and 4 were lost to follow-up. Among 112 patients without capsule retention, treatment was given directly after VCE in 58 patients, while 54 underwent further examinations (**Table 2**), which were negative in 9. Thus, in 67 patients therapy was done solely on the basis of the diagnosis made by VCE, in 35 it was based also on the results of further tests, while 12 underwent operation following capsule retention.

Discussion

Since the introduction of VCE in clinical practice, several studies have shown that the performance of this technique is superior to that of other diagnostic modalities in detecting small-bowel abnormalities including vascular lesions, inflammation and tumors [19 – 22]. Nowadays, it is well accepted that VCE plays a key role in the diagnostic work-up of obscure gastrointestinal bleeding, in the diagnosis of suspected Crohn's disease and in the evaluation of the small bowel in patients with refractory celiac disease, but the role of capsule endoscopy in the diagnosis and management of small-bowel tumors is still debated, despite a growing body of evidence in this field [13 – 18].

The aim of the present study was to describe the frequency, clinical presentation, endoscopic appearance, and diagnostic work-up of small-bowel tumors in a population that was as large as possible of patients undergoing VCE. For this purpose, the study protocol was sent to all members of the European Capsule Endoscopy Group (ECEG) and a specific notice was placed in the home page of the website www.capsuleendoscopy.org. On the one hand, this method of data collection allowed us to create the largest database published so far of small-bowel tumors detected by capsule endoscopy (124 tumors with 5129 capsule procedures performed); on the other hand, this led to the inclusion of a group of centers that were heterogeneous (in terms of number of patients referred for VCE, number of tumors identified, number of capsule examinations performed, and the diagnostic and therapeutic work-up done before and after VCE). The fact that the majority of cases came from Italy and Spain is probably due to the existence in both countries of a capsule endoscopy study group that facilitated case collection.

In our study the frequency of small-bowel tumors (2.4%) was surprisingly and substantially lower than that reported in other studies in which, as in the present one, the authors collected both benign and malignant neoplasms [13 – 16, 18, 19]. The strict patient selection (particularly the requirement for histological confirmation of lesions identified by capsule endoscopy, and

the exclusion of patients with a high pre-test probability of having a tumor) could be a possible explanation for this difference. On the other hand, while our series is comparable to the other published series [13 – 19] in terms of age and gender of the patients and of clinical indication for VCE, an important difference is the number of VCE examinations performed, which in our study is almost ten times larger than that of the largest previously published series. To ascertain whether the number of examinations is in any way related to the frequency of tumor detection, we examined the relationship between frequency of tumors found and number of VCE examinations performed in the centers participating in the study. Indeed, this analysis revealed a significant inverse correlation (Spearman $r = -0.56$, $P < 0.002$; **Fig. 1**), suggesting that the high number of VCEs carried out might in some way be related to the low frequency of tumor detection that we found. Interestingly, our figures are in keeping with those of the second largest existing study, by Pasha et al. [23], that was recently presented at an international meeting; this included 1000 VCE examinations, with a 1.6% frequency of small-bowel tumors. There is no obvious explanation for this. It is possible that centers where fewer examinations were carried out adopted stricter criteria for patient selection than larger centers; however, the characteristics of the patients enrolled in the different participating centers in our study were homogeneous.

In our series, as in other studies, OGIB was the leading indication for capsule endoscopy. This was expected, since OGIB is the indication for VCE in 65% – 100% of cases in all published series [13,18].

The design of the study does not allow estimation of the sensitivity and specificity of capsule endoscopy for small-bowel tumors. Recently published studies with double-balloon enteroscopy (DBE) [24] clearly demonstrated that capsule endoscopy can miss even large malignant masses (a pooled analysis of previously published studies [21] showed a miss rate of up to 18.9%). On the other hand, the difficulties in distinguishing bulges from masses underline the main limitation of capsule endoscopy, i.e. the inability to take biopsies. Furthermore, we could not evaluate the role of DBE in confirming or disproving the diagnoses made by capsule endoscopy, since during the collection of our cases DBE was not widely available at the participating centers. Despite these limitations, our study confirms that nowadays capsule endoscopy is often used as the third examination (in about 50% of patients) after a negative bidirectional endoscopy, especially in patients with obscure gastrointestinal bleeding [19,25,26].

As far as the diagnostic work-up before VCE is concerned, all our patients underwent at least one gastroscopy and one colonoscopy with negative results. In the present study, we focused on the alternative techniques to conventional endoscopy that were included in the work-up of patients before capsule endoscopy, because we were interested in assessing their diagnostic performance for small-bowel tumors. The mean number of examinations performed in 69 patients who underwent other diagnostic tests before VCE, excluding repeated bidirectional endoscopy, is comparable with those reported in other studies [13 – 15,17 – 19] (range 1.28–1.57), as is the percentage of patients in whom other diagnostic techniques failed to identify the neoplasm (about 65%) [15]. Concerning the impact of VCE on diagnosis, we were conservative and counted only the patients in whom VCE showed a tumor undetected by other techniques. Nevertheless, 80% of tumors were identified solely by VCE, and this figure is much higher than that reported by others [17]. Enteroscopy (both push and balloon enteroscopy) was seldom performed before VCE, and had a low diagnostic yield (**Table 2**; 1/11, 9%). On the other hand, when conventional enteroscopy (push or DBE) was performed after a positive VCE examination, the diagnostic yield rose sharply (**Table 2**; 27/35, 77%; $P = < 0.0001$), suggesting that VCE can be useful in indicating the utility of conventional endoscopy for obtaining tissue samples.

As reported in other recently published series [13 – 18], adenocarcinomas, GISTs, carcinoids and lymphomas account for about 90% of small-bowel neoplasms. The most frequent tumor type in our series was GIST. When it is located in the small bowel, it is generally considered to be malignant in about 50% of cases; however, from an oncological standpoint, GISTs form a continuum. In general, size and mitotic activity [27,29] are used to judge the oncologic potential of these tumors. Unfortunately, we do not have mitotic activity data for the GISTs in our series, and therefore we decided not to classify them as malignant or benign. Our study also confirms the high tropism of skin melanoma for the small-bowel mucosa [10,11,13 – 16,18,30,31].

As expected, the majority of small-bowel tumors in our study were lesions that protruded into the lumen located in the jejunum, an area that is difficult to evaluate with other diagnostic techniques. Since confirmation of the diagnosis was obtained in all cases by conventional endoscopic or surgical means, we were able to compare the location of the lesion as assessed by the VCE reviewer with that found at endoscopy or surgery: in patients with a single lesion we found an impressive agreement (92.8%), which is comparable with that reported by Pasha et al. [23]. Among the eight cases in which the location of the lesion was misjudged at capsule endoscopy, in four cases the lesion was more proximal than expected on the basis of the capsule examination and in four cases it was more distal.

We did not use the standard definition of capsule retention [32], i.e. retention of the capsule in the small intestine for more than 15 days, because in some cases the procedure planned to solve the clinical problem was performed earlier than 15 days from ingestion. Therefore, we defined capsule retention as having occurred when the videos showed repetitive images, suggesting stenosis, and the capsule was retrieved at the site of the lesion by surgery or push enteroscopy. According to these criteria, capsule retention occurred in about 10% of cases. This rate is similar to the 11.5% reported by Bailey et al. [15] and substantially higher than those reported by Urbain et al. [17] and Cobrin et al. (0%) [14], while Pasha et al. [23] reported a very high occurrence (about 25%) of capsule retention in patients with small-bowel tumors.

We do not have a obvious explanation for these differences among studies. As reported in several studies [32,34] a negative SBS or enteroclysis performed before VCE does not guarantee the passage of the capsule: in our series five patients with capsule retention had undergone an SBS or enteroclysis with negative findings before the VCE.

Acute obstruction due to capsule retention is a rare complication [35] of capsule endoscopy and capsule retention can be considered, particularly in this subset of patients who often require surgical intervention, as a “positive complication” leading to diagnosis. None of our patients with capsule retention experienced acute obstruction, as reported also by Bailey et al. [15]. We can hypothesize that in patients with small-bowel tumors, the slow growth and the development of pre-stenotic dilatation, often described at the time of surgical intervention, can prevent acute obstruction. The development of a dissolvable capsule to

test bowel patency, by Given Imaging (Yoqneam, Israel) [37 – 41], and the recently achieved possibility of retrieving retained capsules with DBE [36], will probably decrease the occurrence and clinical consequences of this complication.

Capsule endoscopy appears thus to be appropriate as the first step in the diagnostic process of small-bowel tumors, since it may direct further diagnostic procedures (push enteroscopy, DBE) aimed at obtaining tissue: in our series this led to a definitive histologic diagnosis in 77 % – 80 % of cases. In addition, capsule endoscopy has a direct impact on therapy, since 60 % of our patients in whom the capsule was not retained underwent surgical or endoscopic therapy immediately following capsule endoscopy. At present, surgery is by far the most frequently used treatment in these patients [14 – 18, 23].

Whether the timely and widespread use of capsule endoscopy in patients with obscure gastrointestinal bleeding or other unexplained abdominal complaints will lead to the earlier identification of patients with small-bowel tumors, resulting ultimately in a survival benefit for the patients, will have to be clarified in specifically designed studies. Such studies will help define the best diagnostic/therapeutic algorithm for these patients, and the place of capsule endoscopy in such an algorithm.

Acknowledgments

We would like to thank both David Hainovitz (webmaster of the website) and Ms. Maria Pia Curiale, of the Organizing Secretariat of the Italian Club for Capsule Endoscopy for the technical support in data collection, and M. G. Lorenzatto for continuous and essential support.

Competing interests: Roberto de Franchis has a consultancy agreement with Given Imaging.

Institutions

[1] Department of Internal Medicine, Gastroenterology 3 Unit, University of Milan, IRCCS Policlinico, Mangiagalli and Regina Elena Foundation, Milan, Italy
[2] Department of Gastroenterology and Clinical Nutrition, S. Giovanni AS Hospital, Turin, Italy
[3] Endoscopy Unit, Department of Medicine, Malmo University Hospital, Malmo, Sweden
[4] Endoscopy Unit, Gregorio Maranon University Hospital, Madrid, Spain
[5] Unità Operativa di Endoscopia Digestiva e Chirurgica, Dipartimento di Scienze chirurgiche, Policlinico Gemelli, Università Cattolica del Sacro Cuore, Rome, Italy
[6] Department of Surgery and Advanced Technologies, School of Medicine, University of Naples Federico II, Naples, Italy
[7] Unità Operativa di Gastroenterologia ed Endoscopi Digestiva Istituto Tumori Giovanni Paolo II, Bari, Italy
[8] Department of Gastroenterology, Ghent University Hospital, Ghent, Belgium
[9] Gastroenterology Unit, Krankenanstalt Rudolfstiftung, Vienna, Austria
[10] Second Department of Internal Medicine, Faculty of Medicine and Teaching Hospital, Charles University, Hradec Kralove, Czech Republic
[11] Gastroenterology Unit, Department of Internal Medicine, Odense University Hospital, Odense, Denmark
[12] Servicio de Gastroenterologia, Hospital de Egas Moniz, Lisbon, Portugal
[13] Gastroenterology Unit, Institute of Gastroenterology and Hepatology, Iasi, Romania
[14] Department of Gastroenterology, Euromedica Geniki Clinici Thessalonikis Thessalonika, Grece
[15] Unità Operativa di Gastroenterologia, Centro di Riferimento Oncologico, Aviano, Italy
[16] Small Bowel Unit, Hospital Morales Meseguer, Murcia, Spain

References

1 *Neugut AI, Jacobson JS, Suh S et al.* The epidemiology of cancer of the small bowel. Cancer Epidemiol Biomarkers Prev 1998; 7: 243 – 251
2 *Delaunoit T, Neczyporenko F, Limburg PJ et al.* Pathogenesis and risk factors of small bowel adenocarcinoma: a colorectal cancer sibling? Am J Gastroenterol 2005; 100: 703 – 710
3 *Di Sarjo JA, Burt RW, Vargas H et al.* Small bowel cancer: epidemiological and clinical characteristics from a population-based registry. Am J Gastroenterol 1994; 89: 699 – 701
4 *Barclay TC, Schapira DV.* Malignant tumors of the small intestine. Cancer 1983; 51: 878 – 881
5 *Blanchard DK, Budde JM, Hatch GF et al.* Tumors of the small intestine. World J Surg 2000; 24: 421 – 429
6 *O'Riordan BG, Vilor M, Herrera L.* Small bowel tumors: an overview. Dig Dis Sci 1996; 14: 245 – 257
7 *Gill S, Heuman DM, Mihas AA.* Small intestinal neoplasms. J Clin Gastroenterol 2001; 33: 267 – 282
8 *Wilson JM, Melvin DB, Gray GF et al.* Primary malignancies of the small bowel: a report of 96 cases and review of the literature. Ann Surg 1974; 180: 175 – 179
9 *Johnson AM, Harman PK, Hanks JB.* Primary small bowel malignancies. Am Surg 1985; 51: 31 – 36
10 *Das Gupta TK, Brasfield RD.* Metastatic melanoma of the gastrointestinal tract. Arch Surg 1964; 88: 969 – 973
11 *Reintgen DS, Thompson W, Garbutt J, Seigler HF.* Radiologic, endoscopic and surgical considerations of melanoma metastatic to the gastrointestinal tract. Surgery 1984; 95: 635 – 639
12 *Talamonti MS, Goetz LH, Rao S et al.* Primary cancers of the small bowel: analysis of prognostic factors and results of surgical management. Arch Surg 2002; 137: 564 – 570; discussion 570–571
13 *de Franchis R, Rondonotti E, Abbiati C et al.* Small bowel malignancy. Gastrointest Endosc Clin N Am 2004; 14: 139 – 148
14 *Cobrin G, Pittman R, Lewis SB.* Increased diagnostic yield of small bowel tumors with capsule endoscopy. Cancer 2006; 107: 22 – 26
15 *Bailey A, Debinski HS, Appleyard MN et al.* Diagnosis and outcome of small bowel tumors found by capsule endoscopy: a three-center Australian experience. Am J Gastroenterol 2006; 101: 2237 – 2243
16 *Estevez E, Gonzalez-Conde B, Vazquez-Iglesias JL et al.* Incidence of tumoral pathology according to study using capsule endoscopy for patients with obscure gastrointestinal bleeding. Surg Endosc 2007; [Epub ahead of print]
17 *Urbain D, DeLooze D, Demedts I et al.* Video capsule endoscopy in small-bowel malignancy: a multicenter Belgian study. Endoscopy 2006; 38: 408 – 411
18 *Schwartz GD, Barkin JS.* Small-bowel tumors detected by wireless capsule endoscopy. Dig Dis Sci 2007; 52: 1026 – 1030
19 *Triester SL, Leighton JA, Leontiadis GI et al.* A meta-analysis of the yield of capsule endoscopy compared to other diagnostic modalities in patients with obscure gastrointestinal bleeding. Am J Gastroenterol 2005; 100: 2407 – 2418
20 *de Leusse A, Vahedi K, Edery J et al.* Capsule endoscopy for first line exploration of obscure gastrointestinal bleeding. Gastroenterology 2007; 132: 855 – 862
21 *Lewis BS, Eisen GM, Friedman S.* Pooled analysis to evaluate results of capsule endoscopy trials. Endoscopy 2005; 37: 960 – 965
22 *Pennazio M, Santucci R, Rondonotti E et al.* Outcome of patients with obscure gastrointestinal bleeding after capsule endoscopy: report of 100 consecutive cases. Gastroenterology 2004; 126: 643 – 653
23 *Pasha SF, Sharma VK, Carey EJ et al.* Utility of video capsule endoscopy in the detection of small bowel tumors – A single center experience of 1000 consecutive patients. International Conference on Capsule Endoscopy 2007; abstracts: 45
24 *Chong AK, Chin BW, Meredith CG.* Clinically significant small-bowel pathology identified by double-balloon enteroscopy but missed by capsule endoscopy. Gastrointest Endosc 2006; 64: 445 – 449
25 *Concha R, Amaro R, Barkin JS.* Obscure gastrointestinal bleeding: diagnostic and therapeutic approach. J Clin Gastroenterol 2007; 41: 242 – 251
26 *Redondo-Cerezo E, Perez-Vigara G et al.* Diagnostic yield and impact of capsule endoscopy on management of patients with gastrointestinal bleeding of obscure origin. Dig Dis Sci 2007; 52: 1376 – 1381
27 *Miettinen M, Lasota J.* Gastrointestinal stromal tumours: review on morphology, molecular pathology, prognosis and differential diagnosis. Arch Pathol Lab Med 2006; 130: 1446 – 1478

28 *Miettinen M, Sobin LH, Lasota J.* Gastrointestinal stromal tumors of the stomach: a clinicopathologic, immunohistochemical, and molecular genetic study of 1765 cases with long-term follow-up. Am J Surg Pathol 2005; 29: 52 – 68
29 *Miettinen M, Makhlouf HR, Sobin LH et al.* Gastrointestinal stromal tumors (GISTs) of the jejunum and ileum: a clinicopathologic, immunohistochemical and molecular genetic study of 906 cases prior to imatinib with long-term follow-up. Am J Surg Pathol 2006; 30: 477 – 489
30 *Bender GN, Maglinte DD, MCLarney JH et al.* Malignant melanoma: patterns of metastasis to the small bowel, reliability of imaging studies, and clinical relevance. Am J Gastroenterol 2001; 96: 2392 – 2400
31 *De Francesco V, Stoppino G, Tonti P et al.* Ileal metastasis from thoracic melanoma disclosed by video capsule endoscopy: an unusual but not extraordinary source of obscure bleeding. Endoscopy 2007; 39 (Suppl 1): E109 [Epub ahead of print]
32 *Liangpunsakul S, Chadalawada V, Rex DK et al.* Wireless capsule endoscopy detects small bowel ulcers in patients with normal results from state of the art enteroclysis. Am J Gastroenterol 2003; 98: 1295 – 1298
33 *Kalantzis C, Apostolopoulos P, Mavrogiannis P et al.* Capsule endoscopy retention as a helpful tool in the management of a young patient with suspected small-bowel disease. World J Gastroenterol 2007; 13: 1289 – 1291
34 *Cheifetz AS, Kornbluth AA, Legnani P et al.* The risk of retention of the capsule endoscope in patients with known or suspected Crohn's disease. Am J Gastroenterol 2006; 101: 2218 – 2222
35 *Cave D, Legnani P, de Franchis R et al.* ICCE consensus for capsule retention. Endoscopy 2005; 37: 1065 – 1067
36 *Lee B, Choi H, Choi KY et al.* Retrieval of a retained capsule endoscope by double-balloon enteroscopy. Gastrointest Endosc 2005; 62: 463 – 465
37 *Delvaux M, Ben SoussanE, Laurent V et al.* Clinical evaluation of the use of M2A patency capsule system before a capsule endoscopy procedure in patients with known or suspected intestinal stenosis. Endoscopy 2005; 37: 801 – 807
38 *Spada C, Spera G, Riccioni M et al.* A novel diagnostic tool for detecting functional patency of the small bowel: the Given Patency Capsule. Endoscopy 2005; 37: 793 – 800
39 *Gay G, Delvaux M, Laurent V et al.* Temporary intestinal occlusion induced by a patency capsule in a patient with Crohn's disease. Endoscopy 2005; 37: 793 – 800
40 *Signorelli C, Rondonotti E, Villa F et al.* Use of the Given Patency System for the screening of patients at high risk for capsule retention. Dig Liver Dis 2006; 38: 326 – 330
41 *Spada C, Shah SK, Riccioni ME et al.* Video capsule endoscopy in patients with known or suspected small bowel stricture previously tested with the dissolving patency capsule. J Clin Gastroenterol 2007; 41: 576 – 582

Appendix

The following also contributed to the study:

R. Marmo[1], A. Pezzoli[2], C. M. Girelli[3], M. Opezzi[4], L. De Luca[5], P. Pantanella[6], A. Alberani[7], R. Chiaverini[8], B. Gonzalez Suarez[9], S. Fernandez Diez[10], I. Fernandez Urien[11], J. Valle[12], J. Cabriada[13]

Institutions
[1] Division of Gastroenterology, Department of Medicine, Ospedale L. Curto, Polla, Italy
[2] Department of Gastroenterology and Gastrointestinal Endoscopy, Azienda Ospedaliera Universitaria S. Anna, Ferrara, Italy
[3] Servizio di Gastroenterologia ed Endoscopia Digestiva, Unità Operativa Medicina 1, Ospedale do Busto Arsizio, Busto Arsizio, Italy
[4] Servizio di Gastroenterologia ed Endoscopia Digestiva, Ospedale Galliera di Genova, Genoa, Italy
[5] Unità Operativa di Gastroenterologia, Ospedale Pellegrini, Naples, Italy
[6] Unità Operativa di Gastroenterologia, Ospedale S. Camillo Forlanini, Rome, Italy
[7] Unità Operativa di Gastroenterologia, Ospedale Bellaria-Maggiore, Bologna, Italy
[8] Unità Complessa di Chirirgia Generale, Ospedale SS Rosario di Venafro, Venafro, Italy
[9] Hospital de la Santa Creu i Sant Pau, Gastroenterology Unit. Barcelona, Spain
[10] Gastroenterology Unit, Hospital Clinico S. Carlos, Madrid, Spain
[11] Department of Gastroenterology, University of Navarra, Pamplona, Spain
[12] Gastroenterology Unit, Hospital Virgen de la Salud, Toledo, Spain
[13] Gastroenterology Unit, Hospital Galdakao, Bilbao, Spain

World Journal of Gastroenterology

Submit a Manuscript: http://www.f6publishing.com

World J Gastroenterol 2017 September 21; 23(35): 6500-6515

DOI: 10.3748/wjg.v23.i35.6500

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

META-ANALYSIS

Proton pump inhibitors therapy and risk of *Clostridium difficile* infection: Systematic review and meta-analysis

Anca Trifan, Carol Stanciu, Irina Girleanu, Oana Cristina Stoica, Ana Maria Singeap, Roxana Maxim, Stefan Andrei Chiriac, Alin Ciobica, Lucian Boiculese

Anca Trifan, Irina Girleanu, Oana Cristina Stoica, Ana Maria Singeap, Roxana Maxim, Stefan Andrei Chiriac, Institute of Gastroenterology and Hepatology, "St. Spiridon" Hospital, "Grigore T. Popa" University of Medicine and Pharmacy, 700111 Iasi, Romania

Carol Stanciu, Institute of Gastroenterology and Hepatology, "St. Spiridon" Hospital, 700111 Iasi, Romania

Alin Ciobica, Department of Research, Faculty of Biology, "Alexandru Ioan Cuza" University of Iasi, 700506 Iasi, Romania

Lucian Boiculese, Department of Preventive Medicine and Interdisciplinarity, "Grigore. T. Popa" University of Medicine and Pharmacy, 700111 Iasi, Romania

ORCID number: Anca Trifan (0000-0001-9144-5520); Carol Stanciu (0000-0002-6427-4049); Irina Girleanu (0000-0001-5925-1232); Oana Cristina Stoica (0000-0003-4698-1548); Ana Maria Singeap (0000-0001-5621-548X); Roxana Maxim (0000-0001-5905-4546); Stefan Andrei Chiriac (0000-0003-2497-9236); Alin Ciobica (0000-0002-1750-6011); Lucian Boiculese (0000-0001-5825-6189).

Author contributions: Trifan A and Stanciu C designed the study, contributed to the selection of studies, analyzed the data and wrote the manuscript; Stoica OC, Maxim R, Singeap AM, Girleanu I, Chiriac SA and Ciobica A were involved in the acquisition of data and contributed to the analysis and interpretation of data; Girleanu I, Stoica OC, Maxim R and Singeap AM drafted the manuscript; Boiculese L and Girleanu I contributed to the statistical analysis; all authors have read and approved the final version of the manuscript; all authors accept responsibility for its content.

Conflict-of-interest statement: The authors deny any conflict of interest.

Data sharing statement: No additional data are available.

Manuscript source: Unsolicited manuscript

Correspondence to: Carol Stanciu, MD, FRCP, Professor, Institute of Gastroenterology and Hepatology,"St. Spiridon" Hospital, Independentei Street No. 1, 700111 Iasi, Romania. stanciucarol@yahoo.com
Telephone: +40-722-306020
Fax: +40-232-246611

Received: July 13, 2017
Peer-review started: July 17, 2017
First decision: August 10, 2017
Revised: August 11, 2017
Accepted: August 25, 2017
Article in press: August 25, 2017
Published online: September 21, 2017

Abstract

AIM

To perform a systematic review and meta-analysis on proton pump inhibitors (PPIs) therapy and the risk of *Clostridium difficile* infection (CDI).

METHODS

We conducted a systematic search of MEDLINE/PubMed and seven other databases through January 1990 to March 2017 for published studies that evaluated the association between PPIs and CDI. Adult case-control and cohort studies providing information on the association between PPI therapy and the development of CDI were included. Pooled odds ratios (ORs) estimates with 95% confidence intervals (CIs) were calculated using the random effect. Heterogeneity was assessed by I^2 test and Cochran's Q statistic.

Potential publication bias was evaluated *via* funnel plot, and quality of studies by the Newcastle-Otawa Quality Assessment Scale (NOS).

RESULTS

Fifty-six studies (40 case-control and 16 cohort) involving 356683 patients met the inclusion criteria and were analyzed. Both the overall pooled estimates and subgroup analyses showed increased risk for CDI despite substantial statistical heterogeneity among studies. Meta-analysis of all studies combined showed a significant association between PPI users and the risk of CDI (pooled OR = 1.99, CI: 1.73-2.30, $P < 0.001$) as compared with non-users. The association remained significant in subgroup analyses: by design-case-control (OR = 2.00, CI: 1.68-2.38, $P < 0.0001$), and cohort (OR = 1.98, CI: 1.51-2.59, $P < 0.0001$); adjusted (OR = 1.95, CI: 1.67-2.27, $P < 0.0001$) and unadjusted (OR = 2.02, CI: 1.41-2.91, $P < 0.0001$); unicenter (OR = 2.18, CI: 1.72-2.75, $P < 0.0001$) and multicenter (OR = 1.82, CI: 1.51-2.19, $P < 0.0001$); age ≥ 65 years (OR = 1.93, CI: 1.40-2.68, $P < 0.0001$) and < 65 years (OR = 2.06, CI: 1.11-3.81, $P < 0.01$). No significant differences were found in subgroup analyses (test for heterogeneity): $P = 0.93$ for case-control *vs* cohort, $P = 0.85$ for adjusted *vs* unadjusted, $P = 0.24$ for unicenter *vs* multicenter, $P = 0.86$ for age ≥ 65 years and < 65 years. There was significant heterogeneity across studies ($I^2 = 85.4\%$, $P < 0.001$) as well as evidence of publication bias (funnel plot asymmetry test, $P = 0.002$).

CONCLUSION

This meta-analysis provides further evidence that PPI use is associated with an increased risk for development of CDI. Further high-quality, prospective studies are needed to assess whether this association is causal.

Key words: Proton pump inhibitors; *Clostridium difficile* infection; Risk; Systematic review; Meta-analysis

Core tip: A possible association between the use of proton pump inhibitors (PPIs) and the risk of *Clostridium difficile* infection (CDI) have been suggested by several studies. This meta-analysis, including the largest number of studies published to date found the risk of CDI almost two-times higher in PPIs users than in nonusers. Because all the studies analyzed were observational, the causality could not be confirmed. Nevertheless, clinicians should be aware of such potential association and prescribe the PPIs only where they are clearly indicated.

Trifan A, Stanciu C, Girleanu I, Stoica OC, Singeap AM, Maxim R, Chiriac SA, Ciobica A, Boiculese L. Proton pump inhibitors therapy and risk of *Clostridium difficile* infection: Systematic review and meta-analysis. *World J Gastroenterol* 2017; 23(35): 6500-6515 Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i35/6500.htm DOI: http://dx.doi.org/10.3748/wjg.v23.i35.6500

INTRODUCTION

Over the past two decades *Clostridium difficile* (*C. difficile*) infection (CDI) has registered an increasing trend worldwide both in incidence and severity[1-5], with healthcare costs varying between 1.2 and 4.7 billion dollars each year in the United States alone[6-9]. In addition to the broad-spectrum antimicrobial therapy which has been the most prominent causative factor for CDI[10,11], other potential risk factors have been identified such as: advanced age, hospitalization [particularly in intensive care units (ICU)], immunosuppression, renal insufficiency, hypoalbuminemia, lengthy hospital stay, the use of nasogastric tubes, invasive gastrointestinal procedures, chemotherapy, the presence of comorbidities, environment-related factors, and the emergence of a hypervirulent strain of the bacterium known as North American pulso-type 1 in some areas[12-21]. However, there might be some other risk factors for the CDI epidemic in the recent years despite tighter control on the use of antibiotics and stricter control policies on hospital-related infections[17]. A possible association between the use of proton pump inhibitors (PPIs) and the development of CDI has been suggested and numerous studies have examined it, reporting conflicting results[22-43].

Since their release in the late 1980s, PPIs have become some of the most widely prescribed agents both in outpatient and inpatient settings throughout the world[44-53], with sales totalling billions dollars worldwide[54,55]. These drugs have proven effective in the treatment of ulcer disease (including bleeding peptic ulcer), gastroesophageal reflux disease, *Helicobacter pylori* (in combination with antibiotics), Zollinger-Ellison syndrome, in the prophylaxis of upper gastrointestinal complications with nonsteroidal anti-inflammatory drugs (NSAIDs) therapy, stress ulcer prophylaxis in ICU patients, and functional dyspepsia[50,53,56-60]. The widespread use of PPIs during the last 25 years in clinical practice is the result not only of their high efficacy but also of their excellent safety profile, proving to be one of the safest class of medication used in gastroenterology[57,61-64].

Nevertheless, like in the case of other drugs, PPIs are not as safe as it has been thought and more recently, concerns have been raised about their potential association with pneumonia[65-67], bone fractures[68-70], interstitial nephritis and acute kidney injury[71]. More recently, reports of other potential PPIs adverse events such as risk for chronic kidney disease[72,73], dementia[74], spontaneous bacterial peritonitis[75,76], acute myocardial infarction[77,78], micronutrient

deficiency (magnesium, calcium, iron)[79,80] were published, although the quality of evidence for these is consistently low to very low[81].

An association between PPIs use and CDI is, at least theoretically, rational. Thus, intestinal homeostasis is maintained by host defense mechanisms in which gastric acid plays an important role as a barrier to ingested bacteria and bacterial overgrowth[82]. PPIs therapy profoundly inhibits gastric acid production leading to the proliferation of spores and their ability to convert to a vegetative form of *C. difficile* [83]. Moreover, PPIs impair leukocyte function by inhibiting phagocytosis and acidification of phagolysosome[84].

Several systematic reviews and meta-analyses have reported conflicting results regarding the association between PPIs use and increased risk of CDI. Thus, no less than six meta-analyses[85-90] found a significant association between PPIs therapy and increased risk of CDI. These findings were also supported by several studies[19,22-26,39,91-114] which reported a risk for CDI two or three times higher in PPIs users than in nonusers. Moreover, the United States Food and Drug Administration (FDA) informed the public about a possible correlation between PPIs use and CDI[115]. Still, other studies and meta-analyses have failed to associate PPIs use with the development of CDI[11,27,34,38,40-43,116-123]. It should be mentioned that PPIs continue to be among the most used drugs despite the above mentioned concerns about long-term side effects. Furthermore, beside a marked overuse of PPIs, over half of prescriptions are for non-indicated reasons[29]. One study reported that 60.7% of patients with CDI used PPIs, of whom only 47.1% had an evidence-based indication[30].

The aim of this systematic review and meta-analysis is to summarize data on the association between PPIs use and the risk of CDI as presented in the published studies.

MATERIALS AND METHODS

Information sources

A systematic literature search was independently conducted by four study investigators (Girleanu I, Stoica OC, Singeap AM and Chiriac SA) using a variety of databases including MEDLINE/PubMed, Web of Science (ISI Web of Knowledge), Scopus, EMBASE, Science Direct, Cumulative Index to Nursing and Allied Health Literature (CINAHL), Excerpta Medica Database, and Cochrane Library, from January 1990 (the first PPI received FDA approval in 1989) to March 2017. The database searches were performed using the following medical subject heading (MeSH) terms: "proton pump inhibitors", "acid suppressive therapy", "omeprazole", "pantoprazole", "lansoprazole", "rabeprazole", "esomeprazole", combined with "*C. difficile* infection", "*C. difficile*-associated diarrhea", "pseudomembranous colitis". Reference lists of all retrieved papers were hand-searched to identify any additional studies that may have been missed in the computed-assisted literature search. The investigation was limited to studies performed in adult human beings, written and published in English, French, and German, in any geographic region.

Inclusion and exclusion criteria

Selection of the studies. Inclusion and exclusion criteria were established a priori by two authors (Trifan A and Stanciu C). First, duplicate citations were identified and removed, then three of us (Ciobica A, Maxim R and Singeap AM) independently reviewed the titles and abstracts of the studies and excluded those which did not answer the search question. Adult case-control and cohort studies providing information on the association between PPI therapy and the development of CDI were included. Studies conducted on pediatric patients, systematic reviews and meta-analyses, consensus documents, studies using PPIs simultaneously with H2 receptor antagonists (H2RA) or reporting exclusively on H2RA, case reports, editorials, protocols, and studies presented only as abstracts were excluded. There was no restriction related to the type of PPI regimen or diagnostic methods of CDI. Any disagreements about study inclusion were resolved in consensus with a third author (Stanciu C or Trifan A) after the full-text of the potential study had been reviewed; all eligible studies were assessed in full. They were subsequently included in this meta-analysis only if reported odds ratios (ORs) or risk ratios (RRs) for (adjusted or unadjusted) case-control and cohort studies, respectively, or data for their calculation were available.

Data extraction

Extracted data were cross-checked independently by four authors (Girleanu I, Stoica OC, Chiriac SA and Ciobica A) from each included study using a standardized data extracting sheet which included the last name of first author, journal and year of publication, country where the study was carried out, study design, sample size, age (mean or median) and gender distribution of patients, duration of the PPI treatment, effect estimates ORs or RRs, and 95% confidence intervals (CIs) of PPI exposure with and without adjustment for confounding variables. Any disagreement between reviewers was resolved in consensus with a third reviewer (Stanciu C or Trifan A).

Study quality assessment

Assessment of study quality was made independently by two authors (Boiculese L and Girleanu I) using the Newcastle-Ottawa Quality Assessment Scale (NOS; ranging 0-9)[124] as recommended by the Cochrane Handbook for Systematic Reviews of Interventions[125]. The NOS comprises three domains: selection, comparability, and outcome for cohort studies or exposure for case-control studies. A maximum of

four stars were awarded for selection, two stars for comparability, and three stars for exposure/outcome. Studies with cumulative score ⩾ 7 were considered high quality, 6 stars to be of moderate quality, and less than 6 stars to be of low quality. When disagreement, after discussion with the third author (Trifan A or Stanciu C) a consensus was reached. The final analysis included 56 high and moderate quality studies.

As none of the studies was randomized, and all were observational (case-control and cohort), the methods used in our systematic review and meta-analysis followed the MOOSE (Meta-Analysis of Observational Studies in Epidemiology) criteria[126].

Statistical analysis

Meta-analyses were performed both for all studies together and separately for case-control and cohort studies using DerSimonian and Laird[127] random effects model due to expected heterogeneity between studies. Our primary analysis focused on the association between PPIs therapy and the risk for developing CDI and because all of PPIs have similar efficacy we have not performed meta-analyses stratified by type of PPIs. The results are reported as pooled ORs with 95%CIs for primary and subgroup analyses.

Heterogeneity between studies was assessed by I^2 statistic and Cochran's *Q*-statistic. The level of heterogeneity was considered as high when I^2 > 75% or P < 0.10 for the *Q* statistic[128]. I^2 values between 61%-75%, 30%-60%, and < 30% were considered to represent substantial, moderate and low level of heterogeneity, respectively[129]. Seven potential confounders were considered: study design, effect estimate (adjusted *vs* unadjusted), setting (community *vs* inpatient), number of centers (single center *vs* multicenter), age, study quality, and geographical region.

Publication bias was assessed quantitatively using Egger's regression asymmetry test[130] and a P < 0.1 was considered statistically significant for asymmetry, and qualitatively by visual inspection of funnel plots of the logarithmic OR *vs* their standard errors[131]. Asymmetrical funnel plots were regarded to indicate high risk of publication bias.

Number needed to harm (NNH) estimates the number of patients needed to be treated with PPI for one additional person to have a CDI, and was calculated using the pooled OR (95%CI) from the meta-analysis and Patient Expected Event Rate (1.67%)[120].

All statistical tests were two tailed, and results associated with P < 0.05 (except for heterogeneity and publication bias) were considered significant. All analyses were performed using R version 3.2.3 software for the metaphor package 1.9-8, which provides a comprehensive collection of validated functions[132]. The statistical analyses of this study were performed by an expert in biostatistics from "Grigore T. Popa" University of Medicine and Pharmacy, Department of Medical Informatics and Biostatistics.

RESULTS

Search results

The initial online databases search identified 944 studies and 12 more were found from the reference lists of the articles retrieved. After reviewing all titles and abstracts, 216 studies were selected for full-text review, from which 56 studies were found to fulfill the inclusion criteria and were included in meta-analysis. Five of the 56 studies were published after the last meta-analysis (Figure 1).

Characteristics of included studies

The characteristics of the included studies are shown in Table 1. Of the included 56 studies, 40 (71.4%) were case-control, and 16 (28.6%) cohort studies, addressed to hospital-acquired (n = 43), community-acquired (n = 6), and both hospital and community-acquired CDI (n = 7). Most of the studies (n = 31) were single-center. The size of the study population ranged from 40 to 101796. In total, 356683 subjects were included, most of them from North-American and European studies (n = 46).

Quality assessment

The median value of NOS quality assessment was 7, with a mean 6.67 ± 0.74, range 6-8. In studies reporting gender, the proportion of men ranged from 47% to 67%, and from those that reported the age, the average age ranged between 18 and 82.2 years. Thirty-eight studies identified confounding factors (sex, age, antibiotic use, comorbidities) used for adjustment of the association between PPI therapy and risk of CDI. The majority of the studies were retrospective (85.7%) and only 8 were prospective (14.3%). None of the studies was randomized.

Meta-analysis

Meta-analysis of all studies combined. The results of pooled analysis for all 56 studies showed a significant association between PPI therapy and the risk of CDI as compared with non-PPI users (OR = 1.99, CI: 1.73-2.30, P < 0.001) (Figure 2). There was significant heterogeneity of effects across studies (I^2 = 85.41%; P < 0.001).

Subgroup analyses of case-control and cohort studies also showed a significant higher risk of CDI with PPI use (Table 2). There was no significant difference of effects between cohort and case-control studies (P = 0.931). The pooled OR for the cohort studies was 1.98 similar to OR for case-control that was 2.0.

The association remained also significant after limiting meta-analysis to studies with both adjusted (OR = 1.95, CI: 1.67-2.27, P < 0.001) and unadjusted

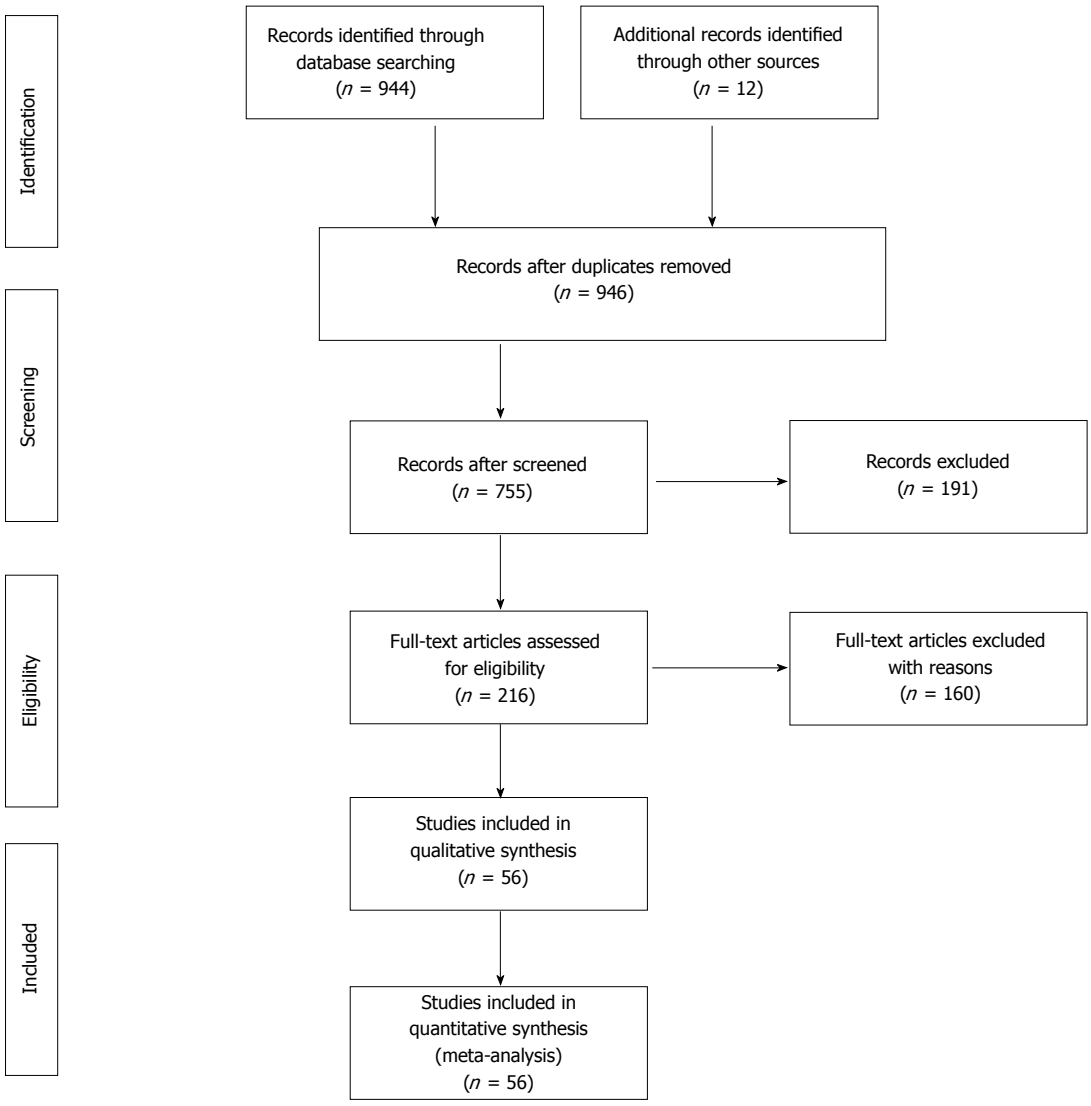


Figure 1 Study selection process.

data (OR = 2.02, CI: 1.41-2.91, $P < 0.001$). There was also no significant difference of effects between adjusted and unadjusted studies ($P = 0.856$).

PPIs use was found to be associated with an increased risk of CDI in both single-center studies (OR = 2.18, 95%CI: 1.72-2.75) and multicenter studies (OR = 1.82, 95%CI: 1.51-2.19).

There was no significant difference between inpatients and outpatients regarding CDI risk ($P = 0.868$). For both inpatients and outpatients the PPIs use almost doubled the risk of CDI (OR = 1.95, OR = 2.10, respectively).

When grouped by region, a direct association was found in the European group (OR = 1.78, 95%CI: 1.35-2.34), the North American group (OR = 2.00, 95%CI: 1.67-2.40), while the highest risk of CDI after PPI treatment was demonstrated in the Asian group (OR = 2.31, 95%CI: 1.96-2.72).

The subgroup of high-quality studies (NOS $\geqslant$ 7) showed a direct association (OR = 1.88, 95%CI: 1.55-2.28) between PPIs and risk of CDI, and this association was also significant in the medium-quality group (OR = 2.11, 95%CI: 1.69-2.62), with no difference between the two groups ($P = 0.441$).

There was no statistical difference regarding the risk for CDI for elderly ($\geqslant$ 65 years) compared with younger group (< 65 years) ($P = 0.860$).

Publication bias

We have drawn the funnel plot for 3 levels of confidence interval (90%, 95% and 99% corresponding to shades white, gray and dark gray) (Figure 3). The Egger's test of asymmetry proved no significance ($Z = 0.3699$, $P = 0.711$).

Number needed to harm

Based on reported incidence of CDI (at 14 d after hospital admission) of 1.67% in patients who have not used PPI, we estimate a NNH of 63 (95%CI: 48-78), if these patients will receive PPIs.

Table 1 Characteristics of studies included in the meta-analysis

Author, yr	Region	Study design	Centers	Setting	Sample size, *n*	Mean age, yr	Identified confounders	OR (95%CI)
Akhtar *et al* Shaheen[91], 2007	America	Case-control	Unicenter	Inpatient	1290		Adjusted for age, sex, comorbidities, antibiotics, chemotherapy	2.1 (1.6-2.7)
Al-Tureihi *et al*[19], 2005	America	Case-control	Unicenter	Inpatient	53		Adjusted for age, antibiotics	3.1 (1.0-9.7)
Aseeri *et al*[23], 2008	America	Case-control	Unicenter	Inpatient	188		Adjusted for admission date, sex, age group, antibiotic use, patient location, and room type	4.4 (2.3-8.2)
Bajaj *et al*[133], 2010	America	Case-control	Multicenter	Mixt	162		Adjusted for antibiotics, PPI	37.6 (6.2-227.6)
Barletta *et al*[92], 2014	Asia	Case-control	Unicenter	Inpatient	408		Adjusted for PPI exposure, antibiotics, immunosuppression	2.1 (1.2-3.8)
Baxter *et al*[93], 2008	America	Case-control	Multicenter	Inpatient	4493		Adjusted for antibiotics, age, hospital stay, other infections	1.2 (1.0-1.4)
Beaulieu *et al*[27], 2007		Cohort	Unicenter	Inpatient	827		Adjusted for age, sex, length of stay, comorbidities, APACHE score, NGT feeding, tracheal tube placement, antibiotics	1.3 (0.9-2.0)
Branch *et al*[94], 2007	America	Case-control	Unicenter	Inpatient	787	66.02	No	13.0 (7.5-22.7)
Buendgens *et al*[95], 2014	Europe	Case-control	Multicenter	Inpatient	3286		Adjusted for age, sex, antibiotics, comorbidities, other treatment	3.1 (1.1-8.7)
Campbell *et al*[38], 2013	America	Case-control	Unicenter	Inpatient	96		Adjusted for antibiotics, hospitalization	2.2 (0.6-8.0)
Cunningham *et al*[96], 2003	Europe	Case-control	Unicenter	Inpatient	320		Adjusted for antibiotics and chemotherapy	2.5 (1.5-4.1)
Dalton *et al*[22], 2009	America	Cohort	Multicenter	Inpatient	14719	74.7	Adjusted for number of medication groups, antibiotic days, age, length of stay, medical service, PPI days	1.9 (1.4-2.7)
Debast *et al*[116], 2009	Europe	Case-control	Unicenter	Inpatient	154		Adjusted for age, hospital stay, comorbidities, antibiotics	1.1 (0.5-2.4)
Dial *et al*[26], 2004 (case-control)	America	Case-control	Multicenter	Inpatient	188		Adjusted for age, antibiotics	2.6 (1.3-5.0)
Dial *et al*[26], 2004 (cohort)	America	Cohort	Multicenter	Inpatient	1187		Adjusted for age, antibiotics	2.1 (1.2-3.5)
Dial *et al*[98], 2005	Europe	Case-control	Multicenter	Outpatient	13563		Adjusted for age, sex, antibiotics	2.9 (2.4-3.5)
Dial *et al*[97], 2006	Europe	Case-control	Multicenter	Outpatient	3484		Adjusted for PPI and antibiotics	3.5 (2.3-5.3)
Dial *et al*[134], 2008	America	Case-control	Multicenter	Outpatient	9196	79.8	Adjusted for age, sex, antibiotics, comorbidities, physician visits, hospital admissions, length of stay	1.6 (1.3-1.9)
Dubberke *et al*[99], 2007	America	Cohort	Unicenter	Inpatient	36086		Adjusted for age, admissions, antibiotics, albumin level, leukemia/lymphoma, mechanical ventilation, antimotility agents	1.6 (1.3-2.1)
Elseviers *et al*[100], 2015	Europe	Case-control	Multicenter	Inpatient	743	71.9	Adjusted for age, co-morbidity, endoscopic procedures	1.9 (1.1-3.4)
Faleck *et al*[42], 2016	America	Cohort	Unicenter	Inpatient	11230	66	Adjusted for age, sex, antibiotics, comorbidities, length of stay	0.6 (0.4-0.8)
Garzotto *et al*[43], 2015	Europe	Case-control	Multicenter	Inpatient	225		No	0.4 (0.2-0.8)
Hebbard *et al*[135], 2017	Asia	Case-control	Unicenter	Inpatient	200	59.7	Adjusted for age, chemotherapy, abdominal surgery, antibiotics	2.4 (1.0-5.7)
Hensgens *et al*[117], 2011	Europe	Case-control	Unicenter	Inpatient	169		Adjusted for age, co-morbidity, antibiotics, ICU stay	1.1 (0.5-2.5)
Howell *et al*[136], 2010	America	Cohort	Unicenter	Inpatient	101796	65.4	Adjusted for age, comorbidities, antibiotics	1.7 (1.3-2.1)
Ingle *et al*[40], 2011	Asia	Cohort	Unicenter	Mixt	99	47	Adjusted for immunosuppression	1.8 (0.4-7.4)
Ingle *et al*[118], 2013	Asia	Case-control	Unicenter	Community	150	45.3	no	2.3 (0.6-9.2)
Jayatilaka *et al*[101], 2007	America	Case-control	Unicenter	Inpatient	366		Adjusted for PPI	2.7 (1.6-4.8)

Kazakova *et al*[102], 2006	America	Case-control	Unicenter	Mixt	195		Adjusted for antibiotics, PPI, length of stay, psychosis, depression	5.0 (1.3-19.3)
Khan *et al*[39], 2012	Asia	Cohort	Unicenter	Inpatient	123		Adjusted for surgery, PPI, antibiotics, hospitalization, Underlying debilitating conditions	3.2 (1.2-8.5)
Khanafer *et al*[119], 2013	Europe	Cohort	Unicenter	Inpatient	40			2.5 (0.6-9.6)
Kuntz *et al*[2], 2011	America	Case-control	Unicenter	Mixt	3344		no	2.3 (1.5-3.3)
Kurti *et al*[3], 2015	Europe	Case-control	Multicenter	Inpatient	979	72.4	Adjusted for antibiotics, PPI, length of stay,	1.6 (1.1-2.2)
Kutty *et al*[41], 2010	America	Case-control	Multicenter	Outpatient	144	62	No	1.7 (0.7-4.0)
Lewis *et al*[103], 2016	America	Cohort	Unicenter	Inpatient	41663		No	6.4 (3.6-11.5)
Lin *et al*[137], 2013	Asia	Case-control	Multicenter	Inpatient	86	59	Age, sex, unit, antibiotics, length of stay	10.1 (1.2-87.4)
Linney *et al*[24], 2010	America	Case-control	Unicenter	Inpatient	284		Age, sex, discharge date and hospital unit, antibiotics, diabetes mellitus, IBD, cancer, enteral feeding, length of stay	2.4 (1.4-4.3)
Loo *et al*[120], 2005	America	Case-control	Multicenter	Inpatient	474		no	1.0 (0.7-1.4)
Loo *et al*[138], 2011	America	Cohort	Multicenter	Inpatient	4143	67.4	Adjusted for age, PPI, antibiotics, chemotherapy	2.6 (1.7-4.0)
Lowe *et al*[121], 2006	America	Case-control	Multicenter	Inpatient	13692	78.7	Adjusted for antibiotics, other medications, and comorbidities	0.9 (0.7-1.0)
McFarland *et al*[122], 2007	America	Case-control	Multicenter	Mixt	368		No	0.8 (0.5-1.4)
Mizui *et al*[104], 2013	Asia	Case-control	Multicenter	Inpatient	2716	71.7	No	3.2 (1.4-7.3)
Modena *et al*[105], 2005	America	Case-control	Unicenter	Inpatient	250		Adjusted for macrolides, ICU, length of stay, infections	3.3 (1.6-6.8)
Mori *et al*[123], 2015	Asia	Case-control	Unicenter	Outpatient	78	58.2	No	0.4 (0.1-2.0)
Muto *et al*[106], 2005	America	Case-control	Multicenter	Inpatient	406		Adjusted for PPI, antibiotics, diabetes mellitus, organ transplantation	2.4 (1.3-4.4)
Pakyz *et al*[107], 2014	America	Case-control	Multicenter	Inpatient	14164		No	1.4 (1.3-1.5)
Peled *et al*[108], 2007	America	Cohort	Unicenter	Inpatient	217		Adjusted for PPI, low albumin level,	3.7 (1.5-9.3)
Pepin *et al*[11], 2005	America	Cohort	Unicenter	Inpatient	5619		Adjusted for age, length of stay, antibiotics	1.0 (0.7-1.2)
Ro *et al*[139], 2016	Asia	Cohort	Unicenter	Inpatient	1005	64.8	Adjusted for age, antibiotics, comorbidities	3.3 (1.5-7.2)
Roughead *et al*[109], 2016	Asia	Cohort	Multicenter	Mixt	54957		Adjusted for antibiotics, PPI, length of stay,	2.4 (1.9-3.1)
Shah *et al*[34], 2000	Europe	Case-control	Unicenter	Inpatient	252		No	0.8 (0.4-1.5)
Southern *et al*[110], 2010	Europe	Cohort	Multicenter	Inpatient	3904	65.5	No	2.3 (1.1-4.5)
Vesteinsdottir *et al*[111], 2012	Europe	Case-control	Multicenter	Mixt	333		No	1.6 (1.0-2.6)
Yang *et al*[112], 2011	Asia	Case-control	Multicenter	Inpatient	1420	67.12	No	1.9 (1.3-2.7)
Yearsley *et al*[25], 2006	Europe	Case-control	Unicenter	Inpatient	308	79.1	Adjusted for PPI, antibiotics, female sex	1.9 (1.1-3.2)
Yip *et al*[140], 2001	America	Case-control	Unicenter	Inpatient	54		No	3.0 (0.8-11.1)

CI: Confidence interval; IBD: Inflammatory bowel disease; ICU: Intensive care unit; PPI: Proton pump inhibitor; NGT: Naso-gastric tube; OR: Odds ratio.

DISCUSSION

This systematic review and meta-analysis which includes 56 studies and 356683 subjects[2,3,11,19,22-27,34,38-43,91-112,116-123,133-140] found a significant association between PPI therapy and the risk for CDI development. Both the overall pooled estimates (OR = 1.99, CI: 1.73-2.30, $P < 0.001$) and subgroup analyses showed a significant increased risk for CDI in patients on PPI therapy compared to nonusers, despite substantial statistical heterogeneity among studies and evidence of publication bias. Thus, in line with previous meta-analyses, our results add further evidence to PPIs use as a risk factor for development of CDI [85-89].

Since 2001, when Yip *et al*[140] first suggested a possible association between PPIs use and the risk of CDI, other studies, systematic reviews, and meta-analyses have reported such an association. It should be mentioned that a decade earlier (1993), Walker *et al*[141] suggested that the H2RAs therapy was a potential risk factor for CDI. In an earlier systematic review which included 11 studies with 126999 patients, Leonard *et al*[113] reported a significant association between PPI therapy and CDI (OR = 2.05, 95%CI: 1.47-2.85) although there was significant heterogeneity among the studies ($\chi^2 = 50.9$, $P <$

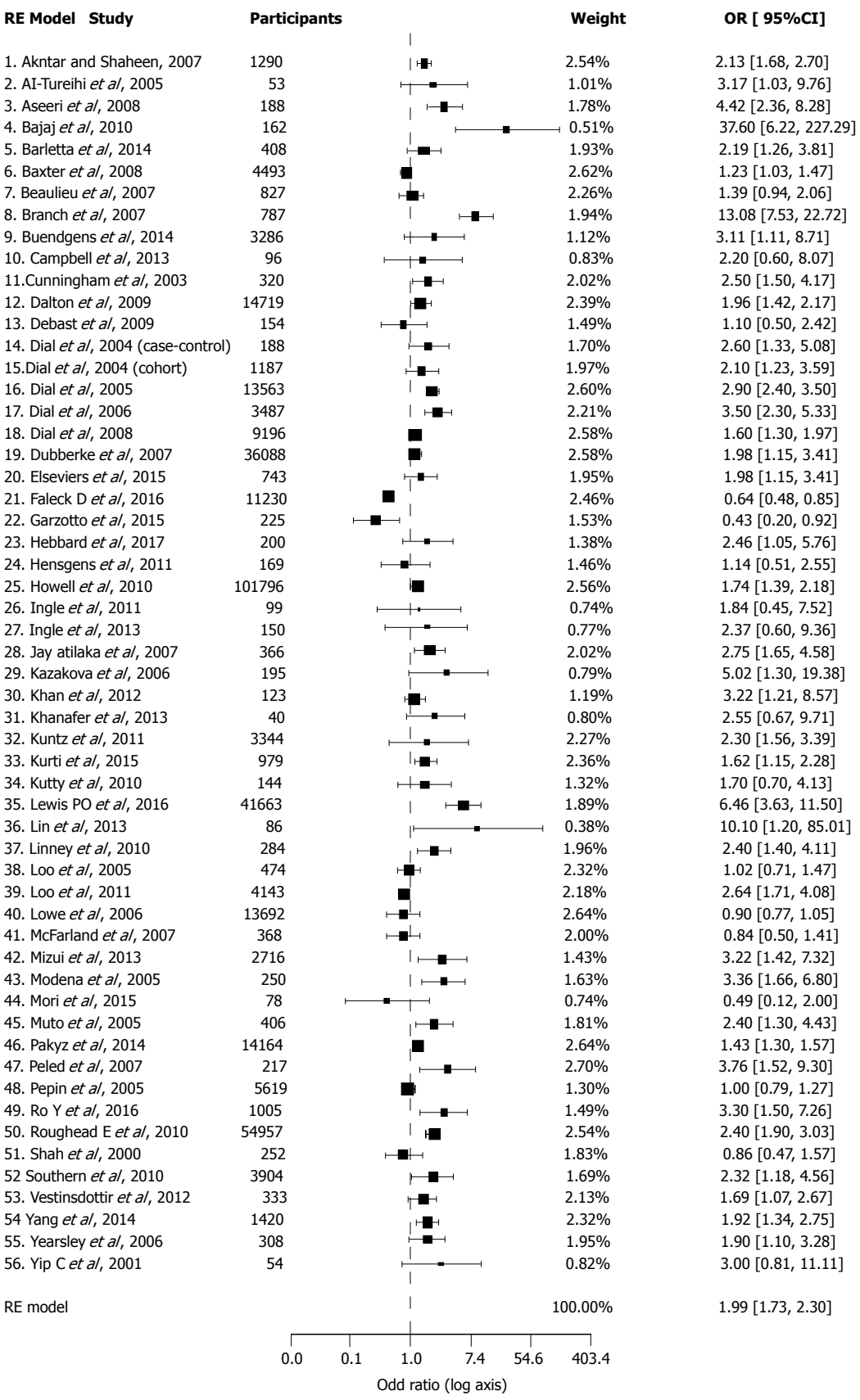


Figure 2 Forest plot of the meta-analysis.

0.0001). During the last years, six meta-analyses have been published on this topic, and all reported a positive association between PPIs use and the risk for CDI. Thus, Janarthanan *et al*[88] in a meta-analysis including

Table 2 Subgroup analysis

Subgroup analysis	No. of studies (n = 56)	ORs	95%CI	Heterogeneity, I^2, %	Heterogeneity between groups, P value
Study design					
Case-control	40	2	1.68-2.38	85.54	0.931
Cohort	16	1.98	1.51-2.59	85.99	
Study type					
Adjusted	38	1.95	1.67-2.27	85.02	0.856
Unadjusted	18	2.02	1.41-2.91	85.58	
Centers					
Unicentric	31	2.18	1.72-2.75	83.99	0.241
Multicentric	25	1.82	1.51-2.19	86.97	
Type					
Inpatient	43	1.95	1.67-2.29	84.99	0.868
Outpatient	6	2.1	1.36-3.24	84.84	
Mixt	7	2.19	1.39-3.45	76.77	
Region					
Europe	14	1.78	1.35-2.34	74.33	0.231
America	31	2	1.67-2.40	88.58	
Asia	11	2.31	1.96-2.72	89.18	
Age					
Age < 65 yr	6	2.06	1.11-3.81	35.39	0.86
Age ⩾ 65 yr	13	1.93	1.40-2.68	92.11	
NOS					
NOS ⩾ 7	26	1.88	1.55-2.28	87.65	0.441
NOS < 7	30	2.11	1.69-2.62	81.98	

CI: Confidence interval; NOS: Newcastle-Ottawa Quality Assessment Scale; ORs: Odds ratio.

23 observational studies with nearly 300000 patients found a 65% increase in the incidence of CDI among PPIs users with an estimated risk of 1.69 and 95%CI from 1.395 to 1.974. In another meta-analysis (30 studies, 202965 patients), Desphande *et al*[85] reported that PPI therapy was associated with a 2-fold increase in risk for CDI, but their study is limited by unadjusted risk estimates. Recently, the same team[90] performed a meta-analysis examining the relationship between PPI therapy and the risk for recurrent CDI, and found a positive association with the pooled risk ratio of 1.58 (95%CI: 1.13-2.21). A third meta-analysis by Kwok *et al*[87] including 42 studies (313000 participants) also found a statistically significant association between PPIs use and the risk for CDI compared with nonusers (OR = 2.51; 95%CI: 1.47-2.85; P = 0.05). Tleyjeh *et al*[86] in a systematic review and meta-analysis including 51 observational studies (37 case-control and 14 cohort) examining healthcare and community-associated CDI, found a very low quality evidence for an association between PPI therapy and CDI not supporting a cause-effect relationship. Authors reported a pooled OR of 1.65 (95%CI: 1.47-1.85) with evidence of publication bias and significant statistical heterogeneity among the studies (I^2 = 89.9%). More recently, Arriola *et al*[89] suggest, in a meta-analysis including only inpatients, that PPIs use significantly increases the risk of hospital-acquired CDI (OR = 1.81). Bavishi *et al*[114] in a systematic review regarding the use of PPI and increased susceptibility to enteric infection found 27 studies evaluating an association between PPI therapy and the risk of CDI, 17 of which reported a significant association. Based on an analysis of 28 studies, US FDA issued a warning on the risk of CDI with PPIs use[115], and similar warnings are found in CDI treatment guidelines[142].

Several studies reported that PPIs use is also a risk factor for community-acquired CDI. Dial *et al*[26], in a study including over 1000 cases of community-acquired CDI, found that patients who had received PPIs within the previous 90 d had a nearly 3-fold increased risk for CDI. A similar result was reported by Kutty *et al*[41] who found a 2-3-fold increased for community-acquired CDI in patients treated with PPIs within the previous 6 mo. Marwick *et al*[143] in a study including patients aged 65 years or older identified all cases of community-acquired CDI and found that patients prescribed PPIs within the previous 6 mo had a 1.7-fold increased risk for CDI compared to matched controls. A study assessing the epidemiology of community-acquired CDI found rates of PPI use of nearly 30% among patients with this infection compared to less than 3% in the general population[144]. These results indicate a similar degree of association between PPIs use and CDI risk, be it community-acquired CDI or hospital-acquired CDI[145].

Nevertheless, the association between PPIs use and the risk for CDI remains to a certain extent controversial despite the results reported above, as several studies failed to find such an association[11,27,34,94,122]. Beaulieu *et al*[27] found that the use of gastric acid-suppression therapy does not predispose to development of CDI, while McFarland *et al*[122] reported no relation between CDI and the use of PPIs. Branch *et al*[94] found that

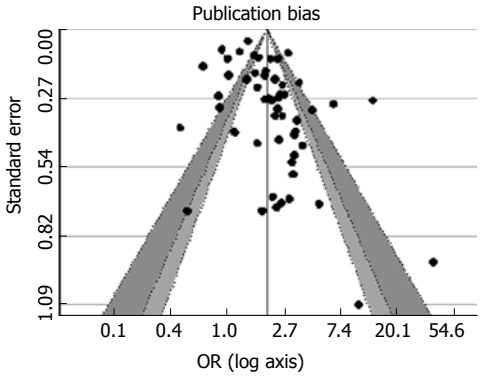


Figure 3 Funnel plot with 95% confidence limits.

PPI use did not increase the incidence of CDI in hospitalized patients.

The mechanism by which PPI therapy contributes to an increased risk of CDI is unclear. It has been proposed that a vegetative form of *C. difficile* survives in conditions of gastric pH greater than 4[114]-the threshold for enteric infections acquisition, including *C. difficile*. Howell *et al*[136] reported that the risk of nosocomial CDI rose with increasing levels of acid suppression. Hegarty *et al*[146] reported that PPI therapy decreased the expression of genes holding an important role in colonocyte integrity, thus favoring the development of CDI. Other studies show that long-term use of PPIs decreases microbial diversity, a condition found in patients with CDI[147].

As we have already mentioned, our subgroup analyses also showed an increased risk for CDI. There were no significant differences of effects between cohort and case-control studies, adjusted and unadjusted data, single-center and multicenter studies, hospitalized-and community-acquired CDI or among geographic regions. Advanced age is a well-known risk factor for CDI. To our surprise, we found no increased risk of CDI in elderly patients (≥ 65 years) using PPIs compared with youngers (OR = 1.93 *vs* OR = 2.06, P = 0.860). A possible explanation is that many of such patients may have atrophic gastritis with low gastric acid output[148] and PPIs use cannot further lower gastric acid secretion, without any additional risk of CDI[32].

As data regarding the association between PPI therapy and risk of CDI are supported only by observational studies, a final estimation of the real risk is not possible. It should be mentioned that randomized placebo-controlled clinical trials evaluating the association of PPIs use and the risk for CDI are ethically unfeasible and therefore, such studies could not be performed in the future. Thus, a weak association between PPI therapy and CDI does not confirm causality and could be the result of bias and uncontrolled confounding (*e.g.*, comorbidities, comedication use, *etc.*) which were lacking in most studies.

Our meta-analysis has some strengths such as the largest number of studies published to date, adjusted effect estimates concerning the association between PPI use and the risk of CDI, and subgroup analyses based on age, region, type, design and quality of the study. However, it also has several limitations: the included studies were observational, influenced by confounding variables despite statistical adjustment, the significant heterogeneity among most of them and lack of information regarding the dose and duration of PPI use as well as patient compliance to PPI therapy.

Although the above presented data from several meta-analyses and many studies demonstrated an association between PPI therapy and the risk for development of CDI, PPIs continue to be overused even in patients who are at high risk of CDI, because they are still considered "safe" drugs by most physicians. There is evidence that over half of PPI users who developed CDI had no valid indications for such therapy[25]. While in many countries PPIs are now totally available as over-the-counter medication, clinicians should inform their patients about the risk of CDI when PPIs are used on the long-term and without valid indication.

In spite of the aforementioned limitations of our and several other meta-analyses, clinicians should be aware of the risk of CDI when prescribing long-term PPI therapy, particularly in patients at high risk (*e.g.*, hospitalized patients on antibiotics). It should be underlined that PPIs remain, on the whole, a safe group of drugs[149], providing enormous benefits when prescribed for well-established indications. Unfortunately, many prescriptions fall outside accepted indications[90].

In conclusion, this systematic review and meta-analysis provides further evidence that PPI use significantly increases the risk for developing CDI, despite the substantial heterogeneity and publication bias present among studies. Due to the fact that all the studies included in our analysis are observational and cannot confirm causality, further large, high quality, prospective studies are needed to assess the association between PPI use and the risk of CDI.

COMMENTS

Background

Proton pump inhibitors (PPIs) are among the most widely prescribed agents by gastroenterologists because of their high efficacy and excellent safety profile. However, more recently, concerns have been raised about association between PPI therapy and several potentially serious adverse events including *Clostridium difficile* (*C. difficile*) infection (CDI). This systematic review and meta-analysis explored the existing evidence regarding the association of PPI therapy and CDI.

Research frontiers

Many observational studies and meta-analyses have reported conflicting results regarding the association between PPI therapy and the risk for CDI.

Innovations and breakthroughs

This systematic review and meta-analysis, including the largest number

of studies published to date, provides further evidence that PPI therapy is associated with an increased risk for development of CDI. Because all the studies analyzed were observational, with inherent limitations, the causality could not be confirmed.

Applications

Although our systematic review and meta-analysis, in line with previous studies and meta-analyses, reported an association between PPI therapy and the risk for development of CDI, such association remains controversial and a final estimation of the real risk has not been established. Further high-quality, prospective studies are needed to assess whether this association is causal. Until then, clinicians should be aware that long-term PPI therapy may be associated with the risk of CDI, and prescribe the PPIs in the lowest effective dose only to patients with a clear indication.

Terminology

PPIs are a group of potent inhibitors of gastric acid secretion. CDI is a symptomatic infection due to the spore-forming bacterium *C. difficile*.

Peer-review

This manuscript is an interesting, informative and well-presented meta-analysis on PPI therapy and risk of *C. difficile* infection.

REFERENCES

1 **Khanna S**, Pardi DS. The growing incidence and severity of Clostridium difficile infection in inpatient and outpatient settings. *Expert Rev Gastroenterol Hepatol* 2010; **4**: 409-416 [PMID: 20678014 DOI: 10.1586/egh.10.48]

2 **Kuntz JL**, Chrischilles EA, Pendergast JF, Herwaldt LA, Polgreen PM. Incidence of and risk factors for community-associated Clostridium difficile infection: a nested case-control study. *BMC Infect Dis* 2011; **11**: 194 [PMID: 21762504 DOI: 10.1186/1471-2334-11-194]

3 **Kurti Z**, Lovasz BD, Mandel MD, Csima Z, Golovics PA, Csako BD, Mohas A, Gönczi L, Gecse KB, Kiss LS, Szathmari M, Lakatos PL. Burden of Clostridium difficile infection between 2010 and 2013: Trends and outcomes from an academic center in Eastern Europe. *World J Gastroenterol* 2015; **21**: 6728-6735 [PMID: 26074711 DOI: 10.3748/wjg.v21.i21.6728]

4 **Honda H**, Dubberke ER. The changing epidemiology of Clostridium difficile infection. *Curr Opin Gastroenterol* 2014; **30**: 54-62 [PMID: 24285002 DOI: 10.1097/MOG.0000000000000018]

5 **Dubberke ER**, Butler AM, Yokoe DS, Mayer J, Hota B, Mangino JE, Khan YM, Popovich KJ, Fraser VJ. Multicenter study of Clostridium difficile infection rates from 2000 to 2006. *Infect Control Hosp Epidemiol* 2010; **31**: 1030-1037 [PMID: 20695799 DOI: 10.1086/656245]

6 **Peery AF**, Dellon ES, Lund J, Crockett SD, McGowan CE, Bulsiewicz WJ, Gangarosa LM, Thiny MT, Stizenberg K, Morgan DR, Ringel Y, Kim HP, Dibonaventura MD, Carroll CF, Allen JK, Cook SF, Sandler RS, Kappelman MD, Shaheen NJ. Burden of gastrointestinal disease in the United States: 2012 update. *Gastroenterology* 2012; **143**: 1179-1187.e1-e3 [PMID: 22885331 DOI: 10.1053/j.gastro.2012.08.002]

7 **Depestel DD**, Aronoff DM. Epidemiology of Clostridium difficile infection. *J Pharm Pract* 2013; **26**: 464-475 [PMID: 24064435 DOI: 10.1177/0897190013499521]

8 **Reveles KR**, Lee GC, Boyd NK, Frei CR. The rise in Clostridium difficile infection incidence among hospitalized adults in the United States: 2001-2010. *Am J Infect Control* 2014; **42**: 1028-1032 [PMID: 25278388 DOI: 10.1016/j.ajic.2014.06.011]

9 **Gabriel L**, Beriot-Mathiot A. Hospitalization stay and costs attributable to Clostridium difficile infection: a critical review. *J Hosp Infect* 2014; **88**: 12-21 [PMID: 24996516 DOI: 10.1016/j.jhin.2014.04.011]

10 **Thomas C**, Stevenson M, Riley TV. Antibiotics and hospital-acquired Clostridium difficile-associated diarrhoea: a systematic review. *J Antimicrob Chemother* 2003; **51**: 1339-1350 [PMID: 12746372 DOI: 10.1093/jac/dkg254]

11 **Pépin J**, Saheb N, Coulombe MA, Alary ME, Corriveau MP, Authier S, Leblanc M, Rivard G, Bettez M, Primeau V, Nguyen M, Jacob CE, Lanthier L. Emergence of fluoroquinolones as the predominant risk factor for Clostridium difficile-associated diarrhea: a cohort study during an epidemic in Quebec. *Clin Infect Dis* 2005; **41**: 1254-1260 [PMID: 16206099 DOI: 10.1086/496986]

12 **Taslim H**. Clostridium difficile infection in the elderly. *Acta Med Indones* 2009; **41**: 148-151 [PMID: 19752488]

13 **Lawrence SJ**, Puzniak LA, Shadel BN, Gillespie KN, Kollef MH, Mundy LM. Clostridium difficile in the intensive care unit: epidemiology, costs, and colonization pressure. *Infect Control Hosp Epidemiol* 2007; **28**: 123-130 [PMID: 17265392 DOI: 10.1086/511793]

14 **Eddi R**, Malik MN, Shakov R, Baddoura WJ, Chandran C, Debari VA. Chronic kidney disease as a risk factor for Clostridium difficile infection. *Nephrology* (Carlton) 2010; **15**: 471-475 [PMID: 20609100 DOI: 10.1111/j.1440-1797.2009.01274.x]

15 **Schneeweiss S**, Korzenik J, Solomon DH, Canning C, Lee J, Bressler B. Infliximab and other immunomodulating drugs in patients with inflammatory bowel disease and the risk of serious bacterial infections. *Aliment Pharmacol Ther* 2009; **30**: 253-264 [PMID: 19438424 DOI: 10.1111/j.1365-2036.2009.04037.x]

16 **Dubberke ER**, Olsen MA, Stwalley D, Kelly CP, Gerding DN, Young-Xu Y, Mahé C. Identification of Medicare Recipients at Highest Risk for Clostridium difficile Infection in the US by Population Attributable Risk Analysis. *PLoS One* 2016; **11**: e0146822 [PMID: 26859403 DOI: 10.1371/journal.pone.0146822]

17 **Ananthakrishnan AN**. Clostridium difficile infection: epidemiology, risk factors and management. *Nat Rev Gastroenterol Hepatol* 2011; **8**: 17-26 [PMID: 21119612 DOI: 10.1038/nrgastro.2010.190]

18 **Raza S**, Baig MA, Russell H, Gourdet Y, Berger BJ. Clostridium difficile infection following chemotherapy. *Recent Pat Antiinfect Drug Discov* 2010; **5**: 1-9 [PMID: 19929843 DOI: 10.2174/157489110790112608]

19 **Al-Tureihi FI**, Hassoun A, Wolf-Klein G, Isenberg H. Albumin, length of stay, and proton pump inhibitors: key factors in Clostridium difficile-associated disease in nursing home patients. *J Am Med Dir Assoc* 2005; **6**: 105-108 [PMID: 15871884 DOI: 10.1016/j.jamda.2005.01.003]

20 **Morfin-Otero R**, Garza-Gonzalez E, Aguirre-Diaz SA, Escobedo-Sanchez R, Esparza-Ahumada S, Perez-Gomez HR, Petersen-Morfin S, Gonzalez-Diaz E, Martinez-Melendez A, Rodriguez-Noriega E; Hospital Civil de Guadalajara, Fray Antonio Alcalde Clostridium difficile Team. Clostridium difficile outbreak caused by NAP1/BI/027 strain and non-027 strains in a Mexican hospital. *Braz J Infect Dis* 2016; **20**: 8-13 [PMID: 26620948 DOI: 10.1016/j.bjid.2015.09.008]

21 **Rotramel A**, Poritz LS, Messaris E, Berg A, Stewart DB. PPI therapy and albumin are better predictors of recurrent Clostridium difficile colitis than choice of antibiotics. *J Gastrointest Surg* 2012; **16**: 2267-2273 [PMID: 23007285 DOI: 10.1007/s11605-012-2037-9]

22 **Dalton BR**, Lye-Maccannell T, Henderson EA, Maccannell DR, Louie TJ. Proton pump inhibitors increase significantly the risk of Clostridium difficile infection in a low-endemicity, non-outbreak hospital setting. *Aliment Pharmacol Ther* 2009; **29**: 626-634 [PMID: 19183143 DOI: 10.1111/j.1365-2036.2008.03924.x]

23 **Aseeri M**, Schroeder T, Kramer J, Zackula R. Gastric acid suppression by proton pump inhibitors as a risk factor for clostridum difficile-associated diarrhea in hospitalized patients. *Am J Gastroenterol* 2008; **103**: 2308-2313 [PMID: 18702653 DOI: 10.1111/j.1572-0241.2008.01975.x]

24 **Linney S**, Fernandes T, Einarson T, Sengar A, Walker JH, Mills A. Association Between Use of Proton Pump Inhibitors and a Clostridium difficile-Associated Disease Outbreak: Case-Control Study. *Can J Hosp Pharm* 2010; **63**: 31-37 [PMID: 22478951 DOI: 10.4212/cjhp.v63i1.866]

25 **Yearsley KA**, Gilby LJ, Ramadas AV, Kubiak EM, Fone DL, Allison MC. Proton pump inhibitor therapy is a risk factor for Clostridium difficile-associated diarrhoea. *Aliment Pharmacol Ther* 2006; **24**: 613-619 [PMID: 16907893 DOI: 10.1111/j.1365-2036.2006.03015.x]

26 **Dial S**, Alrasadi K, Manoukian C, Huang A, Menzies D. Risk of Clostridium difficile diarrhea among hospital inpatients prescribed proton pump inhibitors: cohort and case-control studies. *CMAJ* 2004; **171**: 33-38 [PMID: 15238493 DOI: 10.1503/cmaj.1040876]

27 **Beaulieu M**, Williamson D, Pichette G, Lachaine J. Risk of Clostridium difficile-associated disease among patients receiving proton-pump inhibitors in a Quebec medical intensive care unit. *Infect Control Hosp Epidemiol* 2007; **28**: 1305-1307 [PMID: 17926283 DOI: 10.1086/521664]

28 **Pant C**, Madonia P, Minocha A. Does PPI therapy predispose to Clostridium difficile infection? *Nat Rev Gastroenterol Hepatol* 2009; **6**: 555-557 [PMID: 19713988 DOI: 10.1038/nrgastro.2009.128]

29 **Rashid S**, Rajan D, Iqbal J, Lipka S, Jacob R, Zilberman V, Shah M, Mustacchia P. Inappropriate Use of Gastric Acid Suppression Therapy in Hospitalized Patients with Clostridium difficile-Associated Diarrhea: A Ten-Year Retrospective Analysis. *ISRN Gastroenterol* 2012; **2012**: 902320 [PMID: 22701180 DOI: 10.5402/2012/902320]

30 **Choudhry MN**, Soran H, Ziglam HM. Overuse and inappropriate prescribing of proton pump inhibitors in patients with Clostridium difficile-associated disease. *QJM* 2008; **101**: 445-448 [PMID: 18411220 DOI: 10.1093/qjmed/hcn035]

31 **Patil R**, Blankenship L. Proton Pump Inhibitors and Clostridium Difficile Infection: Are We Propagating an Already Rapidly Growing Healthcare Problem? *Gastroenterology Res* 2013; **6**: 171-173 [PMID: 27785249 DOI: 10.4021/gr575w]

32 **Cunningham R**, Dial S. Is over-use of proton pump inhibitors fuelling the current epidemic of Clostridium difficile-associated diarrhoea? *J Hosp Infect* 2008; **70**: 1-6 [PMID: 18602190 DOI: 10.1016/j.jhin.2008.04.023]

33 **McCarthy DM**. Proton pump inhibitor use and Clostridium difficile colitis: cause or coincidence? *J Clin Gastroenterol* 2012; **46**: 350-353 [PMID: 22495812 DOI: 10.1097/MCG.0b013e31824b228f]

34 **Shah S**, Lewis A, Leopold D, Dunstan F, Woodhouse K. Gastric acid suppression does not promote clostridial diarrhoea in the elderly. *QJM* 2000; **93**: 175-181 [PMID: 10751237 DOI: 10.1093/qjmed/93.3.175]

35 **Naggie S**, Miller BA, Zuzak KB, Pence BW, Mayo AJ, Nicholson BP, Kutty PK, McDonald LC, Woods CW. A case-control study of community-associated Clostridium difficile infection: no role for proton pump inhibitors. *Am J Med* 2011; **124**: 276.e1-276.e7 [PMID: 21396512 DOI: 10.1016/j.amjmed.2010.10.013]

36 **Vindigni SM**, Surawicz CM. C. difficile Infection: Changing Epidemiology and Management Paradigms. *Clin Transl Gastroenterol* 2015; **6**: e99 [PMID: 26158611 DOI: 10.1038/ctg.2015.24]

37 **Rostom A**, Moayyedi P, Hunt R; Canadian Association of Gastroenterology Consensus Group. Canadian consensus guidelines on long-term nonsteroidal anti-inflammatory drug therapy and the need for gastroprotection: benefits versus risks. *Aliment Pharmacol Ther* 2009; **29**: 481-496 [PMID: 19053986 DOI: 10.1111/j.1365-2036.2008.03905.x]

38 **Campbell KA**, Phillips MS, Stachel A, Bosco JA 3rd, Mehta SA. Incidence and risk factors for hospital-acquired Clostridium difficile infection among inpatients in an orthopaedic tertiary care hospital. *J Hosp Infect* 2013; **83**: 146-149 [PMID: 23313026 DOI: 10.1016/j.jhin.2012.11.009]

39 **Khan FY**, Abu-Khattab M, Anand D, Baager K, Alaini A, Siddique MA, Mohamed SF, Ali MI, Al Bedawi MM, Naser MS. Epidemiological features of Clostridium difficile infection among inpatients at Hamad General Hospital in the state of Qatar, 2006-2009. *Travel Med Infect Dis* 2012; **10**: 179-185 [PMID: 22800937 DOI: 10.1016/j.tmaid.2012.06.004]

40 **Ingle M**, Deshmukh A, Desai D, Abraham P, Joshi A, Rodrigues C, Mankeshwar R. Prevalence and clinical course of Clostridium difficile infection in a tertiary-care hospital: a retrospective analysis. *Indian J Gastroenterol* 2011; **30**: 89-93 [PMID: 21553102 DOI: 10.1007/s12664-011-0097-5]

41 **Kutty PK**, Woods CW, Sena AC, Benoit SR, Naggie S, Frederick J, Evans S, Engel J, McDonald LC. Risk factors for and estimated incidence of community-associated Clostridium difficile infection, North Carolina, USA. *Emerg Infect Dis* 2010; **16**: 197-204 [PMID: 20113547 DOI: 10.3201/eid1602.090953]

42 **Faleck DM**, Salmasian H, Furuya EY, Larson EL, Abrams JA, Freedberg DE. Proton Pump Inhibitors Do Not Increase Risk for Clostridium difficile Infection in the Intensive Care Unit. *Am J Gastroenterol* 2016; **111**: 1641-1648 [PMID: 27575714 DOI: 10.1038/ajg.2016.343]

43 **Rodríguez Garzotto A**, Mérida García A, Muñoz Unceta N, Galera Lopez MM, Orellana-Miguel MÁ, Díaz-García CV, Cortijo-Cascajares S, Cortes-Funes H, Agulló-Ortuño MT. Risk factors associated with Clostridium difficile infection in adult oncology patients. *Support Care Cancer* 2015; **23**: 1569-1577 [PMID: 25410088 DOI: 10.1007/s00520-014-2506-7]

44 **Devlin JW**, Welage LS, Olsen KM. Proton pump inhibitor formulary considerations in the acutely ill. Part 2: Clinical efficacy, safety, and economics. *Ann Pharmacother* 2005; **39**: 1844-1851 [PMID: 16204393 DOI: 10.1345/aph.1G176]

45 **Attwood SE**, Ell C, Galmiche JP, Fiocca R, Hatlebakk JG, Hasselgren B, Långström G, Jahreskog M, Eklund S, Lind T, Lundell L. Long-term safety of proton pump inhibitor therapy assessed under controlled, randomised clinical trial conditions: data from the SOPRAN and LOTUS studies. *Aliment Pharmacol Ther* 2015; **41**: 1162-1174 [PMID: 25858519 DOI: 10.1111/apt.13194]

46 **Kyne L**, Hamel MB, Polavaram R, Kelly CP. Health care costs and mortality associated with nosocomial diarrhea due to Clostridium difficile. *Clin Infect Dis* 2002; **34**: 346-353 [PMID: 11774082 DOI: 10.1086/338260]

47 **Jacobson BC**, Ferris TG, Shea TL, Mahlis EM, Lee TH, Wang TC. Who is using chronic acid suppression therapy and why? *Am J Gastroenterol* 2003; **98**: 51-58 [PMID: 12526936 DOI: 10.1111/j.1572-0241.2003.07186.x]

48 **Kelly OB**, Dillane C, Patchett SE, Harewood GC, Murray FE. The Inappropriate Prescription of Oral Proton Pump Inhibitors in the Hospital Setting: A Prospective Cross-Sectional Study. *Dig Dis Sci* 2015; **60**: 2280-2286 [PMID: 25840918 DOI: 10.1007/s10620-015-3642-8]

49 **Craig DG**, Thimappa R, Anand V, Sebastian S. Inappropriate utilization of intravenous proton pump inhibitors in hospital practice--a prospective study of the extent of the problem and predictive factors. *QJM* 2010; **103**: 327-335 [PMID: 20211846 DOI: 10.1093/qjmed/hcq019]

50 **Moayyedi P**, Delaney BC, Vakil N, Forman D, Talley NJ. The efficacy of proton pump inhibitors in nonulcer dyspepsia: a systematic review and economic analysis. *Gastroenterology* 2004; **127**: 1329-1337 [PMID: 15521002 DOI: 10.1053/j.gastro.2004.08.026]

51 **Sebastian SS**, Kernan N, Qasim A, O'Morain CA, Buckley M. Appropriateness of gastric antisecretory therapy in hospital practice. *Ir J Med Sci* 2003; **172**: 115-117 [PMID: 14700112 DOI: 10.1007/BF02914494]

52 **Haroon M**, Yasin F, Gardezi SK, Adeeb F, Walker F. Inappropriate use of proton pump inhibitors among medical inpatients: a questionnaire-based observational study. *JRSM Short Rep* 2013; **4**: 2042533313497183 [PMID: 24040498 DOI: 10.1177/2042533313497183]

53 **Pappas M**, Jolly S, Vijan S. Defining Appropriate Use of Proton-Pump Inhibitors Among Medical Inpatients. *J Gen Intern Med* 2016; **31**: 364-371 [PMID: 26553337 DOI: 10.1007/s11606-015-3536-7]

54 **Ladd AM**, Panagopoulos G, Cohen J, Mar N, Graham R. Potential costs of inappropriate use of proton pump inhibitors. *Am J Med Sci* 2014; **347**: 446-451 [PMID: 24270078 DOI: 10.1097/

MAJ.0b013e31829f87d5]
55 **Shaheen NJ**, Hansen RA, Morgan DR, Gangarosa LM, Ringel Y, Thiny MT, Russo MW, Sandler RS. The burden of gastrointestinal and liver diseases, 2006. *Am J Gastroenterol* 2006; **101**: 2128-2138 [PMID: 16848807 DOI: 10.1111/j.1572-0241.2006.00723.x]
56 **Barkun AN**, Bardou M, Pham CQ, Martel M. Proton pump inhibitors vs. histamine 2 receptor antagonists for stress-related mucosal bleeding prophylaxis in critically ill patients: a meta-analysis. *Am J Gastroenterol* 2012; **107**: 507-520 ; quiz 521 [PMID: 22290403 DOI: 10.1038/ajg.2011.474]
57 **Yachimski PS**, Farrell EA, Hunt DP, Reid AE. Proton pump inhibitors for prophylaxis of nosocomial upper gastrointestinal tract bleeding: effect of standardized guidelines on prescribing practice. *Arch Intern Med* 2010; **170**: 779-783 [PMID: 20458085 DOI: 10.1001/archinternmed.2010.51]
58 **Alhazzani W**, Alenezi F, Jaeschke RZ, Moayyedi P, Cook DJ. Proton pump inhibitors versus histamine 2 receptor antagonists for stress ulcer prophylaxis in critically ill patients: a systematic review and meta-analysis. *Crit Care Med* 2013; **41**: 693-705 [PMID: 23318494 DOI: 10.1097/CCM.0b013e3182758734]
59 **Xiao YL**, Peng S, Tao J, Wang AJ, Lin JK, Hu PJ, Chen MH. Prevalence and symptom pattern of pathologic esophageal acid reflux in patients with functional dyspepsia based on the Rome III criteria. *Am J Gastroenterol* 2010; **105**: 2626-2631 [PMID: 20823838 DOI: 10.1038/ajg.2010.351]
60 **Cohen ME**, Hathway JM, Salmasian H, Liu J, Terry M, Abrams JA, Freedberg DE. Prophylaxis for Stress Ulcers With Proton Pump Inhibitors Is Not Associated With Increased Risk of Bloodstream Infections in the Intensive Care Unit. *Clin Gastroenterol Hepatol* 2017; **15**: 1030-1036.e1 [PMID: 28110095 DOI: 10.1016/j.cgh.2016.12.035]
61 **Yang YX**, Metz DC. Safety of proton pump inhibitor exposure. *Gastroenterology* 2010; **139**: 1115-1127 [PMID: 20727892 DOI: 10.1053/j.gastro.2010.08.023]
62 **Heidelbaugh JJ**, Goldberg KL, Inadomi JM. Overutilization of proton pump inhibitors: a review of cost-effectiveness and risk [corrected]. *Am J Gastroenterol* 2009; **104** Suppl 2: S27-S32 [PMID: 19262544 DOI: 10.1038/ajg.2009.49]
63 **Brunner G**, Athmann C, Schneider A. Long-term, open-label trial: safety and efficacy of continuous maintenance treatment with pantoprazole for up to 15 years in severe acid-peptic disease. *Aliment Pharmacol Ther* 2012; **36**: 37-47 [PMID: 22531114 DOI: 10.1111/j.1365-2036.2012.05106.x]
64 **Haastrup PF**, Paulsen MS, Christensen RD, Søndergaard J, Hansen JM, Jarbøl DE. Medical and non-medical predictors of initiating long-term use of proton pump inhibitors: a nationwide cohort study of first-time users during a 10-year period. *Aliment Pharmacol Ther* 2016; **44**: 78-87 [PMID: 27137875 DOI: 10.1111/apt.13649]
65 **Lambert AA**, Lam JO, Paik JJ, Ugarte-Gil C, Drummond MB, Crowell TA. Risk of community-acquired pneumonia with outpatient proton-pump inhibitor therapy: a systematic review and meta-analysis. *PLoS One* 2015; **10**: e0128004 [PMID: 26042842 DOI: 10.1371/journal.pone.0128004]
66 **de Groot MC**, Klungel OH, Leufkens HG, van Dijk L, Grobbee DE, van de Garde EM. Sources of heterogeneity in case-control studies on associations between statins, ACE-inhibitors, and proton pump inhibitors and risk of pneumonia. *Eur J Epidemiol* 2014; **29**: 767-775 [PMID: 25154551 DOI: 10.1007/s10654-014-9941-0]
67 **Eurich DT**, Sadowski CA, Simpson SH, Marrie TJ, Majumdar SR. Recurrent community-acquired pneumonia in patients starting acid-suppressing drugs. *Am J Med* 2010; **123**: 47-53 [PMID: 20102991 DOI: 10.1016/j.amjmed.2009.05.032]
68 **Cai D**, Feng W, Jiang Q. Acid-suppressive medications and risk of fracture: an updated meta-analysis. *Int J Clin Exp Med* 2015; **8**: 8893-8904 [PMID: 26309543]
69 **Leontiadis GI**, Moayyedi P. Proton pump inhibitors and risk of bone fractures. *Curr Treat Options Gastroenterol* 2014; **12**: 414-423 [PMID: 25209137 DOI: 10.1007/s11938-014-0030-y]
70 **Eom CS**, Park SM, Myung SK, Yun JM, Ahn JS. Use of acid-suppressive drugs and risk of fracture: a meta-analysis of observational studies. *Ann Fam Med* 2011; **9**: 257-267 [PMID: 21555754 DOI: 10.1370/afm.1243]
71 **Sierra F**, Suarez M, Rey M, Vela MF. Systematic review: Proton pump inhibitor-associated acute interstitial nephritis. *Aliment Pharmacol Ther* 2007; **26**: 545-553 [PMID: 17661758 DOI: 10.1111/j.1365-2036.2007.03407.x]
72 **Arora P**, Gupta A, Golzy M, Patel N, Carter RL, Jalal K, Lohr JW. Proton pump inhibitors are associated with increased risk of development of chronic kidney disease. *BMC Nephrol* 2016; **17**: 112 [PMID: 27487959 DOI: 10.1186/s12882-016-0325-4]
73 **Arora S**, Dellon ES. PPIs and Chronic Kidney Disease: Another Association to Worry About? *Gastroenterology* 2016; **151**: 366-368 [PMID: 27371880 DOI: 10.1053/j.gastro.2016.06.028]
74 **Wijarnpreecha K**, Thongprayoon C, Panjawatanan P, Ungprasert P. Proton pump inhibitors and risk of dementia. *Ann Transl Med* 2016; **4**: 240 [PMID: 27429966 DOI: 10.21037/atm.2016.06.14]
75 **Dam G**, Vilstrup H, Watson H, Jepsen P. Proton pump inhibitors as a risk factor for hepatic encephalopathy and spontaneous bacterial peritonitis in patients with cirrhosis with ascites. *Hepatology* 2016; **64**: 1265-1272 [PMID: 27474889 DOI: 10.1002/hep.28737]
76 **Xu HB**, Wang HD, Li CH, Ye S, Dong MS, Xia QJ, Zhang AQ, Pan K, Ge XL, Dong JH. Proton pump inhibitor use and risk of spontaneous bacterial peritonitis in cirrhotic patients: a systematic review and meta-analysis. *Genet Mol Res* 2015; **14**: 7490-7501 [PMID: 26214428 DOI: 10.4238/2015.July.3.25]
77 **Zhu W**, Hong K. Potential Cardiovascular Risks of Proton Pump Inhibitors in the General Population. *Int Heart J* 2017; **58**: 163-166 [PMID: 28321021 DOI: 10.1536/ihj.16-208]
78 **Shah NH**, LePendu P, Bauer-Mehren A, Ghebremariam YT, Iyer SV, Marcus J, Nead KT, Cooke JP, Leeper NJ. Proton Pump Inhibitor Usage and the Risk of Myocardial Infarction in the General Population. *PLoS One* 2015; **10**: e0124653 [PMID: 26061035 DOI: 10.1371/journal.pone.0124653]
79 **Heidelbaugh JJ**. Proton pump inhibitors and risk of vitamin and mineral deficiency: evidence and clinical implications. *Ther Adv Drug Saf* 2013; **4**: 125-133 [PMID: 25083257 DOI: 10.1177/2042098613482484]
80 **Hashimoto R**, Matsuda T, Chonan A. Iron-deficiency anemia caused by a proton pump inhibitor. *Intern Med* 2014; **53**: 2297-2299 [PMID: 25318791 DOI: 10.2169/internalmedicine.53.2743]
81 **Freedberg DE**, Kim LS, Yang YX. The Risks and Benefits of Long-term Use of Proton Pump Inhibitors: Expert Review and Best Practice Advice From the American Gastroenterological Association. *Gastroenterology* 2017; **152**: 706-715 [PMID: 28257716 DOI: 10.1053/j.gastro.2017.01.031]
82 **Thorens J**, Froehlich F, Schwizer W, Saraga E, Bille J, Gyr K, Duroux P, Nicolet M, Pignatelli B, Blum AL, Gonvers JJ, Fried M. Bacterial overgrowth during treatment with omeprazole compared with cimetidine: a prospective randomised double blind study. *Gut* 1996; **39**: 54-59 [PMID: 8881809 DOI: 10.1136/gut.39.1.54]
83 **Amir I**, Konikoff FM, Oppenheim M, Gophna U, Half EE. Gastric microbiota is altered in oesophagitis and Barrett's oesophagus and further modified by proton pump inhibitors. *Environ Microbiol* 2014; **16**: 2905-2914 [PMID: 24112768 DOI: 10.1111/1462-2920.12285]
84 **Zedtwitz-Liebenstein K**, Wenisch C, Patruta S, Parschalk B, Daxböck F, Graninger W. Omeprazole treatment diminishes intra- and extracellular neutrophil reactive oxygen production and bactericidal activity. *Crit Care Med* 2002; **30**: 1118-1122 [PMID: 12006811 DOI: 10.1097/00003246-200205000-00026]
85 **Deshpande A**, Pant C, Pasupuleti V, Rolston DD, Jain A, Deshpande N, Thota P, Sferra TJ, Hernandez AV. Association between proton pump inhibitor therapy and Clostridium difficile infection in a meta-analysis. *Clin Gastroenterol Hepatol* 2012; **10**: 225-233 [PMID: 22019794 DOI: 10.1016/j.cgh.2011.09.030]
86 **Tleyjeh IM**, Bin Abdulhak AA, Riaz M, Alasmari FA, Garbati MA, AlGhamdi M, Khan AR, Al Tannir M, Erwin PJ, Ibrahim T, Allehibi A, Baddour LM, Sutton AJ. Association between proton pump inhibitor therapy and clostridium difficile infection:

a contemporary systematic review and meta-analysis. *PLoS One* 2012; **7**: e50836 [PMID: 23236397 DOI: 10.1371/journal.pone.0050836]
87 **Kwok CS**, Arthur AK, Anibueze CI, Singh S, Cavallazzi R, Loke YK. Risk of Clostridium difficile infection with acid suppressing drugs and antibiotics: meta-analysis. *Am J Gastroenterol* 2012; **107**: 1011-1019 [PMID: 22525304 DOI: 10.1038/ajg.2012.108]
88 **Janarthanan S**, Ditah I, Adler DG, Ehrinpreis MN. Clostridium difficile-associated diarrhea and proton pump inhibitor therapy: a meta-analysis. *Am J Gastroenterol* 2012; **107**: 1001-1010 [PMID: 22710578 DOI: 10.1038/ajg.2012.179]
89 **Arriola V**, Tischendorf J, Musuuza J, Barker A, Rozelle JW, Safdar N. Assessing the Risk of Hospital-Acquired Clostridium Difficile Infection With Proton Pump Inhibitor Use: A Meta-Analysis. *Infect Control Hosp Epidemiol* 2016; **37**: 1408-1417 [PMID: 27677811 DOI: 10.1017/ice.2016.194]
90 **Deshpande A**, Pasupuleti V, Thota P, Pant C, Rolston DD, Hernandez AV, Donskey CJ, Fraser TG. Risk factors for recurrent Clostridium difficile infection: a systematic review and meta-analysis. *Infect Control Hosp Epidemiol* 2015; **36**: 452-460 [PMID: 25626326 DOI: 10.1017/ice.2014.88]
91 **Akhtar AJ**, Shaheen M. Increasing incidence of clostridium difficile-associated diarrhea in African-American and Hispanic patients: association with the use of proton pump inhibitor therapy. *J Natl Med Assoc* 2007; **99**: 500-504 [PMID: 17534007]
92 **Barletta JF**, Sclar DA. Proton pump inhibitors increase the risk for hospital-acquired Clostridium difficile infection in critically ill patients. *Crit Care* 2014; **18**: 714 [PMID: 25540023 DOI: 10.1186/s13054-014-0714-7]
93 **Baxter R**, Ray GT, Fireman BH. Case-control study of antibiotic use and subsequent *Clostridium difficile*-associated diarrhea in hospitalized patients. *Infect Control Hosp Epidemiol* 2008; **29**: 44-50 [PMID: 18171186 DOI: 10.1086/524320]
94 **Branch K,** Yahl V, Kier K, Mertz N, Marques S. Gastric acid suppression by proton pump inhibitors as an independent risk factor for-associated diarrhea. *P&T* 2007; **32**: 432-437
95 **Buendgens L**, Bruensing J, Matthes M, Dückers H, Luedde T, Trautwein C, Tacke F, Koch A. Administration of proton pump inhibitors in critically ill medical patients is associated with increased risk of developing Clostridium difficile-associated diarrhea. *J Crit Care* 2014; **29**: 696.e11-696.e15 [PMID: 24674763 DOI: 10.1016/j.jcrc.2014.03.002]
96 **Cunningham R**, Dale B, Undy B, Gaunt N. Proton pump inhibitors as a risk factor for Clostridium difficile diarrhoea. *J Hosp Infect* 2003; **54**: 243-245 [PMID: 12855243 DOI: 10.1016/S0195-6701(03)00088-4]
97 **Dial S**, Delaney JA, Schneider V, Suissa S. Proton pump inhibitor use and risk of community-acquired Clostridium difficile-associated disease defined by prescription for oral vancomycin therapy. *CMAJ* 2006; **175**: 745-748 [PMID: 17001054 DOI: 10.1503/cmaj.060284]
98 **Dial S**, Delaney JA, Barkun AN, Suissa S. Use of gastric acid-suppressive agents and the risk of community-acquired Clostridium difficile-associated disease. *JAMA* 2005; **294**: 2989-2995 [PMID: 16414946 DOI: 10.1001/jama.294.23.2989]
99 **Dubberke ER**, Reske KA, Olsen MA, McMullen KM, Mayfield JL, McDonald LC, Fraser VJ. Evaluation of Clostridium difficile-associated disease pressure as a risk factor for C difficile-associated disease. *Arch Intern Med* 2007; **167**: 1092-1097 [PMID: 17533213 DOI: 10.1001/archinte.167.10.1092]
100 **Elseviers MM**, Van Camp Y, Nayaert S, Duré K, Annemans L, Tanghe A, Vermeersch S. Prevalence and management of antibiotic associated diarrhea in general hospitals. *BMC Infect Dis* 2015; **15**: 129 [PMID: 25888351 DOI: 10.1186/s12879-015-0869-0]
101 **Jayatilaka S**, Shakov R, Eddi R, Bakaj G, Baddoura WJ, DeBari VA. Clostridium difficile infection in an urban medical center: five-year analysis of infection rates among adult admissions and association with the use of proton pump inhibitors. *Ann Clin Lab Sci* 2007; **37**: 241-247 [PMID: 17709687]
102 **Kazakova SV**, Ware K, Baughman B, Bilukha O, Paradis A, Sears S, Thompson A, Jensen B, Wiggs L, Bessette J, Martin J, Clukey J, Gensheimer K, Killgore G, McDonald LC. A hospital outbreak of diarrhea due to an emerging epidemic strain of Clostridium difficile. *Arch Intern Med* 2006; **166**: 2518-2524 [PMID: 17159019 DOI: 10.1001/archinte.166.22.2518]
103 **Lewis PO**, Litchfield JM, Tharp JL, Garcia RM, Pourmorteza M, Reddy CM. Risk and Severity of Hospital-Acquired Clostridium difficile Infection in Patients Taking Proton Pump Inhibitors. *Pharmacotherapy* 2016; **36**: 986-993 [PMID: 27455386 DOI: 10.1002/phar.1801]
104 **Mizui T**, Teramachi H, Tachi T, Tamura K, Shiga H, Komada N, Umeda M, Koda A, Aoyama S, Goto C, Tsuchiya T. Risk factors for Clostridium difficile-associated diarrhea and the effectiveness of prophylactic probiotic therapy. *Pharmazie* 2013; **68**: 706-710 [PMID: 24020129]
105 **Modena S**, Bearelly D, Swartz K, Friedenberg FK. Clostridium difficile among hospitalized patients receiving antibiotics: a case-control study. *Infect Control Hosp Epidemiol* 2005; **26**: 685-690 [PMID: 16156324 DOI: 10.1086/502603]
106 **Muto CA**, Pokrywka M, Shutt K, Mendelsohn AB, Nouri K, Posey K, Roberts T, Croyle K, Krystofiak S, Patel-Brown S, Pasculle AW, Paterson DL, Saul M, Harrison LH. A large outbreak of Clostridium difficile-associated disease with an unexpected proportion of deaths and colectomies at a teaching hospital following increased fluoroquinolone use. *Infect Control Hosp Epidemiol* 2005; **26**: 273-280 [PMID: 15796280 DOI: 10.1086/502539]
107 **Pakyz AL**, Jawahar R, Wang Q, Harpe SE. Medication risk factors associated with healthcare-associated Clostridium difficile infection: a multilevel model case-control study among 64 US academic medical centres. *J Antimicrob Chemother* 2014; **69**: 1127-1131 [PMID: 24327619 DOI: 10.1093/jac/dkt489]
108 **Peled N**, Pitlik S, Samra Z, Kazakov A, Bloch Y, Bishara J. Predicting Clostridium difficile toxin in hospitalized patients with antibiotic-associated diarrhea. *Infect Control Hosp Epidemiol* 2007; **28**: 377-381 [PMID: 17385141 DOI: 10.1086/513723]
109 **Roughead EE**, Chan EW, Choi NK, Griffiths J, Jin XM, Lee J, Kimura M, Kimura T, Kubota K, Lai EC, Man KK, Nguyen TA, Ooba N, Park BJ, Sato T, Shin JY, Wang T, Wong IC, Yang YK, Pratt NL. Proton pump inhibitors and risk of Clostridium difficile infection: a multi-country study using sequence symmetry analysis. *Expert Opin Drug Saf* 2016; **15**: 1589-1595 [PMID: 27645304 DOI: 10.1080/14740338.2016.1238071]
110 **Southern WN**, Rahmani R, Aroniadis O, Khorshidi I, Thanjan A, Ibrahim C, Brandt LJ. Postoperative Clostridium difficile-associated diarrhea. *Surgery* 2010; **148**: 24-30 [PMID: 20116817 DOI: 10.1016/j.surg.2009.11.021]
111 **Vesteinsdottir I**, Gudlaugsdottir S, Einarsdottir R, Kalaitzakis E, Sigurdardottir O, Bjornsson ES. Risk factors for Clostridium difficile toxin-positive diarrhea: a population-based prospective case-control study. *Eur J Clin Microbiol Infect Dis* 2012; **31**: 2601-2610 [PMID: 22441775 DOI: 10.1007/s10096-012-1603-0]
112 **Yang BK**, Do BJ, Kim EJ, Lee JU, Kim MH, Kang JG, Kim HS, Kim KH, Jang MK, Lee JH, Kim HY, Shin WG. The simple predictors of pseudomembranous colitis in patients with hospital-acquired diarrhea: a prospective observational study. *Gut Liver* 2014; **8**: 41-48 [PMID: 24516700 DOI: 10.5009/gnl.2014.8.1.41]
113 **Leonard J**, Marshall JK, Moayyedi P. Systematic review of the risk of enteric infection in patients taking acid suppression. *Am J Gastroenterol* 2007; **102**: 2047-2056 [PMID: 17509031 DOI: 10.1111/j.1572-0241.2007.01275.x]
114 **Bavishi C**, Dupont HL. Systematic review: the use of proton pump inhibitors and increased susceptibility to enteric infection. *Aliment Pharmacol Ther* 2011; **34**: 1269-1281 [PMID: 21999643 DOI: 10.1111/j.1365-2036.2011.04874.x]
115 **US Food and Drug Administration.** Clostridium difficile-associated diarrhea can be associated with stomach acid drugs known as proton pump inhibitors (PPIs). RDA Drug Safety Communication; 2012 Available from: URL: http://www.fda.gov/Drugs/DrugSafety/ucm 290510.htm
116 **Debast SB**, Vaessen N, Choudry A, Wiegers-Ligtvoet EA, van

den Berg RJ, Kuijper EJ. Successful combat of an outbreak due to Clostridium difficile PCR ribotype 027 and recognition of specific risk factors. *Clin Microbiol Infect* 2009; **15**: 427-434 [PMID: 19416295 DOI: 10.1111/j.1469-0691.2009.02713.x]

117 **Hensgens MP**, Goorhuis A, van Kinschot CM, Crobach MJ, Harmanus C, Kuijper EJ. Clostridium difficile infection in an endemic setting in the Netherlands. *Eur J Clin Microbiol Infect Dis* 2011; **30**: 587-593 [PMID: 21194003 DOI: 10.1007/s10096-010-1127-4]

118 **Ingle M**, Deshmukh A, Desai D, Abraham P, Joshi A, Gupta T, Rodrigues C. Clostridium difficile as a cause of acute diarrhea: a prospective study in a tertiary care center. *Indian J Gastroenterol* 2013; **32**: 179-183 [PMID: 23526401 DOI: 10.1007/s12664-013-0303-8]

119 **Khanafer N**, Touré A, Chambrier C, Cour M, Reverdy ME, Argaud L, Vanhems P. Predictors of Clostridium difficile infection severity in patients hospitalised in medical intensive care. *World J Gastroenterol* 2013; **19**: 8034-8041 [PMID: 24307797 DOI: 10.3748/wjg.v19.i44.8034]

120 **Loo VG**, Poirier L, Miller MA, Oughton M, Libman MD, Michaud S, Bourgault AM, Nguyen T, Frenette C, Kelly M, Vibien A, Brassard P, Fenn S, Dewar K, Hudson TJ, Horn R, René P, Monczak Y, Dascal A. A predominantly clonal multi-institutional outbreak of Clostridium difficile-associated diarrhea with high morbidity and mortality. *N Engl J Med* 2005; **353**: 2442-2449 [PMID: 16322602 DOI: 10.1056/NEJMoa051639]

121 **Lowe DO**, Mamdani MM, Kopp A, Low DE, Juurlink DN. Proton pump inhibitors and hospitalization for Clostridium difficile-associated disease: a population-based study. *Clin Infect Dis* 2006; **43**: 1272-1276 [PMID: 17051491 DOI: 10.1086/508453]

122 **McFarland LV**. Update on the changing epidemiology of Clostridium difficile-associated disease. *Nat Clin Pract Gastroenterol Hepatol* 2008; **5**: 40-48 [PMID: 18174906 DOI: 10.1038/ncpgasthep1029]

123 **Mori N**, Aoki Y. Clinical characteristics and risk factors for community-acquired Clostridium difficile infection: A retrospective, case-control study in a tertiary care hospital in Japan. *J Infect Chemother* 2015; **21**: 864-867 [PMID: 26482373 DOI: 10.1016/j.jiac.2015.09.004]

124 **Wells G,** Shea B, O'connell D, Peterson J, Welch V, Losos M, Tugwell P. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. Ottawa: Ottawa Hospital Research Institute; Accessed on 2017-3-15 Available from: URL: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp

125 **Higgins JPT,** Green S. Cochrane Handbook for Systematic Reviews of Interventions: Version 5.1.0. The Cochrane Collaboration 2011. Available from: URL: http://www.cochrane-handbook.org

126 **Stroup DF**, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, Moher D, Becker BJ, Sipe TA, Thacker SB. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA* 2000; **283**: 2008-2012 [PMID: 10789670 DOI: 10.1001/jama.283.15.2008]

127 **DerSimonian R**, Laird N. Meta-analysis in clinical trials. *Control Clin Trials* 1986; **7**: 177-188 [PMID: 3802833 DOI: 10.1016/0197-2456(86)90046-2]

128 **Higgins JP**, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ* 2003; **327**: 557-560 [PMID: 12958120 DOI: 10.1136/bmj.327.7414.557]

129 **Higgins JP**, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med* 2002; **21**: 1539-1558 [PMID: 12111919 DOI: 10.1002/sim.1186]

130 **Egger M**, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997; **315**: 629-634 [PMID: 9310563 DOI: 10.1136/bmj.315.7109.629]

131 **Easterbrook PJ**, Berlin JA, Gopalan R, Matthews DR. Publication bias in clinical research. *Lancet* 1991; **337**: 867-872 [PMID: 1672966 DOI: 10.1016/0140-6736(91)90201-Y]

132 **Viechtbauer W**. Conducting meta-analyses in R with the metafor package. *J Stat Softw* 2010; **36**: 1-48 Available from: URL: https://doi.org/10.18637/jss.v036.i03

133 **Bajaj JS**, Ananthakrishnan AN, Hafeezullah M, Zadvornova Y, Dye A, McGinley EL, Saeian K, Heuman D, Sanyal AJ, Hoffmann RG. Clostridium difficile is associated with poor outcomes in patients with cirrhosis: A national and tertiary center perspective. *Am J Gastroenterol* 2010; **105**: 106-113 [PMID: 19844204 DOI: 10.1038/ajg.2009.615]

134 **Dial S**, Kezouh A, Dascal A, Barkun A, Suissa S. Patterns of antibiotic use and risk of hospital admission because of Clostridium difficile infection. *CMAJ* 2008; **179**: 767-772 [PMID: 18838451 DOI: 10.1503/cmaj.071812]

135 **Hebbard AIT**, Slavin MA, Reed C, Trubiano JA, Teh BW, Haeusler GM, Thursky KA, Worth LJ. Risks factors and outcomes of Clostridium difficile infection in patients with cancer: a matched case-control study. *Support Care Cancer* 2017; **25**: 1923-1930 [PMID: 28155020 DOI: 10.1007/s00520-017-3606-y]

136 **Howell MD**, Novack V, Grgurich P, Soulliard D, Novack L, Pencina M, Talmor D. Iatrogenic gastric acid suppression and the risk of nosocomial Clostridium difficile infection. *Arch Intern Med* 2010; **170**: 784-790 [PMID: 20458086 DOI: 10.1001/archinternmed.2010.89]

137 **Lin YC**, Huang YT, Lee TF, Lee NY, Liao CH, Lin SY, Ko WC, Hsueh PR. Characteristics of patients with Clostridium difficile infection in Taiwan. *Epidemiol Infect* 2013; **141**: 2031-2038 [PMID: 23218131 DOI: 10.1017/S0950268812002749]

138 **Loo VG**, Bourgault AM, Poirier L, Lamothe F, Michaud S, Turgeon N, Toye B, Beaudoin A, Frost EH, Gilca R, Brassard P, Dendukuri N, Béliveau C, Oughton M, Brukner I, Dascal A. Host and pathogen factors for Clostridium difficile infection and colonization. *N Engl J Med* 2011; **365**: 1693-1703 [PMID: 22047560 DOI: 10.1056/NEJMoa1012413]

139 **Ro Y**, Eun CS, Kim HS, Kim JY, Byun YJ, Yoo KS, Han DS. Risk of Clostridium difficile Infection with the Use of a Proton Pump Inhibitor for Stress Ulcer Prophylaxis in Critically Ill Patients. *Gut Liver* 2016; **10**: 581-586 [PMID: 27021503 DOI: 10.5009/gnl15324]

140 **Yip C**, Loeb M, Salama S, Moss L, Olde J. Quinolone use as a risk factor for nosocomial Clostridium difficile-associated diarrhea. *Infect Control Hosp Epidemiol* 2001; **22**: 572-575 [PMID: 11732787 DOI: 10.1086/501954]

141 **Walker KJ**, Gilliland SS, Vance-Bryan K, Moody JA, Larsson AJ, Rotschafer JC, Guay DR. Clostridium difficile colonization in residents of long-term care facilities: prevalence and risk factors. *J Am Geriatr Soc* 1993; **41**: 940-946 [PMID: 8104968 DOI: 10.1111/j.1532-5415.1993.tb06759.x]

142 **Surawicz CM**, Brandt LJ, Binion DG, Ananthakrishnan AN, Curry SR, Gilligan PH, McFarland LV, Mellow M, Zuckerbraun BS. Guidelines for diagnosis, treatment, and prevention of Clostridium difficile infections. *Am J Gastroenterol* 2013; **108**: 478-498; quiz 499 [PMID: 23439232 DOI: 10.1038/ajg.2013.4]

143 **Marwick CA**, Yu N, Lockhart MC, McGuigan CC, Wiuff C, Davey PG, Donnan PT. Community-associated Clostridium difficile infection among older people in Tayside, Scotland, is associated with antibiotic exposure and care home residence: cohort study with nested case-control. *J Antimicrob Chemother* 2013; **68**: 2927-2933 [PMID: 23825381 DOI: 10.1093/jac/dkt257]

144 **Chitnis AS**, Holzbauer SM, Belflower RM, Winston LG, Bamberg WM, Lyons C, Farley MM, Dumyati GK, Wilson LE, Beldavs ZG, Dunn JR, Gould LH, MacCannell DR, Gerding DN, McDonald LC, Lessa FC. Epidemiology of community-associated Clostridium difficile infection, 2009 through 2011. *JAMA Intern Med* 2013; **173**: 1359-1367 [PMID: 23780507 DOI: 10.1001/jamainternmed.2013.7056]

145 **Freedberg DE**, Salmasian H, Friedman C, Abrams JA. Proton pump inhibitors and risk for recurrent Clostridium difficile infection among inpatients. *Am J Gastroenterol* 2013; **108**: 1794-1801 [PMID: 24060760 DOI: 10.1038/ajg.2013.333]

146 **Hegarty JP**, Sangster W, Harris LR 3rd, Stewart DB. Proton pump

inhibitors induce changes in colonocyte gene expression that may affect Clostridium difficile infection. *Surgery* 2014; **156**: 972-978 [PMID: 25151556 DOI: 10.1016/j.surg.2014.06.074]

147 **Seto CT**, Jeraldo P, Orenstein R, Chia N, DiBaise JK. Prolonged use of a proton pump inhibitor reduces microbial diversity: implications for Clostridium difficile susceptibility. *Microbiome* 2014; **2**: 42 [PMID: 25426290 DOI: 10.1186/2049-2618-2-42]

148 **Husebye E**, Skar V, Høverstad T, Melby K. Fasting hypochlorhydria with gram positive gastric flora is highly prevalent in healthy old people. *Gut* 1992; **33**: 1331-1337 [PMID: 1446855 DOI: 10.1136/gut.33.10.1331]

149 **Scarpignato C**, Gatta L, Zullo A, Blandizzi C; SIF-AIGO-FIMMG Group; Italian Society of Pharmacology, the Italian Association of Hospital Gastroenterologists, and the Italian Federation of General Practitioners. Effective and safe proton pump inhibitor therapy in acid-related diseases - A position paper addressing benefits and potential harms of acid suppression. *BMC Med* 2016; **14**: 179 [PMID: 27825371 DOI: 10.1186/s12916-016-0718-z]

P- Reviewer: Chiba T, Garcia-Olmo D, Mohammad RN
S-Editor: Wei LJ **L- Editor**: A **E- Editor**: Ma YJ

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v22.i1.369

World J Gastroenterol 2016 January 7; 22(1): 369-378
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

REVIEW

Capsule endoscopy: The road ahead

Ana-Maria Singeap, Carol Stanciu, Anca Trifan

Ana-Maria Singeap, Anca Trifan, Gastroenterology and Hepatology Institute, "Gr. T. Popa" University of Medicine and Pharmacy, "St. Spiridon" Emergency Hospital, 700111 Iasi, Romania

Carol Stanciu, Gastroenterology and Hepatology Institute, "St. Spiridon" Emergency Hospital, 700111 Iasi, Romania

Author contributions: Singeap AM, Stanciu C and Trifan A contributed equally to the conception and design of the review; Singeap AM performed acquisition of data; Stanciu C analyzed the data, coordinated the manuscript drafting and revised it critically; Trifan A participated in the analysis and interpretation of data, and critically revised the manuscript for important intellectual content; all authors read and approved the final version of the manuscript.

Conflict-of-interest statement: There is no conflict of interest associated with any of the senior author or other coauthors contributed their efforts in this manuscript.

Correspondence to: Carol Stanciu, MD, FRCP, Professor, Gastroenterology and Hepatology Institute, "St. Spiridon" Emergency Hospital, Independentei 1, 700111 Iasi, Romania. stanciucarol@yahoo.com
Telephone: +40-732-402860
Fax: +40-232-246611

Received: May 5, 2015
Peer-review started: May 9, 2015
First decision: July 19, 2015
Revised: August 4, 2015
Accepted: September 30, 2015
Article in press: September 30, 2015
Published online: January 7, 2016

Abstract

Since its introduction into clinical practice 15 years ago, capsule endoscopy (CE) has become the first-line investigation procedure in some small bowel pathologies, and more recently, dedicated esophageal and colon CE have expanded the fields of application to include the upper and lower gastrointestinal disorders. During this time, CE has become increasingly popular among gastroenterologists, with more than 2 million capsule examinations performed worldwide, and nearly 3000 PubMed-listed studies on its different aspects published. This huge interest in CE may be explained by its non-invasive nature, patient comfort, safety, and access to anatomical regions unattainable *via* conventional endoscopy. However, CE has several limitations which impede its wider clinical applications, including the lack of therapeutic capabilities, inability to obtain biopsies and control its locomotion. Several research groups are currently working to overcome these limitations, while novel devices able to control capsule movement, obtain high quality images, insufflate the gut lumen, perform chromoendoscopy, biopsy of suspect lesions, or even deliver targeted drugs directly to specific sites are under development. Overlooking current limitations, especially as some of them have already been successfully surmounted, and based on the tremendous progress in technology, it is expected that, by the end of next 15 years, CE able to perform both diagnostic and therapeutic procedures will remain the major form of digestive endoscopy. This review summarizes the literature that prognosticates about the future developments of CE.

Key words: Capsule endoscopy; Biopsy; Drug delivery systems; Capsule endoscope locomotion; Capsule localization

Core tip: Since its introduction into clinical practice 15 years ago, small bowel capsule endoscopy (CE) has

revolutionized direct endoscopic imaging of the gut. During this time, CE has gained tremendous popularity among gastroenterologists, and a vast research pertaining to its different aspects has been published. Dedicated esophageal and colon CE have expanded the field of application to upper and lower gastrointestinal disorders. However, besides its recognized advantages, CE also has several limitations such as the lack of therapeutic capabilities, the inability to obtain biopsies and control its locomotion. Active research is in progress to overcome the current limitations, while the latest advances in CE technology enable us to look forward to a next generation CE capable of performing both diagnostic and therapeutic procedures. This review summarizes the literature that prognosticates about the future of CE.

Singeap AM, Stanciu C, Trifan A. Capsule endoscopy: The road ahead. *World J Gastroenterol* 2016; 22(1): 369-378 Available from: URL: http://www.wjgnet.com/1007-9327/full/v22/i1/369.htm DOI: http://dx.doi.org/10.3748/wjg.v22.i1.369

INTRODUCTION

Fifteen years have passed since small bowel capsule endoscopy (CE) was launched[1], revolutionizing noninvasive direct visualization of the small bowel, considered until then the "black box" of the gastrointestinal (GI) tract. During this time, CE has been used extensively, with more than 2 million capsules swallowed worldwide[2] and nearly 3000 PubMed-listed studies pertaining to its different aspects published[3]. Technical progress led to the introduction of some updated versions (2nd and 3rd generations) of CE for the small bowel and the manufacturing of the CE designed for esophagus and colon. In just a few years, CE has evolved very rapidly, becoming an invaluable tool for examination of almost the entire GI tract, and its diagnostic achievements have by far exceeded early expectations. Still, CE is not an ideal tool, as it has several limitations, including the lack of therapeutic capabilities, inability to control its locomotion and thus, to revisualize critical areas and obtain biopsies. The objective of many research groups worldwide is to overcome these limitations and develop a new generation of CE with higher diagnostic yield and therapeutic capabilities. Of course, it is very difficult to predict the future in medicine, and would be for CE. However, based on the extraordinary developments seen in just 15 years since its emergence, and the tremendous progresses of modern technology, it can be anticipated that, by the end of next 15 years, the new generation of CE able to perform both diagnostic and therapeutic procedures in a noninvasive, painless, and elegant manner will remain the major form of digestive endoscopy, covering the entire GI tract from mouth to anus, as its inventors have dreamed. This review summarizes available literature that prognosticates about the future developments in CE.

BRIEF LOOK BACK AND THE CURRENT STATUS

The first model of CE called M2A (meaning "mouth to anus") was launched in 2000 by Given Diagnostic Imaging, Yoqneam, Israel[4], and the merits for its design belonged, in a similar degree, to the Israeli engineer Gavriel Iddan and the British gastroenterologist Paul Swain[1,4]. A year later, M2A was approved for clinical use in Europe and the United States, and after the advent of esophageal CE, M2A changed its name into PillCam SB (meaning "small bowel"). Several other companies have also developed small bowel endoscopic capsules: EndoCapsule (Olympus Corp., Tokyo, Japan)[5], OMOM capsule (Jinshan Science and Technology Company, Chongqing, China)[6], Mirocam (IntroMedic Co., Seoul, South Korea)[7], and CapsoCam SV1 (CapsoVision, Saratoga, CA, United States)[8], all having many similar characteristics and diagnostic performances to PillCam SB, but differing with regard to image acquisition rate, field of view, battery life, dimensions, and technology for transmission of images. Given Imaging has also developed PillCam ESO and PillCam COLON for the evaluation of esophageal and colonic diseases, respectively[9,10]. Improvements in technology have led to the development of 2nd and 3rd generation CEs which overcome some limitations of the 1st generation CE by increasing the view angle, extending the effective battery life, and including several others systems which offer superior image quality, tissue coverage, and interpretation efficiency[11-13].

In only 15 years since the introduction of CE into clinical practice, its achievements have exceeded what was previously thought as possible. Thus, CE has revolutionized the evaluation of obscure gastrointestinal bleeding (OGIB) and unexplained iron deficiency anemia (IDA)[13-15], becoming the first-line modality in the diagnosis of both. The role of CE in OGIB/IDA is supported mainly by its diagnostic performance, which is superior to other diagnostic modalities (push enteroscopy, intraoperative enteroscopy, small bowel barium radiography, CT-enterography, CT-angiography, MR-enterography), as well as by its positive impact on patient management and outcome[14,16-21]. When CE was compared to double-balloon enteroscopy, a similar diagnostic accuracy for OGIB was reported[22]. CE examination leads to therapeutic endoscopic or surgical interventions and, consequently, to bleeding being stopped and outcomes improved[23,24].

Thanks to its capacity to directly visualize mucosa of the entire small bowel, CE has undoubtedly contributed substantially to progress in diagnosis, therapeutic

decision, and outcome in Crohn's disease (CD). Reviews of existent literature on CE diagnostic yield, for both suspected and known small bowel CD, show it to be superior to other diagnostic techniques such as small bowel follow-through, enteroclysis, push-enteroscopy, ileo-colonoscopy, and CT-enterography[25-27]. CE is superior to MR-enterography in identifying small bowel mucosal lesions, while MR-enterography is superior to CE in diagnosing mural and extra-enteric lesions[28]. In patients with known CD, an important treatment goal is mucosal healing which can be reliably assessed by CE[29-31].

CE has an 8-fold magnification capacity and a minimum size of lesion detection of 0.1-0.2 mm, so that villi can be easily observed during a procedure; therefore, it may be a useful noninvasive diagnostic tool in patients with suspected or established celiac disease[32,33]. However, CE is actually an alternative to endoscopy with biopsy only in patients clinically suspected of celiac disease unable or unwilling to undergo conventional endoscopy.

CE has become the procedure of choice for detecting small bowel polyps in hereditary polyposis syndromes like Peutz-Jegher syndrome and familial adenomatous polyposis[34,35]. In addition, widespread use of CE has more than doubled the diagnosis rate in small bowel tumors[36-41].

Esophageal capsule endoscopy, although at 3rd generation, has limited role in clinical practice and it is still under evaluation[42]. Colon capsule is also under evaluation, and is currently recommended in case of incomplete colonoscopy and in patients unwilling or unable to perform colonoscopy[43,44].

Limitations of current capsule endoscopy

Although CE has seen tremendous advances in a short period of time since its introduction in clinical practice, it has several limitations. Thus, CE remains a purely visual technique with no ability to obtain biopsy specimens or perform therapeutic maneuvers. The most obvious drawback is the operator's inability to control its locomotion through GI tract. The capsules presently on the market are unable to localize or mark the location of detected lesions. Visualization may be impaired by the presence of food materials or bubbles and, in contrast with conventional endoscopy, CE cannot perform flushing, suctioning, or air insufflation to obtain better images. All capsules for clinical use are powered by limited-life batteries which may be depleted before the examination is complete. The rate of missed lesions is still high for those located in the duodenum and proximal jejunum, where the transit is more rapid than in the distal segment of the small bowel. Reading time for interpretation is another shortcoming of CE, as it takes more than 1 h to read a full 8-h examination. Finally, the costs are still high.

FUTURE EXPECTATIONS IN CAPSULE ENDOSCOPY

The future of CE is difficult to predict ("Prediction is very difficult, especially about the future" - Niels Bohr, Nobel Prize winner, 1885-1962), although novel technologies may lead to developments which today seem almost unimaginable. Improvements achieved in just 15 years since the introduction of CE in clinical practice go beyond what was previously thought as possible. GI endoscopy has had a similar history: initially limited only to viewing the esophagus/gastric lumen, it has improved progressively over a few decades, developing into an accurate diagnostic and therapeutic technique. CE also started as a tool for visualizing only the "black box" (small bowel) which has long been the final frontier of the GI endoscopy, and it evolved very rapidly to become a non-invasive endoscopic tool in the examination of almost the entire GI tract.

Most likely, over the next 15 years, CE will slowly replace diagnostic standard endoscopy and take over most therapeutic procedures with no pain and no need for sedation. We know that several research groups throughout the world are working to develop new multifunctional capsules with diagnostic and therapeutic capabilities extending far beyond our imagination. What we do not yet know is whether the future CE will be "universal", containing both diagnostic and therapeutic modules (an "ideal" CE)[45] or "specific", for diagnosis or therapy[11].

Maneuverable capsules

In contrast to standard endoscopy, the movement of the current capsule endoscopes through the GI tract is passive, ensured by peristaltic motion, the operator being unable to control the endoscopic navigation (right and left, back and forth) in a given area. It is of the upmost importance to solving the CE's maneuvering limitation so as to increase its diagnostic yield and allow targeted biopsy and even drug delivery. Besides enhancing diagnostic yield, a capsule whose locomotion can be controlled will reduce the amount of energy consumed, examination time, as well as the rate of capsule retention. Even more, an active control of the endoscopic capsule would allow us to examine the stomach, and finally, the entire GI tract[46].

Systems that can be used to propel or steer the capsule are under development. There are two locomotion systems: an internal one, integrated on-board the capsule, and an external one (outside the capsule), most frequently based on magnetic fields. Some proposed internal systems consist of legged-like mechanisms (propellers/paddles) that can be deployed by the capsule to resist peristaltic movements, while the external locomotion systems usually use a capsule

covered with a magnetic shield which can interact with external magnetic fields created by an electromagnet or permanent magnet. Electromagnets require bulkier equipment by comparison to permanent magnets[47-50].

The legged-like device approach consists of providing the capsule with propellers/paddles which will start functioning on demand during capsule navigation through various segments of the GI tract. A four-legged capsule, two in the front and two in the rear, has been proposed, an eight-legged capsule was also suggested to be feasible, and even a twelve-legged locomotion capsule was designed to improve propulsion and reduce tissue injury[47,51-53]. However, several technical drawbacks such as insufficient space available within the capsule and high power consumption should be overcome. In addition, a failure in the synchronization of the legs may cause damage to the GI tissue.

Magnetic control appears to be the most attractive and promising approach. It is based on the principle that a large external magnetic field created by a permanent magnet or electromagnet near the patient interacts with a small internal magnet component integrated into the capsule to provide an active control of the endoscopic capsule[48]. Given Imaging has incorporated a magnet inside one of the domes of a standard PillCam colon capsule, which can be manipulated with an external handheld magnet moved on the patient's abdomen[54,55]. Using such magnetically maneuverable capsule, one study reported > 75% of gastric mucosa visualized and no adverse events[55]. Siemens (Siemens Medical, Erlangen, Germany) and Olympus (Olympus America, Center Valley, PA, United States) have recently tested the prototype of a magnetically guided capsule endoscope that uses a three-dimensional, external magnetic field which interacts with the magnet inside the capsule, allowing the capsule to be moved forward or backward[56,57]. Rey *et al*[58,59] made the first blinded comparative clinical trial on gastric examination in humans, comparing a magnetically guided capsule endoscope with a conventional high-definition gastroscope, and found a similar diagnostic yield for both methods. Rahman *et al*[60], using the Intromedic MiroCam-Navi system, reported a high degree of visualization, control, and maneuverability with this system. A robotic magnetic navigation system used in cardiology (Niobe, Stereotaxis Inc., St. Louis, MO, United States) has been suggested for CE but has been tested only in plastic phantoms[61]. Several other versions of endoscopic capsules magnetically propelled by a robotic arm have been proposed[62].

Two research projects funded by the European Union aim to develop a self-propelling minirobot pill. One is *VECTOR* (Versatile Endoscopic Capsule for gastrointestinal Tumor Recognition and Therapy) for early diagnosis and treatment of GI cancer[63], and the other is *NEMO* (Nano-based capsule Endoscopy with Molecular imaging and Optical biopsy) which designed to combine optical, nano, and maneuvering technologies in a new capsule with different diagnostic and therapeutic capabilities[64].

A videocapsule endoscope called Compact Photonic Explorer (CPE), measuring 5 mm in size, has been developed at the City University and City College of New York. It can be manipulated externally by remote controlled radio signal and may be used in the future for diagnostic and therapeutic means[65]. Recently, a mathematical model of an electrically propelled capsule endoscopy has been proposed, using double pairs of electrodes, and which is able to move the capsule forward and backward at a speed of 2.91 mm/s and 2.23 mm/s, respectively[66].

To summarize, the development of propelled/steerable capsules will represent a major advance of capsule technology, which will open a myriad of possibilities, including a more detailed evaluation of affected areas and prelevation of biopsy specimens, endoscopic targeted therapy, examination of the stomach, thus the entire GI tract becoming virtually as accessible as the skin[67]!

Biopsy

Once a maneuverable capsule is developed, the next step is to obtain a tissue sample. Several biopsy devices have been developed and used on animal models. A spring-loaded device similar to the Crosby capsule, guided by real-time imaging and RF-controlled remote manipulation, and a capsule using Micro-Electro-Mechanical-Systems (MEMS) technology have been successfully tested[68]. Both NEMO and VECTOR projects develop capsules designed for virtual biopsies and drug delivery[63,64]. The rotational Micro Biopsy Capsule Device (Seoul, South Korea) which contains a triggering part with paraffin block and a rotational tissue-cutting razor (biopsy part) has been tested[69]. A tethered capsule endomicroscopy of the esophagus, which uses optical frequency domain imaging technology and enables 3D imaging of esophagus in microscopic detail, has also been developed[70]. This capsule endomicroscope is able to differentiate Barrett's esophagus from normal esophageal mucosa. Other magnetic capsules using untethered microgrippers to grab tissue samples or magnetic torsion spring mechanism have been designed[71,72].

Optical enhancing techniques could lead to optical "biopsy", which refers to a method of obtaining a morphological diagnosis without biopsy specimens, and prototype endoscopic capsules with such technology have been developed, including the wireless spectroscopic compact photonic explorer for diagnostic optical imaging to detect microscopic malignancy[65]. One research group integrated near-infrared fluorescent probe in CE to enhance optical diagnosis of neoplasia, which proved able to distinguish adenomatous tissue in

experimental colitis in mice[73].

Power source

At present, available endoscopic capsules use two coin-shaped, silver-oxide batteries that can generate 20 mW of energy, far too little to accomplish the multiple diagnostic and therapeutic tasks of the future capsule, most of them requiring power consumption. In addition, batteries occupy most of the space in an endoscopic capsule. Therefore, increased power supply and reduced size of batteries, to leave enough space to incorporate diagnostic/therapeutic components into the capsule, are essential for further developments in CE technology. A solution may be lithium ion microbattery technology which could provide a power density up to 2000-times higher than other microbatteries[74]. Recently, Rathore *et al*[75] using Ultrascale FinFET 16 nm technology for manufacturing endoscopic capsules (instead of 18 μm used for conventional endoscopic capsules) have reported an increased battery life, reduced power consumption with up to 50%, and a reduced size of the capsule by 12% compared to traditional capsules. An alternative method to reduce battery consumption is to use low complexity video compression technology that saves radiofrequency (RF) transmitting power[76].

External rechargeable batteries (from an extra-corporeal power supply) using RF, microwave or electric induction, and even "battery free" CE using wireless power transmission (WPT) technology are created. An excellent overview of the development of emergency WPT technique for application in CE has been recently published by Basar *et al*[77]. WPT system employs a transmitting coil positioned outside the human body and a receiving coil installed within the CE, thereby eliminating the need for an internal battery[78]. Thus, the RF System Lab (Nagano, Japan) was the first to use WPT technology in their Sayaka and Norika capsules[79], and several publications centered on WPT technology for the endoscopic capsule[77,80]. Jia *et al*[80], using WPT technology, have reported on its ability to transmit 500 mW of electricity, which is significantly higher than the amount generated by current batteries used for the endoscopic capsules available on the market.

An alternative solution will be the development of three-dimensional microbattery technology for geometrical energy and power density of battery[81,82], and many research groups are working in the field, still progressing in several laboratories.

Targeted drug delivery

Unfortunately, none of the current capsules is able to perform therapy. New capsule devices are under development in order to enable drug delivery in specific diseased areas of the GI tract. A number of clinical situations can benefit from targeted drug delivery such as the use of hemostatic spray to an active bleeding lesion or localized application of steroid/immunomodulation for CD. One capsule prototype is able to deliver an injection of 1 mL of targeted medication while using a holding mechanism[83]. To achieve this, an accurate control mechanism of capsule positioning and a drug release mechanism should be incorporated into a capsule endoscope. As future capsules will most likely be smaller, space limitation within the capsule is an important impediment when incorporating such mechanisms[84].

Philips company (Philips Research, Eindhoven, The Netherlands) has launched an "intelligent" pill (iPill) measuring 11 mm × 26 mm and incorporating a microprocessor, battery, pH sensor, temperature sensor, radiofrequency transceiver, fluid pump, and a drug reservoir[85]. Tracking of the iPill in the GI tract is based on information regarding pH change and gut transit time. Once tracked in the aimed area, the iPill will open and deliver the drug under the control of the microprocessor. The iPill is under trial in CD and colorectal cancer[68,85,86].

Several other wireless capsules, such as the Gastro-target telemetric capsule (Gastrotarget, Tonawanda, NY, United States), High-frequency capsule (Battelle-Institute V, Frankfurt, Germany), Telemetric capsule (INSERM UG1, Strasbourg, Cedex, France), Enterion capsule (Pheaton Research, Nottingham, United Kingdom), and the IntelliSite capsule (Innovative Devices, Raleigh, NC, United States), have been developed for targeted drug delivery in specific areas of the GI tract[68]. However, capsule tracking is inaccurate due to lack of an anchoring mechanism and thus, drug release cannot be fully controlled. Two therapeutic capsule endoscopes have recently been proposed for bioadhesive patch release and targeted drug delivery, respectively, both capsules being controlled by an external permanent magnetic source[83,87]. A soft magnetically actuated capsule, capable of multimodal gradual or sudden drug release, has also been developed[88].

Even with a new CE designed for targeted drug delivery, several other problems should be taken into consideration. Thus, in some diseases of GI tract such as CD, drug delivery is required on a daily basis, for several days or even weeks. To overcome this problem, a pre-programmed non-viewing capsule for targeted drug delivery has been proposed[89].

Luminal insufflation

CE visualization, especially of the colonic mucosa, is limited as the capsule is unable to provide insufflation in order to distend the intestine and expose all mucosal surfaces for examination. This shortcoming is a potential cause for CE's high false-negative diagnostic rate in the colon. Experiments and insufflation capsule prototypes show the feasibility of generating large volumes of gas from a small volume of liquid hydrogen peroxide, weak acids and bases in a capsule to provide wireless insufflation for enhancing visualization[90,91].

Recently, a method of controlled colonic insufflation (CO_2) *via* an untethered capsule *in vivo* has been reported[67].

Shorter reading time

Future CE should allow shorter reading time for interpretation of images acquired by capsule, and this may be achieved by development of more efficient software[92]. A computer-aided lesion detection will significantly reduce reading time.

Home procedure

In the near future, CE (small bowel capsule endoscopy and especially colon capsule endoscopy) will become a home procedure that could be done on weekends, thus avoiding work absence[93,94].

Accurate location of detected lesion

A tagging module consisting of a micro tag, compressed spring and thermal ignitor can be integrated within future CEs; when activated by an external signal, the micro tag is impaled into the mucosa to mark the precise location of a lesion for the following endoscopic therapy[95]. Location of lesion and estimation of its size is possible by using Rapid 6 system of software developed by Given Imaging[96].

Automated capsule localization

Automated capsule localization with a software using color image analysis to discriminate between different segments of GI tract (esophagus, stomach, small bowel, colon) identified CE passage across the pylorus in 93% of cases[97-99]. The next step will be development of the software program to increase the frame rate while CE is traversing the duodenum, in order to improve identification of the ampulla of Vater and detect more lesions in the periampullary region[100,101] which is poorly visualized by CE, CT- and MR-enterography[102,103].

Entire GI tract visualization

An ideal CE would be able to visualize the entire GI tract, from mouth to anus, during a single procedure. Currently available capsules cannot be used for this purpose because of the significant physiological differences of the various segments of the GI tract, and therefore, only specific esophageal, small bowel, and colon capsules are available. However, the colon capsule (PillCam COLON 2, Given Imaging) developed for evaluation of the colon, can also be used to visualize almost the entire GI tract. This capsule is provided with two cameras able to record video images from both ends, with an adaptive frame acquisition rate (between 4 and 35 frames per second). Thus, it may visualize the esophagus, examine the stomach and duodenum with an external maneuvering system to control capsule locomotion, then the small intestine and, finally, the colon. Preliminary studies have already concluded that GI tract evaluation with PillCam COLON 2 is feasible, especially for small bowel, although other segments (esophagus, stomach) need technical improvements to obtain a good visualization[46]. In the near future, a pan-endoscopy with CE may be a reality[29,104,105].

CONCLUSION

Undoubtedly, CE has opened a new era in endoscopic diagnosis for gastroenterologists and has set a milestone in the evolution of endoscopic examination of the GI tract without discomfort or need for sedation, or the risks implied by conventional endoscopy. During a relatively short period of time (15 years), CE has proven its high diagnostic yield in multiple pathologies of the GI tract such as obscure GI bleeding, CD, celiac disease, as well as in small and large bowel tumors. Nevertheless, the endoscopic capsules currently available are diagnostic tools only, and still have several limitations (passive locomotion, inability to perform biopsy or deliver therapy, *etc*). Modern technology continues to make tremendous progress in CE, helping it overcome the above mentioned limitations. Although it is difficult to make predictions about the future, we believe that in the next 15 years, our dreams of an efficient diagnostic and therapeutic CE for the diverse pathologies of the entire GI tract will become a reality.

REFERENCES

1 **Iddan G**, Meron G, Glukhovsky A, Swain P. Wireless capsule endoscopy. *Nature* 2000; **405**: 417 [PMID: 10839527 DOI: 10.1038/35013140]

2 **Eliakim R**. Video capsule endoscopy of the small bowel. *Curr Opin Gastroenterol* 2013; **29**: 133-139 [PMID: 23221650 DOI: 10.1097/MOG.0b013e32835bdc03]

3 **Koulaouzidis A**, Rondonotti E, Karargyris A. Small-bowel capsule endoscopy: a ten-point contemporary review. *World J Gastroenterol* 2013; **19**: 3726-3746 [PMID: 23840112 DOI: 10.3748/wjg.v19.i24.3726]

4 **Iddan GJ**, Swain CP. History and development of capsule endoscopy. *Gastrointest Endosc Clin N Am* 2004; **14**: 1-9 [PMID: 15062374 DOI: 10.1016/j.giec.2003.10.022]

5 **Rey JF**, Kuznetsov K, Vazquez-Ballesteros E. Olympus capsule endoscope for small and large bowel exploration. *Gastrointest Endosc* 2006; **63**: AB176 [DOI: 10.1016/j.gie.2006.03.381]

6 **Li CY**, Zhang BL, Chen CX, Li YM. OMOM capsule endoscopy in diagnosis of small bowel disease. *J Zhejiang Univ Sci B* 2008; **9**: 857-862 [PMID: 18988304 DOI: 10.1631/jzus.B0820034]

7 **Bang S**, Park JY, Jeong S, Kim YH, Shim HB, Kim TS, Lee DH, Song SY. First clinical trial of the "MiRo" capsule endoscope by using a novel transmission technology: electric-field propagation. *Gastrointest Endosc* 2009; **69**: 253-259 [PMID: 18640676 DOI: 10.1016/j.gie.2008.04.033]

8 **Friedrich K**, Gehrke S, Stremmel W, Sieg A. First clinical trial of a newly developed capsule endoscope with panoramic side view for small bowel: a pilot study. *J Gastroenterol Hepatol* 2013; **28**: 1496-1501 [PMID: 23701674]

9 **Eliakim R**, Yassin K, Shlomi I, Suissa A, Eisen GM. A novel diagnostic tool for detecting oesophageal pathology: the PillCam oesophageal video capsule. *Aliment Pharmacol Ther* 2004; **20**: 1083-1089 [PMID:

15569110 DOI: 10.1111/j.1365-2036.2004.02206.x]
10 **Eliakim R**, Fireman Z, Gralnek IM, Yassin K, Waterman M, Kopelman Y, Lachter J, Koslowsky B, Adler SN. Evaluation of the PillCam Colon capsule in the detection of colonic pathology: results of the first multicenter, prospective, comparative study. *Endoscopy* 2006; **38**: 963-970 [PMID: 17058158 DOI: 10.1055/s-2006-944832]
11 **Eliakim R**. Capsule endoscopy - Where are we at 2011 and where are we headed? *Intest Res* 2012; **10**: 235-243 [DOI: 10.5217/jr.2012.10.3.235]
12 **Spada C**, Hassan C, Galmiche JP, Neuhaus H, Dumonceau JM, Adler S, Epstein O, Gay G, Pennazio M, Rex DK, Benamouzig R, de Franchis R, Delvaux M, Devière J, Eliakim R, Fraser C, Hagenmuller F, Herrerias JM, Keuchel M, Macrae F, Munoz-Navas M, Ponchon T, Quintero E, Riccioni ME, Rondonotti E, Marmo R, Sung JJ, Tajiri H, Toth E, Triantafyllou K, Van Gossum A, Costamagna G. Colon capsule endoscopy: European Society of Gastrointestinal Endoscopy (ESGE) Guideline. *Endoscopy* 2012; **44**: 527-536 [PMID: 22389230 DOI: 10.1055/s-0031-1291717]
13 **Wang A**, Banerjee S, Barth BA, Bhat YM, Chauhan S, Gottlieb KT, Konda V, Maple JT, Murad F, Pfau PR, Pleskow DK, Siddiqui UD, Tokar JL, Rodriguez SA. Wireless capsule endoscopy. *Gastrointest Endosc* 2013; **78**: 805-815 [PMID: 24119509 DOI: 10.1016/j.gie.2013.06.026]
14 **Appleyard M**, Glukhovsky A, Swain P. Wireless-capsule diagnostic endoscopy for recurrent small-bowel bleeding. *N Engl J Med* 2001; **344**: 232-233 [PMID: 11188844 DOI: 10.1056/NEJM200101183440316]
15 **Kopylov U**, Seidman EG. Clinical applications of small bowel capsule endoscopy. *Clin Exp Gastroenterol* 2013; **6**: 129-137 [PMID: 23983481 DOI: 10.2147/CEG.S48005]
16 **Laine L**, Sahota A, Shah A. Does capsule endoscopy improve outcomes in obscure gastrointestinal bleeding? Randomized trial versus dedicated small bowel radiography. *Gastroenterology* 2010; **138**: 1673-1680.e1; quiz e11-12 [PMID: 20138043 DOI: 10.1053/j.gastro.2010.01.047]
17 **Triester SL**, Leighton JA, Leontiadis GI, Fleischer DE, Hara AK, Heigh RI, Shiff AD, Sharma VK. A meta-analysis of the yield of capsule endoscopy compared to other diagnostic modalities in patients with obscure gastrointestinal bleeding. *Am J Gastroenterol* 2005; **100**: 2407-2418 [PMID: 16279893 DOI: 10.1111/j.1572-0241.2005.00274.x]
18 **Saperas E**, Dot J, Videla S, Alvarez-Castells A, Perez-Lafuente M, Armengol JR, Malagelada JR. Capsule endoscopy versus computed tomographic or standard angiography for the diagnosis of obscure gastrointestinal bleeding. *Am J Gastroenterol* 2007; **102**: 731-737 [PMID: 17397406 DOI: 10.1111/j.1572-0241.2007.01058.x]
19 **Koulaouzidis A**, Rondonotti E, Giannakou A, Plevris JN. Diagnostic yield of small-bowel capsule endoscopy in patients with iron-deficiency anemia: a systematic review. *Gastrointest Endosc* 2012; **76**: 983-992 [PMID: 23078923 DOI: 10.1016/j.gie.2012.07.035]
20 **Hartmann D**, Schmidt H, Bolz G, Schilling D, Kinzel F, Eickhoff A, Huschner W, Möller K, Jakobs R, Reitzig P, Weickert U, Gellert K, Schultz H, Guenther K, Hollerbuhl H, Schoenleben K, Schulz HJ, Riemann JF. A prospective two-center study comparing wireless capsule endoscopy with intraoperative enteroscopy in patients with obscure GI bleeding. *Gastrointest Endosc* 2005; **61**: 826-832 [PMID: 15933683 DOI: 10.1016/S0016-5107(05)00372-X]
21 **de Leusse A**, Vahedi K, Edery J, Tiah D, Fery-Lemonnier E, Cellier C, Bouhnik Y, Jian R. Capsule endoscopy or push enteroscopy for first-line exploration of obscure gastrointestinal bleeding? *Gastroenterology* 2007; **132**: 855-862; quiz 1164-1165 [PMID: 17324401 DOI: 10.1053/j.gastro.2006.12.002]
22 **Pasha SF**, Leighton JA, Das A, Harrison ME, Decker GA, Fleischer DE, Sharma VK. Double-balloon enteroscopy and capsule endoscopy have comparable diagnostic yield in small-bowel disease: a meta-analysis. *Clin Gastroenterol Hepatol* 2008; **6**: 671-676 [PMID: 18356113 DOI: 10.1016/j.cgh.2008.01.005]
23 **Cañas-Ventura A**, Márquez L, Bessa X, Dedeu JM, Puigvehí M, Delgado-Aros S, Ibáñez IA, Seoane A, Barranco L, Bory F, Andreu M, González-Suárez B. Outcome in obscure gastrointestinal bleeding after capsule endoscopy. *World J Gastrointest Endosc* 2013; **5**: 551-558 [PMID: 24255747 DOI: 10.4253/wjge.v5.i11.551]
24 **Min YW**, Kim JS, Jeon SW, Jeen YT, Im JP, Cheung DY, Choi MG, Kim JO, Lee KJ, Ye BD, Shim KN, Moon JS, Kim JH, Hong SP, Chang DK. Long-term outcome of capsule endoscopy in obscure gastrointestinal bleeding: a nationwide analysis. *Endoscopy* 2014; **46**: 59-65 [PMID: 24254387 DOI: 10.1055/s-0033-1358803]
25 **Leighton JA**, Gralnek IM, Cohen SA, Toth E, Cave DR, Wolf DC, Mullin GE, Ketover SR, Legnani PE, Seidman EG, Crowell MD, Bergwerk AJ, Peled R, Eliakim R. Capsule endoscopy is superior to small-bowel follow-through and equivalent to ileocolonoscopy in suspected Crohn's disease. *Clin Gastroenterol Hepatol* 2014; **12**: 609-615 [PMID: 24075891 DOI: 10.1016/j.cgh.2013.09.028]
26 **Triester SL**, Leighton JA, Leontiadis GI, Gurudu SR, Fleischer DE, Hara AK, Heigh RI, Shiff AD, Sharma VK. A meta-analysis of the yield of capsule endoscopy compared to other diagnostic modalities in patients with non-stricturing small bowel Crohn's disease. *Am J Gastroenterol* 2006; **101**: 954-964 [PMID: 16696781 DOI: 10.1111/j.1572-0241.2006.00506.x]
27 **Dionisio PM**, Gurudu SR, Leighton JA, Leontiadis GI, Fleischer DE, Hara AK, Heigh RI, Shiff AD, Sharma VK. Capsule endoscopy has a significantly higher diagnostic yield in patients with suspected and established small-bowel Crohn's disease: a meta-analysis. *Am J Gastroenterol* 2010; **105**: 1240-1248; quiz 1249 [PMID: 20029412 DOI: 10.1038/ajg.2009.713]
28 **Crook DW**, Knuesel PR, Froehlich JM, Eigenmann F, Unterweger M, Beer HJ, Kubik-Huch RA. Comparison of magnetic resonance enterography and video capsule endoscopy in evaluating small bowel disease. *Eur J Gastroenterol Hepatol* 2009; **21**: 54-65 [PMID: 19086147 DOI: 10.1097/MEG.0b013e32830ce7a7]
29 **Hall B**, Holleran G, McNamara D. Current applications and potential future role of wireless capsule technology in Crohn's disease. *Scand J Gastroenterol* 2014; **49**: 1275-1284 [PMID: 25260016 DOI: 10.3109/00365521.2014.962606]
30 **Cotter J**, Dias de Castro F, Moreira MJ, Rosa B. Tailoring Crohn's disease treatment: the impact of small bowel capsule endoscopy. *J Crohns Colitis* 2014; **8**: 1610-1615 [PMID: 24631311 DOI: 10.1016/j.crohns.2014.02.018]
31 **Niv E**, Fishman S, Kachman H, Arnon R, Dotan I. Sequential capsule endoscopy of the small bowel for follow-up of patients with known Crohn's disease. *J Crohns Colitis* 2014; **8**: 1616-1623 [PMID: 24666976 DOI: 10.1016/j.crohns.2014.03.003]
32 **Rondonotti E**, Spada C, Cave D, Pennazio M, Riccioni ME, De Vitis I, Schneider D, Sprujevnik T, Villa F, Langelier J, Arrigoni A, Costamagna G, de Franchis R. Video capsule enteroscopy in the diagnosis of celiac disease: a multicenter study. *Am J Gastroenterol* 2007; **102**: 1624-1631 [PMID: 17459022 DOI: 10.1111/j.1572-0241.2007.01238.x]
33 **Rokkas T**, Niv Y. The role of video capsule endoscopy in the diagnosis of celiac disease: a meta-analysis. *Eur J Gastroenterol Hepatol* 2012; **24**: 303-308 [PMID: 22266837 DOI: 10.1097/MEG.0b013e32834fa914]
34 **Brown G**, Fraser C, Schofield G, Taylor S, Bartram C, Phillips R, Saunders B. Video capsule endoscopy in peutz-jeghers syndrome: a blinded comparison with barium follow-through for detection of small-bowel polyps. *Endoscopy* 2006; **38**: 385-390 [PMID: 16680639]
35 **Koornstra JJ**. Small bowel endoscopy in familial adenomatous polyposis and Lynch syndrome. *Best Pract Res Clin Gastroenterol* 2012; **26**: 359-368 [PMID: 22704577 DOI: 10.1016/j.bpg.2012.01.022]
36 **Schwartz GD**, Barkin JS. Small-bowel tumors detected by wireless capsule endoscopy. *Dig Dis Sci* 2007; **52**: 1026-1030 [PMID: 17380403 DOI: 10.1007/s10620-006-9483-8]

37 **Urgesi R**, Riccioni ME, Bizzotto A, Cianci R, Spada C, Pelecca G, Ricci R, Costamagna G. Increased diagnostic yield of small bowel tumors with PillCam: the role of capsule endoscopy in the diagnosis and treatment of gastrointestinal stromal tumors (GISTs). Italian single-center experience. *Tumori* 2012; **98**: 357-363 [PMID: 22825512 DOI: 10.1700/1125.12405]

38 **Girelli CM**, Porta P, Colombo E, Lesinigo E, Bernasconi G. Development of a novel index to discriminate bulge from mass on small-bowel capsule endoscopy. *Gastrointest Endosc* 2011; **74**: 1067-1074; quiz 1115.e1-5 [PMID: 21907982 DOI: 10.1016/j.gie.2011.07.022]

39 **Rondonotti E**, Pennazio M, Toth E, Menchen P, Riccioni ME, De Palma GD, Scotto F, De Looze D, Pachofsky T, Tacheci I, Havelund T, Couto G, Trifan A, Kofokotsios A, Cannizzaro R, Perez-Quadrado E, de Franchis R. Small-bowel neoplasms in patients undergoing video capsule endoscopy: a multicenter European study. *Endoscopy* 2008; **40**: 488-495 [PMID: 18464193 DOI: 10.1055/s-2007-995783]

40 **Cheung DY**, Lee IS, Chang DK, Kim JO, Cheon JH, Jang BI, Kim YS, Park CH, Lee KJ, Shim KN, Ryu JK, Do JH, Moon JS, Ye BD, Kim KJ, Lim YJ, Choi MG, Chun HJ. Capsule endoscopy in small bowel tumors: a multicenter Korean study. *J Gastroenterol Hepatol* 2010; **25**: 1079-1086 [PMID: 20594222 DOI: 10.1111/j.1440-1746.2010.06292.x]

41 **Trifan A**, Singeap AM, Cojocariu C, Sfarti C, Stanciu C. Small bowel tumors in patients undergoing capsule endoscopy: a single center experience. *J Gastrointestin Liver Dis* 2010; **19**: 21-25 [PMID: 20361070]

42 **Nakamura T**, Terano A. Capsule endoscopy: past, present, and future. *J Gastroenterol* 2008; **43**: 93-99 [PMID: 18306982 DOI: 10.1007/s00535-007-2153-6]

43 **Spada C**, Hassan C, Barbaro B, Iafrate F, Cesaro P, Petruzziello L, Minelli Grazioli L, Senore C, Brizi G, Costamagna I, Alvaro G, Iannitti M, Salsano M, Ciolina M, Laghi A, Bonomo L, Costamagna G. Colon capsule versus CT colonography in patients with incomplete colonoscopy: a prospective, comparative trial. *Gut* 2015; **64**: 272-281 [PMID: 24964317 DOI: 10.1136/gutjnl-2013-306550]

44 **Negreanu L**, Babiuc R, Bengus A, Sadagurschi R. PillCam Colon 2 capsule in patients unable or unwilling to undergo colonoscopy. *World J Gastrointest Endosc* 2013; **5**: 559-567 [PMID: 24255748 DOI: 10.4253/wjge.v5.i11.559]

45 **Roberts-Thomson IC**, Singh R, Teo E, Nguyen NQ, Lidums I. The future of endoscopy. *J Gastroenterol Hepatol* 2010; **25**: 1051-1057 [PMID: 20594218 DOI: 10.1111/j.1440-1746.2010.06333.x]

46 **Remes-Troche JM**, Jiménez-García VA, García-Montes JM, Hergueta-Delgado P, Roesch-Dietlen F, Herrerías-Gutiérrez JM. Application of colon capsule endoscopy (CCE) to evaluate the whole gastrointestinal tract: a comparative study of single-camera and dual-camera analysis. *Clin Exp Gastroenterol* 2013; **6**: 185-192 [PMID: 24068872 DOI: 10.2147/CEG.S45215]

47 **Quirini M**, Menciassi A, Scapellato S, Dario P, Rieber F, Ho CN, Schostek S, Schurr MO. Feasibility proof of a legged locomotion capsule for the GI tract. *Gastrointest Endosc* 2008; **67**: 1153-1158 [PMID: 18513557 DOI: 10.1016/j.gie.2007.11.052]

48 **Sliker LJ**, Ciuti G. Flexible and capsule endoscopy for screening, diagnosis and treatment. *Expert Rev Med Devices* 2014; **11**: 649-666 [PMID: 25148269 DOI: 10.1586/17434440.2014.941809]

49 **Kim HM**, Yang S, Kim J, Park S, Cho JH, Park JY, Kim TS, Yoon ES, Song SY, Bang S. Active locomotion of a paddling-based capsule endoscope in an in vitro and in vivo experiment (with videos). *Gastrointest Endosc* 2010; **72**: 381-387 [PMID: 20497903 DOI: 10.1016/j.gie.2009.12.058]

50 **Ciuti G**, Menciassi A, Dario P. Capsule endoscopy: from current achievements to open challenges. *IEEE Rev Biomed Eng* 2011; **4**: 59-72 [PMID: 22273791 DOI: 10.1109/RBME.2011.2171182]

51 **Quirini M**, Menciassi A, Scapellato S, Stefanini C, Dario P. Design and fabrication of a motor legged capsule for the active exploration of the gastrointestinal tract. *IEEE/ASME Trans Mechatron* 2008; **13**: 169-179 [DOI: 10.11009/TMECH.2008.918491]

52 **Valdastri P**, Webster RJ, Quaglia C, Quirini M, Menciassi A, Dario P. A new mechanism for mesoscale legged locomotion in compliant tubular environments. *IEEE Trans Robot* 2009; **25**: 1047-1057 [DOI: 10.1109/TRO.2009.2014127]

53 **Quaglia C**, Buselli E, Webster RJ, Valdastri P, Menciassi A, Dario P. An endoscopic capsule robot: A meso-scale engineering case study. *J Micromech Microeng* 2009; **19**: 1-11 [DOI: 10.1088/0960-1317/19/10/105007]

54 **Swain P**, Toor A, Volke F, Keller J, Gerber J, Rabinovitz E, Rothstein RI. Remote magnetic manipulation of a wireless capsule endoscope in the esophagus and stomach of humans (with videos). *Gastrointest Endosc* 2010; **71**: 1290-1293 [PMID: 20417507 DOI: 10.1016/j.gie.2010.01.064]

55 **Keller J**, Fibbe C, Volke F, Gerber J, Mosse AC, Reimann-Zawadzki M, Rabinovitz E, Layer P, Schmitt D, Andresen V, Rosien U, Swain P. Inspection of the human stomach using remote-controlled capsule endoscopy: a feasibility study in healthy volunteers (with videos). *Gastrointest Endosc* 2011; **73**: 22-28 [PMID: 21067740 DOI: 10.1016/j.gie.2010.08.053]

56 **Rey JF**, Ogata H, Hosoe N, Ohtsuka K, Ogata N, Ikeda K, Aihara H, Pangtay I, Hibi T, Kudo S, Tajiri H. Feasibility of stomach exploration with a guided capsule endoscope. *Endoscopy* 2010; **42**: 541-545 [PMID: 20593331 DOI: 10.1055/s-0030-1255521]

57 **Siemens and Olympus Healthcare**. Magnetically guided capsule endoscope system for comfortable examination of the stomach - More than 50 participants in the fisrt successful study. Available from: URL: http://www.siemens.com/press/en/materials/healthcare/2010-10-Kapselendoskopie.php

58 **Rey JF**. The future of capsule endoscopy. *Keio J Med* 2013; **62**: 41-46 [PMID: 23708295 DOI: 10.2302/kjm.2012-0011-RE]

59 **Rey JF**, Ogata H, Hosoe N, Ohtsuka K, Ogata N, Ikeda K, Aihara H, Pangtay I, Hibi T, Kudo SE, Tajiri H. Blinded nonrandomized comparative study of gastric examination with a magnetically guided capsule endoscope and standard videoendoscope. *Gastrointest Endosc* 2012; **75**: 373-381 [PMID: 22154417 DOI: 10.1016/j.gie.2011.09.030]

60 **Rahman I**, Pioche M, Shim CS, Sung IK, Saurin JC, Patel P. Magnet assisted capsule endoscopy (MACE) in the upper GI tract is feasible: first human series using the novel Mirocam-Navi System. *Gastrointest Endosc* 2014; **79**: AB122 [DOI: 10.1016/j.gie.2014.02.059]

61 **Carpi F**, Pappone C. Stereotaxis Niobe magnetic navigation system for endocardial catheter ablation and gastrointestinal capsule endoscopy. *Expert Rev Med Devices* 2009; **6**: 487-498 [PMID: 19751121 DOI: 10.1586/erd.09.32]

62 **Ciuti G**, Donlin R, Valdastri P, Arezzo A, Menciassi A, Morino M, Dario P. Robotic versus manual control in magnetic steering of an endoscopic capsule. *Endoscopy* 2010; **42**: 148-152 [PMID: 20017088 DOI: 10.1055/s-0029-1243808]

63 **Schostek S**, Schurr MO. European research on wireless endoscopy--the VECTOR project. *Stud Health Technol Inform* 2013; **189**: 193-199 [PMID: 23739381]

64 **Community Research and Development Information Service**. Nano based capsule-Endoscopy with molecular imaging and optical biopsy. Available from: URL: http://www.cordis.europa.eu/project/rcn/84969_en.html

65 **Wang L**, Zhang G, Luo JC, Zeng F, Wang QZ, Alfano SA, Katz A, Zevallos M, Alfano RR. Wireless spectroscopic compact photonic explorer for diagnostic optical imaging. *Biomed Microdevices* 2005; **7**: 111-115 [PMID: 15940423 DOI: 10.1007/s10544-005-1588-x]

66 **Woo SH**, Kim TW, Mohy-Ud-Din Z, Park IY, Cho JH. Small intestinal model for electrically propelled capsule endoscopy. *Biomed Eng Online* 2011; **10**: 108 [PMID: 22177218 DOI: 10.1186/1475-925X-10-108]

67 **Pasricha T**, Smith BF, Mitchell VR, Fang B, Brooks ER, Gerding JS, Washington MK, Valdastri P, Obstein KL. Controlled colonic insufflation by a remotely triggered capsule for improved mucosal

visualization. *Endoscopy* 2014; **46**: 614-618 [PMID: 24845802 DOI: 10.1055/s-0034-1365497]
68 **Sharma VK**. The future is wireless: advances in wireless diagnostic and therapeutic technologies in gastroenterology. *Gastroenterology* 2009; **137**: 434-439 [PMID: 19545570 DOI: 10.1053/j.gastro.2009.06.029]
69 **Kong K**, Cha J, Jeon D, Cho D. A rotational micro biopsy device for the capsule endoscope. In: IROS 2005: IEEE/RSJ International Conference on Intelligent Robots and Systems. Alberta, Canada: Edmonton, 2005: 1839-1843
70 **Ray K**. Endoscopy: Tethered capsule endomicroscopy of the oesophagus--an easy pill to swallow. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 129 [PMID: 23358395 DOI: 10.1038/nrgastro.2013.15]
71 **Yim S**, Gultepe E, Gracias DH, Sitti M. Biopsy using a magnetic capsule endoscope carrying, releasing, and retrieving untethered microgrippers. *IEEE Trans Biomed Eng* 2014; **61**: 513-521 [PMID: 24108454]
72 **Simi M**, Gerboni G, Menciassi A, Valdastri P. Magnetic torsion spring mechanism for a wireless biopsy capsule. *J Med Device* 2013; **7**: 041009-9 [DOI: 10.1115/1.4025185]
73 **Zhang H**, Morgan D, Cecil G, Burkholder A, Ramocki N, Scull B, Lund PK. Biochromoendoscopy: molecular imaging with capsule endoscopy for detection of adenomas of the GI tract. *Gastrointest Endosc* 2008; **68**: 520-527 [PMID: 18499106 DOI: 10.1016/j.gie.2008.02.023]
74 **Pikul JH**, Gang Zhang H, Cho J, Braun PV, King WP. High-power lithium ion microbatteries from interdigitated three-dimensional bicontinuous nanoporous electrodes. *Nat Commun* 2013; **4**: 1732 [PMID: 23591899 DOI: 10.1038/ncomms2747]
75 **Rathore P**, Tiwary D, Kumar N. A methodology for very low power and small size capsule manufacturing for wireless capsule endoscopy. *Int J Comput Appl T* 2014; 95: 10-14 [DOI: 10.5120/16725-6905]
76 **Wu J**, Li Y. Low-complexity video compression for capsule endoscope based on compressed sensing theory. *Conf Proc IEEE Eng Med Biol Soc* 2009; **2009**: 3727-3730 [PMID: 19965013 DOI: 10.1109/IEMBS.2009.5334819]
77 **Basar MR**, Ahmad MY, Cho J, Ibrahim F. Application of wireless power transmission systems in wireless capsule endoscopy: an overview. *Sensors* (Basel) 2014; **14**: 10929-10951 [PMID: 24949645 DOI: 10.3390/s140610929]
78 **Goodman RR**, Torres RA, McMurtry JG. Acoustic schwannoma and epidermoid cyst occurring as a single cerebellopontine angle mass. *Neurosurgery* 1991; **28**: 433-436 [PMID: 2011228 DOI: 10.1002/rcs.298]
79 **Donnelly PK**, Simpson AR. Cytotoxic cross-matching for organ transplantation. *Lancet* 1991; **337**: 1553-1554 [PMID: 1675410 DOI: 10.1080/13645700310014933]
80 **Jia Z**, Yan G, Liu H, Wang Z, Jiang P, Shi Y. The optimization of wireless power transmission: design and realization. *Int J Med Robot* 2012; **8**: 337-347 [PMID: 22508580 DOI: 10.1002/rcs.1428]
81 **Basar MR**, Malek F, Juni Khairudi M, Shaharom Idris M, Iskandar M. Saleh M. Ingestible Wireless Capsule Technology: A Review of Development and Future Indication. *Inter J of Antenna and Propagation* 2012; **2012**: 807165 [DOI: 10.1155/2012/807165]
82 **Roberts M**, Johns P, Owen J, Brandell D, Edstrom K, El Enany G, Guery C, Golodnitsky D, Lacey M, Lecoeur C, Mazor H, Peled E, Perre E, Shaijumon M, Simon P, Taberna PL. 3D lithium ion batteries - from fundamentals to fabrication. *J Mater Chem* 2011; **21**: 9876-9890
83 **Woods SP**, Constandinou TG. Wireless capsule endoscope for targeted drug delivery: mechanics and design considerations. *IEEE Trans Biomed Eng* 2013; **60**: 945-953 [PMID: 23192476 DOI: 10.1109/TBME.2012.2228647]
84 **Munoz F**, Alici G, Li W. A review of drug delivery systems for capsule endoscopy. *Adv Drug Deliv Rev* 2014; **71**: 77-85 [PMID: 24384373 DOI: 10.1016/j.addr.2013.12.007]
85 **Phillips Technology**. Phillips Intelligent Pill Technology. Available from: URL: http://www.research.philips.com/newscenter/backgrounders/081111-ipill.html
86 **Hale MF**, Sidhu R, McAlindon ME. Capsule endoscopy: current practice and future directions. *World J Gastroenterol* 2014; **20**: 7752-7759 [PMID: 24976712 DOI: 10.3748/wjg.v20.i24.7752]
87 **Quaglia C**, Tognarelli S, Sinibaldi E, Funaro N, Dario P, Menciassi A. Wireless robotic capsule for releasing bioadhesive patches in the gastrointestinal tract. *J Med Device* 2013; **8**: 014503-3 [DOI: 10.1115/1.4025450]
88 **Yim S**, Goyal K, Sitti M. Magnetically Actuated Soft Capsule With the Multimodal Drug Release Function. *IEEE ASME Trans Mechatron* 2013; **18**: 1413-1418 [PMID: 25378896 DOI: 10.1109/TMECH.2012.2235077]
89 **Fireman Z**. Capsule endoscopy: Future horizons. *World J Gastrointest Endosc* 2010; **2**: 305-307 [PMID: 21160761 DOI: 10.4253/wjge.v2.i9.305]
90 **Toennies JL**, Ciuti G, Smith BF, Menciassi A, Valdastri P, Webster RJ. Toward tetherless insufflation of the GI Tract. *Conf Proc IEEE Eng Med Biol Soc* 2010; **2010**: 1946-1949 [PMID: 21097004 DOI: 10.1109/IEMBS.2010.5627793]
91 **Gorlewicz JL**, Battaglia S, Smith BF, Ciuti G, Gerding J, Menciassi A, Obstein KL, Valdastri P, Webster RJ. Wireless insufflation of the gastrointestinal tract. *IEEE Trans Biomed Eng* 2013; **60**: 1225-1233 [PMID: 23212312 DOI: 10.1109/TBME.2012.2230631]
92 **Koulaouzidis A**, Iakovidis DK, Karargyris A, Plevris JN. Optimizing lesion detection in small-bowel capsule endoscopy: from present problems to future solutions. *Expert Rev Gastroenterol Hepatol* 2015; **9**: 217-235 [PMID: 25169106 DOI: 10.1586/17474124.2014.952281]
93 **Adler SN**, Hassan C, Metzger Y, Sompolinsky Y, Spada C. Second-generation colon capsule endoscopy is feasible in the out-of-clinic setting. *Surg Endosc* 2014; **28**: 570-575 [PMID: 24043646 DOI: 10.1007/s00464-013-3206-y]
94 **Bouchard S**, Ibrahim M, Van Gossum A. Video capsule endoscopy: perspectives of a revolutionary technique. *World J Gastroenterol* 2014; **20**: 17330-17344 [PMID: 25516644 DOI: 10.3748/wjg.v20.i46.17330]
95 **Chandrappan J**, Ruiqi L, Su N, Qiang TS, Vaidyanathan K. Tagging module for lesion localization in capsule endoscopy. *Conf Proc IEEE Eng Med Biol Soc* 2010; **2010**: 1890-1893 [PMID: 21096425 DOI: 10.1109/IEMBS.2010.5627090]
96 **Goenka MK**, Majumder S, Goenka U. Capsule endoscopy: Present status and future expectation. *World J Gastroenterol* 2014; **20**: 10024-10037 [PMID: 25110430 DOI: 10.3748/wjg.v20.i29.10024]
97 **Woo SH**, Mohy-Ud-Din Z, Cho JH. Duodenum identification mechanism for capsule endoscopy. *IEEE Trans Biomed Eng* 2011; **58**: 905-912 [PMID: 21134813 DOI: 10.1109/TBME.2010.2095849]
98 **Fisher LR**, Hasler WL. New vision in video capsule endoscopy: current status and future directions. *Nat Rev Gastroenterol Hepatol* 2012; **9**: 392-405 [PMID: 22565098 DOI: 10.1038/nrgastro.2012.88]
99 **Ibrahim M**, Van Gossum A. Novel imaging enhancements in capsule endoscopy. *Gastroenterol Res Pract* 2013; **2013**: 304723 [PMID: 23878532 DOI: 10.1155/2013/304723]
100 **Selby WS**, Prakoso E. The inability to visualize the ampulla of Vater is an inherent limitation of capsule endoscopy. *Eur J Gastroenterol Hepatol* 2011; **23**: 101-103 [PMID: 21030868 DOI: 10.1097/MEG.0b013e3283410210]
101 **Koulaouzidis A**, Douglas S, Plevris JN. Identification of the ampulla of Vater during oesophageal capsule endoscopy: two heads and viewing speed make a difference. *Eur J Gastroenterol Hepatol* 2011; **23**: 361; author reply 362 [PMID: 21430448 DOI: 10.1097/MEG.0b013e3283440443]
102 **Gupta A**, Postgate AJ, Burling D, Ilangovan R, Marshall M, Phillips RK, Clark SK, Fraser CH. A prospective study of MR enterography versus capsule endoscopy for the surveillance of adult patients with Peutz-Jeghers syndrome. *AJR Am J Roentgenol* 2010; **195**: 108-116 [PMID: 20566803 DOI: 10.2214/AJR.09.3174]
103 **Clarke JO**, Giday SA, Magno P, Shin EJ, Buscaglia JM, Jagannath

SB, Mullin GE. How good is capsule endoscopy for detection of periampullary lesions? Results of a tertiary-referral center. *Gastrointest Endosc* 2008; **68**: 267-272 [PMID: 18378233 DOI: 10.1016/j.gie.2007.11.055]

104 **Romero-Vázquez J**, Argüelles-Arias F, García-Montes JM, Caunedo-Álvarez Á, Pellicer-Bautista FJ, Herrerías-Gutiérrez JM. Capsule endoscopy in patients refusing conventional endoscopy. *World J Gastroenterol* 2014; **20**: 7424-7433 [PMID: 24966612 DOI: 10.3748/wjg.v20.i23.7424]

105 **Hosoe N**, Naganuma M, Ogata H. Current status of capsule endoscopy through a whole digestive tract. *Dig Endosc* 2015; **27**: 205-215 [PMID: 25208463 DOI: 10.1111/den.12380]

P- Reviewer: Cerwenka HR, de'Angelis GL, Inamori M, Naito Y
S- Editor: Ma YJ **L- Editor**: Filopodia **E- Editor**: Ma S

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

ISSN 1007-9327

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v18.i39.5514

World J Gastroenterol 2012 October 21; 18(39): 5514-5520
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

GUIDELINES FOR CLINICAL PRACTICE

Checkmate to liver biopsy in chronic hepatitis C?

Anca Trifan, Carol Stanciu

Anca Trifan, Carol Stanciu, "Gr. T. Popa" University of Medicine and Pharmacy, "St. Spiridon" University Hospital, Independenţei 1, 700111 Iaşi, Romania
Author contributions: Trifan A and Stanciu C equally participated in the conception, design and drafting of this article; both authors revised the article critically for important intellectual content and gave the final approval for the version to be published.
Correspondence to: Carol Stanciu, MD, FRCP, Gastroenterology and Hepatology Center, "Sf. Spiridon" University Hospital, Independenţei 1, 700111 Iaşi,
Romania. stanciucarol@yahoo.com
Telephone: +40-72-2306020 Fax: +40-23-2264411
Received: March 13, 2012 Revised: July 6, 2012
Accepted: July 18, 2012
Published online: October 21, 2012

Abstract

Liver biopsy (LB) has traditionally been considered the gold standard for pretreatment evaluation of liver fibrosis in patients with chronic hepatitis C (CHC). However, LB is an invasive procedure with several shortcomings (intra- and interobserver variability of histopathological interpretation, sampling errors, high cost) and the risk of rare but potentially life-threatening complications. In addition, LB is poorly accepted by patients and it is not suitable for repeated evaluation. Furthermore, the prevalence of CHC makes LB unrealistic to be performed in all patients with this disease who are candidates for antiviral therapy. The above-mentioned drawbacks of LB have led to the development of noninvasive methods for the assessment of liver fibrosis. Several noninvasive methods, ranging from serum marker assays to advanced imaging techniques, have proved to be excellent tools for the evaluation of liver fibrosis in patients with CHC, whereas the value of LB as a gold standard for staging fibrosis prior to antiviral therapy has become questionable for clinicians. Despite significant resistance from those in favor of LB, noninvasive methods for pretreatment assessment of liver fibrosis in patients with CHC have become part of routine clinical practice. With protease inhibitors-based triple therapy already available and substantial improvement in sustained virological response, the time has come to move forward to noninvasiveness, with no risks for the patient and, thus, no need for LB in the assessment of liver fibrosis in the decision making for antiviral therapy in CHC.

Key words: Liver biopsy; Fibrosis; Noninvasive methods

Peer reviewers: Giuseppe Montalto, Professor, Clinical Medicine and Emerging Diseases, University of Palermo, via del Vespro, 141, 90100 Palermo, Italy; Masahito Uemura, MD, Associate Professor, Third Department of Internal Medicine, Nara Medical University, Shijo-cho, 840, Kashihara, Nara 634-8522, Japan; Dr. Bernardo Frider, MD, Professor, Department of Hepatology, Hospital General de Agudos Cosme Argerich, Alte Brown 240, Buenos Aires 1155, Argentina

Trifan A, Stanciu C. Checkmate to liver biopsy in chronic hepatitis C? *World J Gastroenterol* 2012; 18(39): 5514-5520 Available from: URL: http://www.wjgnet.com/1007-9327/full/v18/i39/5514.htm DOI: http://dx.doi.org/10.3748/wjg.v18.i39.5514

INTRODUCTION

Chronic hepatitis C (CHC) is a major public health concern, with around 180 million individuals affected worldwide[1]. Liver fibrosis and its end-point cirrhosis are the main causes of morbidity and mortality in patients with CHC[2]. Information on the stage of liver fibrosis is useful in patients with CHC not only for estimation of prognosis, but also for indication of antiviral therapy. Early international guidelines, consensus statements and expert panel opinions on the management of CHC unanimously recommended that decisions on treatment should be made only after performing a liver biopsy (LB) for pretreatment evaluation of the disease[3-5]. Consequently, antiviral treatment for patients with CHC has been indicated only for those with moderate to severe

stages of fibrosis (Metavir F2, F3 or F4), while patients with no or minimal fibrosis (Metavir F0, F1) have not been treated[6]. The rationale of such a strategy was to treat all patients with advanced fibrosis to halt disease progression and prevent complications, rather than those with no or minimal fibrosis who may await better treatments considering the slowly progressing natural history of CHC[7]. The recommendations mentioned above led to the routine performance of LB in nearly all patients who were newly diagnosed with CHC and potential candidates for antiviral therapy. More recent guidelines[8] still recommend LB in making treatment decisions, although it has been recognized that it is not necessary in patients with genotype 2 or 3, who can have as high as a 80% sustained virological response (SVR) rate.

For several decades, LB has been widely regarded as the gold standard for the staging of liver fibrosis[9]. However, LB is an invasive procedure and it is sometimes associated with rare but severe complications[10]. In addition, LB has several drawbacks (intra- and interobserver variability in histopathological interpretation, sampling errors, variable accessibility, high cost) which raises questions about its value for pretreatment assessment of liver fibrosis in patients with CHC[11,12]. Nowadays, many clinicians no longer cite LB as the gold standard but, at best, it can only be considered an imperfect standard for the staging of liver fibrosis[13]. It was this context that, in recent years, triggered a huge interest in the noninvasive assessment of liver fibrosis in patients with CHC. The introduction of a noninvasive methodology for the assessment of liver fibrosis as an alternative to LB in patients with CHC represents a major advancement in clinical hepatology[14]. Many of the noninvasive methods demonstrated accuracy to a considerable degree in identifying significant fibrosis, particularly cirrhosis, and consequently, noninvasive assessment of fibrosis is already a reality in patients with CHC[15]. Obviously, with the recent therapeutic development in CHC and reliable noninvasive diagnostic procedures available, LB has lost both its monopoly in the pretreatment assessment of fibrosis and the influence on decision making for antiviral therapy in patients with CHC.

CASE AGAINST LB

For the last 50 years, LB has been considered the gold standard for the staging of liver fibrosis in spite of its several shortcomings: intra- and interobserver variability in histopathological interpretation[16,17], sampling errors[18,19], and potentially life-threatening complications[20,21]. In clinical practice, we frequently encounter the intra- and interobserver variability in the staging of liver fibrosis[16,17]. Diagnostic errors made by nonspecialist pathologists were reported in > 25% of patients undergoing LB in academic centers[22,23]. According to a recent study[24], community pathologists understaged liver fibrosis in > 70% of cases with CHC. Several studies have shown that sampling errors occur when the LB specimen size is too small for an accurate estimation of fibrosis[18,19]. Both the length and the diameter of the biopsy core may affect the accuracy of fibrosis stage evaluation in patients with CHC[25,26]. Obviously, the shorter and thinner the samples are, the greater is the number of misclassifications of liver fibrosis. There is some controversy among pathologists in defining an adequate LB sample for an accurate staging of liver fibrosis. Some investigators[27] suggest that a sample of at least 15 mm in length and containing more than five portal tracts is adequate, while others recommend biopsy samples of 20 mm containing at least 11 portal tracts[26] or even larger samples, up to 25 mm[18]. Bigger is better[28], but at the price of an increased risk of severe complications[10,18]. However, it should be noted that, in clinical practice, few LB specimens reach an adequate length of 20 mm[29]. Furthermore, LB only samples an extremely small part of the whole organ (1/50 000) and therefore, there is a risk in the evaluation of lesions that are heterogeneously distributed throughout the entire liver[21]. LB may underestimate the amount of fibrosis, and cirrhosis could be missed in 10%-30% of cases[30]. Studies concerning fibrosis staging have also shown differences in one third of cases with CHC between LB samples obtained from the right and left lobes of the liver during laparoscopy[19]. Data on LB complications are heterogenous and contain wide variations in reported rate from one study to another[10,20,21,31-34]. Major complications include bleeding and bile peritonitis, with a reported mortality rate ranging from 0.03% to 0.1%[10,20,31,32,34]. It is worthwhile mentioning that both the transjugular route and ultrasound guidance approaches to LB do not significantly reduce the rate of major complications[35,36]. Complication rates are higher when LB is performed by less-experienced physicians[31,37]. In addition, LB is costly, variably available, poorly accepted by patients, and not suitable for repeated evaluation. The cost of an LB in the United States, United Kingdom and Australia varies between 1000 and 2000 USD, and it could go over 3000 USD if complications occur[12,38-40]. LB is not welcomed by patients and it may be refused by more than half of those with CHC[41]. LB is inappropriate for a dynamic evaluation of liver fibrosis over time, and recommendation to repeat biopsy every 3-5 years to follow up disease progression is certainly unrealistic, mainly due to patient nonadherence[40]. LB is contraindicated in the presence of coagulopathy and thrombocytopenia. Last but not least, the prevalence of CHC makes LB impossible in all patients with CHC who are candidates for antiviral therapy. It is these drawbacks of LB that have led to the development of noninvasive methods for the assessment of liver fibrosis in patients with CHC and, hopefully, to a major change in hepatology practice.

Nevertheless, LB has some well-recognized advantages for assessing fibrosis in CHC, such as direct measuring of liver fibrosis, well-established staging system, and evaluation of associated lesions (steatosis, iron deposition, inflammation, alcoholic liver disease, nonalcoholic fatty liver disease, metabolic syndrome), although these diagnostic advantages are counterbalanced by the aforementioned disadvantages.

CASE IN FAVOR OF NONINVASIVE METHODS

Noninvasive methods for detecting liver fibrosis may be divided in two main groups: serum markers of fibrosis and transient elastography (Fibroscan).

Serum markers for liver fibrosis are commonly divided into direct serum markers, which are directly linked to the modifications in extracellular matrix turnover produced by hepatic stellate cells during the process of fibrogenesis in the liver, and indirect serum markers which reflect alterations of the hepatic functions. The direct markers include glycoproteins (hyaluronate, laminin, YKL-40), collagen family (procollagen Ⅲ, type Ⅳ collagen), collagenases and their inhibitors (matrix metalloproteases, tissue inhibitory metalloprotease-1), and they are not routinely available in most clinical laboratories. The indirect markers are biochemical parameters determined in routine blood tests [platelet count, prothrombin time, aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio]. Serum markers for liver fibrosis may be used singly[42-45] or combining panels of direct or indirect serum markers and demographic parameters[46-55], with the aim of increasing the accuracy of single parameters. Some of them are patent-protected and commercially available: FibroTest® (Biopredictive, Paris, France) licensed under the name of Fibrosure® in the United States (LabCorp, Burlington, NC, United States)[51], Fibrometer® (BioLiveScale, Angers, France)[52], Hepascore (PathWest, University of Western Australia, Australia)[53], ELF® (Enhanced Liver Fibrosis Test, iQur Ltd, Southampton, United Kingdom)[54], and FibroSpect Ⅱ® (Promotheus Laboratory Inc. San Diego, Ca, United States)[55]. Among these, Fibrotest [α-2-macroglobulin, γ-glutamyl transpeptidase (GT), apolipoprotein A1, haptoglobin, total bilirubin, age, sex] is the most widely used and was validated by several studies on patients with CHC[56-63]. The reported accuracy of Fibrotest for significant fibrosis/cirrhosis expressed as area under receiving operating characteristic curve (AUROC) ranges from 0.74% to 0.87%[46,51]. To improve the performance of Fibrotest, its combination with Fibroscan has been suggested; with such a combination, one study reported AUROC of 0.88 for at least F2 (stage in the Metavir scoring system) and 0.95 for F3 or F4[56]. The sensitivity and specificity of serum-marker-based tests could also be improved by combining them using sequential algorithms. Thus, Sebastiani *et al*[64] combined AST/platelets ratio (APRI) with Fibrotest - a combination known as sequential algorithm for fibrosis evaluation biopsy - and found it to have an accuracy of 92.5% in the detection of fibrosis in CHC, obviating 81.5% of liver biopsies. APRI has a slightly lower performance than Fibrotest, with an accuracy between 60% and 82% for significant fibrosis and 60% and 88% for cirrhosis[46,64], but it is a simple cost-free readily available test in all hospital settings. Both Fibrometer (platelet count, hyaluronate, AST, α-2-macroglobulin, international normalized ratio, urea, age) and Hepascore (bilirubin, γGT, α-2-macroglobulin, hyaluronic acid, age, sex) showed good performance for detection of significant fibrosis[52,53,65].

There are several advantages of serum markers such as high applicability, with no risk for the patient and no contraindication; they can be performed and repeated in outpatient clinics; widespread availability; and inter-laboratory reproducibility[66]. However, there are some limitations of serum markers: none is liver specific; results are unreliable in comorbidities (hemolysis, Gilbert syndrome, rheumatoid arthritis); and they have poor performance in the diagnosis of intermediate stages of liver fibrosis[66]. Nevertheless, it is important to note that the performance of each noninvasive marker is evaluated against LB which is an imperfect gold standard, and the apparent failure of noninvasive markers to make an accurate distinction between different stages of intermediate fibrosis could be the consequence of misclassifications from biopsy[67,68].

Transient elastography (Fibroscan®, Echosens, Paris, France) measures liver stiffness in a volume at least 100 times greater than a standard LB sample, and therefore, may be more representative of the entire liver. Fibroscan is composed of an ultrasound transducer probe mounted on the axis of a vibrator; vibration is transmitted to induce an elastic shear wave that propagates through the liver. Pulse-echo ultrasound acquisition is used to measure the velocity of the shear wave, which is directly related to liver stiffness: the stiffer the liver, the faster the shear wave propagates. Results are expressed in kPa, and values range from 2.5 kPa to 75 kPa, with normal values < 5.5 kPa[69]. According to several studies, a cutoff value of 7.2-8.7 kPa defines significant fibrosis, and cirrhosis is diagnosed by a cutoff value of 12.5-14.5 kPa[70,71]. Fibroscan seems to be a reliable method for the diagnosis of significant fibrosis (AUROC 0.84) and cirrhosis (AUROC 0.95)[72,73]. Its combination with serum-based tests (Fibrotest, Fibrometer) increases the performance (but also the costs) for the diagnosis of significant fibrosis[56,71,72]. Among noninvasive methods for diagnosis of cirrhosis, Fibroscan has the highest level of performance[62,72,73], and its combination with serum markers does not increase accuracy[63,72].

Fibroscan has several advantages: it is painless; quick (< 5 min); highly reproducible, with results immediately available; inexpensive; and easy to perform in the outpatient clinic and at the bedside[66]. In addition, Fibroscan can be repeated for longitudinal disease monitoring, which is difficult, if not impossible, with LB. In cirrhotic patients, Fibroscan values correlate with portal pressure (based on the hepatic venous pressure gradient measurement), which is a reliable predictor of clinical outcomes[74-77], disease severity[78], and the risk of hepatocellular carcinoma[79]. Finally, Fibroscan and serum markers are well accepted by patients, therefore, they could be used as screening methods for the detection of liver fibrosis/cirrhosis in at-risk groups[80] and even in general population[81], while LB is unacceptable for screening purposes. Fibroscan measurement failure and unreliable results are due to limited operator experience[82], narrowed intercostal spaces[82], and obesity[82,83], although

this last problem seems to be overcome by a new specially designed probe[84-86]. Results are influenced by ALT flares[87,88], extrahepatic cholestasis[89,90], and congestive heart failure[91].

DISCUSSION

In the past, expert consensus guidelines on the management of CHC unanimously recommended routine LB before initiation of antiviral therapy[3-5,92,93]. Based on LB findings, treatment has often been advocated only for patients with at least moderate to severe stages of fibrosis (Metavir F2, F3 or F4), and withheld for those with no or minimal fibrosis (F0, F1)[6,93]. As a consequence, tens of thousands of patients were most likely denied proper antiviral therapy. More recent guidelines[8,94] recommend LB only in patients with CHC genotype 1 (SVR rate < 50%) in treatment decision making, and consider it unnecessary in those with genotype 2 or 3 who may have an SVR rate as high as 80%. The primary endpoint of antiviral therapy for CHC is achieving SVR - defined as undetectable serum HCV RNA at 24 wk after discontinuation of therapy. Viral eradication prevents disease progression, improves survival, and reduces health care costs associated with the management of complications. Thus, if viral clearance is the aim of antiviral therapy in CHC, then to what degree does an exact histopathological fibrosis stage established through biopsy still matter? With the new protease inhibitor (PI)-based triple therapy (addition of telaprevir or boceprevir to pegylated interferon and ribavirin) available and SVR rates approaching 75% in patients with CHC genotype 1[95,96], it is clear that LB has lost its importance in the recommendation of antiviral therapy.

During the past 10 years, an intensive debate has taken place between those in favor of LB and those who promote noninvasive methods for pretreatment assessment of liver fibrosis in patients with CHC. There is extensive literature showing the pros and cons of LB or noninvasive methods. As in chess, winning does not come easy for a supporter of noninvasive methods against a supporter of LB with a firmly rooted preference. Step by step, those in favor of non-invasive methods have gained ground, waiting for the final move: checkmate! Today, several noninvasive methods, ranging from serum marker assays to advanced imaging techniques, have proved to be excellent tools for the evaluation of liver fibrosis in patients with CHC. According to the latest European Association of the Study of the Liver clinical practice guidelines[97] and United Kingdom consensus guidelines[98] recommendations, noninvasive methods can be used instead of LB in patients with CHC to assess liver disease severity prior to antiviral therapy. It is therefore surprising that many experts in the field of hepatology and the most recent American Association for the Study of Liver Diseases 2011 practice guidelines[99] favor LB before therapy initiation, despite substantial improvement in treatment success rate for genotype 1 patients with PI-based triple therapy. The main reason against noninvasive methods for evaluation of liver fibrosis is their apparent failure to make an accurate distinction between different stages of intermediate fibrosis. It is important to note that the performance of each noninvasive method was evaluated in all studies by calculating the AUROC using LB as a reference standard. As LB is an imperfect standard, a perfect noninvasive method will never reach the maximum value (1.0)[100], and therefore, noninvasive methods are as inaccurate as LB for the assessment of fibrosis stage. Thus, the failure of noninvasive methods to discriminate between different stages of intermediate fibrosis could be the consequence of classification errors from histopathological findings of biopsy[67,68]. For clinicians, it is more important to know if their patients have no/mild or advanced fibrosis/cirrhosis, rather than the exact pathological scoring system through LB, and this could be easily achieved by means of noninvasive methods. Taking into account that all recent international guidelines[97-99] recommend treatment with PI-based triple therapy in all patients with CHC genotype 1, provided that they have no contraindications to peg-interferon and ribavirin, the need to stage liver fibrosis accurately is decreasing in treatment decisions.

The final move - checkmate to LB - is, therefore, possible once the rate of SVR has reached 75% with PI-based triple therapy for patients with CHC genotype 1. Consequently, it is clear that in the era of PI-based triple therapy and other new potent direct-acting agents in the pipeline, the information obtainable from LB has little, if any, influence on treatment decisions. It should be underlined that in this article, checkmate to LB in patients with CHC refers strictly to cases with no need for this invasive and risky procedure in therapeutic decision making. With PI-based triple therapy already available in many countries, and an allocation system probably based mainly on medical need (therapy for those likely to develop complications in the next few years), noninvasive methods with the highest accuracy for detecting severe fibrosis/cirrhosis used as an alternative to LB for pretreatment assessment of liver fibrosis in patients with CHC are now part of routine clinical practice. Fibroscan or any patented biomarkers (Fibrotest, Fibrometer and Hepascore) have recently been recommended for first-line staging of liver fibrosis[101] before deciding on antiviral therapy. However, the adoption rates of noninvasive methods by hepatologists differ from country to country. In France, a survey of 546 hepatologists revealed that 81% of them used noninvasive methods[102], while in the United States, despite the aforementioned shortcomings of LB, there is still significant resistance to accepting noninvasive methods as an alternative to biopsy. We believe that sooner or later this will change, and the requirement of LB prior to starting antiviral therapy in patients with CHC will be reassessed.

In conclusion, in the era of PI-based triple therapy and other new potent direct-acting agents on the horizon that can achieve SVR rates approaching 100%, the time has come to move forward to risk-free noninvasive

methods for the patient, leaving LB behind in the evaluation of liver fibrosis in decision making for CHC antiviral therapy. In other words, checkmate to LB?

REFERENCES

1 **Lavanchy D**. The global burden of hepatitis C. *Liver Int* 2009; **29** Suppl 1: 74-81
2 **Marcellin P**, Asselah T, Boyer N. Fibrosis and disease progression in hepatitis C. *Hepatology* 2002; **36**: S47-S56
3 EASL International Consensus Conference on Hepatitis C. Paris, 26-28, February 1999, Consensus Statement. European Association for the Study of the Liver. *J Hepatol* 1999; **30**: 956-961
4 NIH Consensus Statement on Management of Hepatitis C: 2002. *NIH Consens State Sci Statements* 2002; **19**: 1-46
5 **Strader DB**, Wright T, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C. *Hepatology* 2004; **39**: 1147-1171
6 **Dienstag JL**, McHutchison JG. American Gastroenterological Association technical review on the management of hepatitis C. *Gastroenterology* 2006; **130**: 231-264
7 **Poynard T**, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. *Lancet* 1997; **349**: 825-832
8 **Ghany MG**, Strader DB, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* 2009; **49**: 1335-1374
9 **Bravo AA**, Sheth SG, Chopra S. Liver biopsy. *N Engl J Med* 2001; **344**: 495-500
10 **McGill DB**, Rakela J, Zinsmeister AR, Ott BJ. A 21-year experience with major hemorrhage after percutaneous liver biopsy. *Gastroenterology* 1990; **99**: 1396-1400
11 **Reiss G**, Keeffe EB. Role of liver biopsy in the management of chronic liver disease: selective rather than routine. *Rev Gastroenterol Disord* 2005; **5**: 195-205
12 **Crockett SD**, Kaltenbach T, Keeffe EB. Do we still need a liver biopsy? Are the serum fibrosis tests ready for prime time? *Clin Liver Dis* 2006; **10**: 513-534
13 **Sanai FM**, Keeffe EB. Liver biopsy for histological assessment: The case against. *Saudi J Gastroenterol* 2010; **16**: 124-132
14 **Castera L**, Pinzani M. Non-invasive assessment of liver fibrosis: are we ready? *Lancet* 2010; **375**: 1419-1420
15 **Martínez SM**, Crespo G, Navasa M, Forns X. Noninvasive assessment of liver fibrosis. *Hepatology* 2011; **53**: 325-335
16 **The French METAVIR Cooperative Study Group**. Intraobserver and interobserver variations in liver biopsy interpretation in patients with chronic hepatitis C. *Hepatology* 1994; **20**: 15-20
17 **Westin J**, Lagging LM, Wejstål R, Norkrans G, Dhillon AP. Interobserver study of liver histopathology using the Ishak score in patients with chronic hepatitis C virus infection. *Liver* 1999; **19**: 183-187
18 **Bedossa P**, Dargère D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. *Hepatology* 2003; **38**: 1449-1457
19 **Regev A**, Berho M, Jeffers LJ, Milikowski C, Molina EG, Pyrsopoulos NT, Feng ZZ, Reddy KR, Schiff ER. Sampling error and intraobserver variation in liver biopsy in patients with chronic HCV infection. *Am J Gastroenterol* 2002; **97**: 2614-2618
20 **Thampanitchawong P**, Piratvisuth T. Liver biopsy: complications and risk factors. *World J Gastroenterol* 1999; **5**: 301-304
21 **Cadranel JF**, Rufat P, Degos F. Practices of liver biopsy in France: results of a prospective nationwide survey. For the Group of Epidemiology of the French Association for the Study of the Liver (AFEF). *Hepatology* 2000; **32**: 477-481
22 **Bejarano PA**, Koehler A, Sherman KE. Second opinion pathology in liver biopsy interpretation. *Am J Gastroenterol* 2001; **96**: 3158-3164
23 **Hahm GK**, Niemann TH, Lucas JG, Frankel WL. The value of second opinion in gastrointestinal and liver pathology. *Arch Pathol Lab Med* 2001; **125**: 736-739
24 **Robert M**, Sofair AN, Thomas A, Bell B, Bialek S, Corless C, Van Ness G, Huie-White S, Stabach N, Zaman A. A comparison of hepatopathologists' and community pathologists' review of liver biopsy specimens from patients with hepatitis C. *Clin Gastroenterol Hepatol* 2009; **7**: 335-338
25 **Colloredo G**, Guido M, Sonzogni A, Leandro G. Impact of liver biopsy size on histological evaluation of chronic viral hepatitis: the smaller the sample, the milder the disease. *J Hepatol* 2003; **39**: 239-244
26 **Guido M**, Rugge M. Liver biopsy sampling in chronic viral hepatitis. *Semin Liver Dis* 2004; **24**: 89-97
27 **Afdhal NH**, Nunes D. Evaluation of liver fibrosis: a concise review. *Am J Gastroenterol* 2004; **99**: 1160-1174
28 **Scheuer PJ.** Liver biopsy size matters in chronic hepatitis: bigger is better. *Hepatology* 2003; **38**: 1356-1358
29 **Röcken C**, Meier H, Klauck S, Wolff S, Malfertheiner P, Roessner A. Large-needle biopsy versus thin-needle biopsy in diagnostic pathology of liver diseases. *Liver* 2001; **21**: 391-397
30 **Poniachik J**, Bernstein DE, Reddy KR, Jeffers LJ, Coelho-Little ME, Civantos F, Schiff ER. The role of laparoscopy in the diagnosis of cirrhosis. *Gastrointest Endosc* 1996; **43**: 568-571
31 **Gilmore IT**, Burroughs A, Murray-Lyon IM, Williams R, Jenkins D, Hopkins A. Indications, methods, and outcomes of percutaneous liver biopsy in England and Wales: an audit by the British Society of Gastroenterology and the Royal College of Physicians of London. *Gut* 1995; **36**: 437-441
32 **Montalto G**, Soresi M, Carroccio A, Bascone F, Tripi S, Aragona F, Di Gaetano G, Notarbartolo A. Percutaneous liver biopsy: a safe outpatient procedure? *Digestion* 2001; **63**: 55-60
33 **Myers RP**, Fong A, Shaheen AA. Utilization rates, complications and costs of percutaneous liver biopsy: a population-based study including 4275 biopsies. *Liver Int* 2008; **28**: 705-712
34 **Firpi RJ**, Soldevila-Pico C, Abdelmalek MF, Morelli G, Judah J, Nelson DR. Short recovery time after percutaneous liver biopsy: should we change our current practices? *Clin Gastroenterol Hepatol* 2005; **3**: 926-929
35 **Kalambokis G**, Manousou P, Vibhakorn S, Marelli L, Cholongitas E, Senzolo M, Patch D, Burroughs AK. Transjugular liver biopsy--indications, adequacy, quality of specimens, and complications--a systematic review. *J Hepatol* 2007; **47**: 284-294
36 **Stone MA**, Mayberry JF. An audit of ultrasound guided liver biopsies: a need for evidence-based practice. *Hepatogastroenterology* 1996; **43**: 432-434
37 **Froehlich F**, Lamy O, Fried M, Gonvers JJ. Practice and complications of liver biopsy. Results of a nationwide survey in Switzerland. *Dig Dis Sci* 1993; **38**: 1480-1484
38 **Pokorny CS**, Waterland M. Short-stay, out-of-hospital, radiologically guided liver biopsy. *Med J Aust* 2002; **176**: 67-69
39 **Chalmers RJ**, Kirby B, Smith A, Burrows P, Little R, Horan M, Hextall JM, Smith CH, Klaber M, Rogers S. Replacement of routine liver biopsy by procollagen III aminopeptide for monitoring patients with psoriasis receiving long-term methotrexate: a multicentre audit and health economic analysis. *Br J Dermatol* 2005; **152**: 444-450
40 **Wong JB**, Koff RS. Watchful waiting with periodic liver biopsy versus immediate empirical therapy for histologically mild chronic hepatitis C. A cost-effectiveness analysis. *Ann Intern Med* 2000; **133**: 665-675
41 **Bonny C**, Rayssiguier R, Ughetto S, Aublet-Cuvelier B, Baranger J, Blanchet G, Delteil J, Hautefeuille P, Lapalus F, Montanier P, Bommelaer G, Abergel A. Medical practices and expectations of general practitioners in relation to hepa-

titis C virus infection in the Auvergne region. *Gastroenterol Clin Biol* 2003; **27**: 1021-1025
42 **McHutchison JG**, Blatt LM, de Medina M, Craig JR, Conrad A, Schiff ER, Tong MJ, The Consensus Interferon Study Group. Measurement of serum hyaluronic acid in patients with chronic hepatitis C and its relationship to liver histology. *J Gastroenterol Hepatol* 2000; **15**: 945-951
43 **Murawaki Y**, Ikuta Y, Okamoto K, Koda M, Kawasaki H. Diagnostic value of serum markers of connective tissue turnover for predicting histological staging and grading in patients with chronic hepatitis C. *J Gastroenterol* 2001; **36**: 399-406
44 **Murawaki Y**, Koda M, Okamoto K, Mimura K, Kawasaki H. Diagnostic value of serum type IV collagen test in comparison with platelet count for predicting the fibrotic stage in patients with chronic hepatitis C. *J Gastroenterol Hepatol* 2001; **16**: 777-781
45 **Boeker KH**, Haberkorn CI, Michels D, Flemming P, Manns MP, Lichtinghagen R. Diagnostic potential of circulating TIMP-1 and MMP-2 as markers of liver fibrosis in patients with chronic hepatitis C. *Clin Chim Acta* 2002; **316**: 71-81
46 **Sebastiani G**, Alberti A. Non invasive fibrosis biomarkers reduce but not substitute the need for liver biopsy. *World J Gastroenterol* 2006; **12**: 3682-3694
47 **Forns X**, Ampurdanès S, Llovet JM, Aponte J, Quintó L, Martínez-Bauer E, Bruguera M, Sánchez-Tapias JM, Rodés J. Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. *Hepatology* 2002; **36**: 986-992
48 **Lok AS**, Ghany MG, Goodman ZD, Wright EC, Everson GT, Sterling RK, Everhart JE, Lindsay KL, Bonkovsky HL, Di Bisceglie AM, Lee WM, Morgan TR, Dienstag JL, Morishima C. Predicting cirrhosis in patients with hepatitis C based on standard laboratory tests: results of the HALT-C cohort. *Hepatology* 2005; **42**: 282-292
49 **Vallet-Pichard A**, Mallet V, Nalpas B, Verkarre V, Nalpas A, Dhalluin-Venier V, Fontaine H, Pol S. FIB-4: an inexpensive and accurate marker of fibrosis in HCV infection. comparison with liver biopsy and fibrotest. *Hepatology* 2007; **46**: 32-36
50 **Koda M**, Matunaga Y, Kawakami M, Kishimoto Y, Suou T, Murawaki Y. FibroIndex, a practical index for predicting significant fibrosis in patients with chronic hepatitis C. *Hepatology* 2007; **45**: 297-306
51 **Imbert-Bismut F**, Ratziu V, Pieroni L, Charlotte F, Benhamou Y, Poynard T. Biochemical markers of liver fibrosis in patients with hepatitis C virus infection: a prospective study. *Lancet* 2001; **357**: 1069-1075
52 **Calès P**, Oberti F, Michalak S, Hubert-Fouchard I, Rousselet MC, Konaté A, Gallois Y, Ternisien C, Chevailler A, Lunel F. A novel panel of blood markers to assess the degree of liver fibrosis. *Hepatology* 2005; **42**: 1373-1381
53 **Adams LA**, Bulsara M, Rossi E, DeBoer B, Speers D, George J, Kench J, Farrell G, McCaughan GW, Jeffrey GP. Hepascore: an accurate validated predictor of liver fibrosis in chronic hepatitis C infection. *Clin Chem* 2005; **51**: 1867-1873
54 **Rosenberg WM**, Voelker M, Thiel R, Becka M, Burt A, Schuppan D, Hubscher S, Roskams T, Pinzani M, Arthur MJ. Serum markers detect the presence of liver fibrosis: a cohort study. *Gastroenterology* 2004; **127**: 1704-1713
55 **Patel K**, Gordon SC, Jacobson I, Hézode C, Oh E, Smith KM, Pawlotsky JM, McHutchison JG. Evaluation of a panel of non-invasive serum markers to differentiate mild from moderate-to-advanced liver fibrosis in chronic hepatitis C patients. *J Hepatol* 2004; **41**: 935-942
56 **Castéra L**, Vergniol J, Foucher J, Le Bail B, Chanteloup E, Haaser M, Darriet M, Couzigou P, De Lédinghen V. Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. *Gastroenterology* 2005; **128**: 343-350
57 **Halfon P**, Bourliere M, Deydier R, Botta-Fridlund D, Renou C, Tran A, Portal I, Allemand I, Bertrand JJ, Rosenthal-Allieri A, Rotily M, Sattonet C, Benderitter T, Saint Paul MC, Bonnot HP, Penaranda G, Degott C, Masseyeff MF, Ouzan D. Independent prospective multicenter validation of biochemical markers (fibrotest-actitest) for the prediction of liver fibrosis and activity in patients with chronic hepatitis C: the fibropaca study. *Am J Gastroenterol* 2006; **101**: 547-555
58 **Parkes J**, Guha IN, Roderick P, Rosenberg W. Performance of serum marker panels for liver fibrosis in chronic hepatitis C. *J Hepatol* 2006; **44**: 462-474
59 **Shaheen AA**, Wan AF, Myers RP. FibroTest and FibroScan for the prediction of hepatitis C-related fibrosis: a systematic review of diagnostic test accuracy. *Am J Gastroenterol* 2007; **102**: 2589-2600
60 **Leroy V**, Hilleret MN, Sturm N, Trocme C, Renversez JC, Faure P, Morel F, Zarski JP. Prospective comparison of six non-invasive scores for the diagnosis of liver fibrosis in chronic hepatitis C. *J Hepatol* 2007; **46**: 775-782
61 **Calès P**, de Ledinghen V, Halfon P, Bacq Y, Leroy V, Boursier J, Foucher J, Bourlière M, de Muret A, Sturm N, Hunault G, Oberti F. Evaluating the accuracy and increasing the reliable diagnosis rate of blood tests for liver fibrosis in chronic hepatitis C. *Liver Int* 2008; **28**: 1352-1362
62 **Degos F**, Perez P, Roche B, Mahmoudi A, Asselineau J, Voitot H, Bedossa P. Diagnostic accuracy of FibroScan and comparison to liver fibrosis biomarkers in chronic viral hepatitis: a multicenter prospective study (the FIBROSTIC study). *J Hepatol* 2010; **53**: 1013-1021
63 **Zarski JP**, Sturm N, Guechot J, Paris A, Zafrani ES, Asselah T, Boisson RC, Bosson JL, Guyader D, Renversez JC, Bronowicki JP, Gelineau MC, Tran A, Trocme C, De Ledinghen V, Lasnier E, Poujol-Robert A, Ziegler F, Bourliere M, Voitot H, Larrey D, Rosenthal-Allieri MA, Fouchard Hubert I, Bailly F, Vaubourdolle M. Comparison of nine blood tests and transient elastography for liver fibrosis in chronic hepatitis C: the ANRS HCEP-23 study. *J Hepatol* 2012; **56**: 55-62
64 **Sebastiani G**, Vario A, Guido M, Noventa F, Plebani M, Pistis R, Ferrari A, Alberti A. Stepwise combination algorithms of non-invasive markers to diagnose significant fibrosis in chronic hepatitis C. *J Hepatol* 2006; **44**: 686-693
65 **Becker L**, Salameh W, Sferruzza A, Zhang K, ng Chen R, Malik R, Reitz R, Nasser I, Afdhal NH. Validation of hepascore, compared with simple indices of fibrosis, in patients with chronic hepatitis C virus infection in United States. *Clin Gastroenterol Hepatol* 2009; **7**: 696-701
66 **Castera L**. Noninvasive methods to assess liver disease in patients with hepatitis B or C. *Gastroenterology* 2012; **142**: 1293-1302.e4
67 **Poynard T**, Munteanu M, Imbert-Bismut F, Charlotte F, Thabut D, Le Calvez S, Messous D, Thibault V, Benhamou Y, Moussalli J, Ratziu V. Prospective analysis of discordant results between biochemical markers and biopsy in patients with chronic hepatitis C. *Clin Chem* 2004; **50**: 1344-1355
68 **Liu S**, Schwarzinger M, Carrat F, Goldhaber-Fiebert JD. Cost effectiveness of fibrosis assessment prior to treatment for chronic hepatitis C patients. *PLoS One* 2011; **6**: e26783
69 **Castera L**, Forns X, Alberti A. Non-invasive evaluation of liver fibrosis using transient elastography. *J Hepatol* 2008; **48**: 835-847
70 **Ziol M**, Handra-Luca A, Kettaneh A, Christidis C, Mal F, Kazemi F, de Lédinghen V, Marcellin P, Dhumeaux D, Trinchet JC, Beaugrand M. Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with chronic hepatitis C. *Hepatology* 2005; **41**: 48-54
71 **Poynard T**, Ingiliz P, Elkrief L, Munteanu M, Lebray P, Morra R, Messous D, Bismut FI, Roulot D, Benhamou Y, Thabut D, Ratziu V. Concordance in a world without a gold standard: a new non-invasive methodology for improving accuracy of fibrosis markers. *PLoS One* 2008; **3**: e3857
72 **Boursier J**, de Ledinghen V, Zarski JP, Rousselet MC, Sturm N, Foucher J, Leroy V, Fouchard-Hubert I, Bertrais S, Gallois Y, Oberti F, Dib N, Calès P. A new combination of blood

test and fibroscan for accurate non-invasive diagnosis of liver fibrosis stages in chronic hepatitis C. *Am J Gastroenterol* 2011; **106**: 1255-1263

73 **Castéra L**, Le Bail B, Roudot-Thoraval F, Bernard PH, Foucher J, Merrouche W, Couzigou P, de Lédinghen V. Early detection in routine clinical practice of cirrhosis and oesophageal varices in chronic hepatitis C: comparison of transient elastography (FibroScan) with standard laboratory tests and non-invasive scores. *J Hepatol* 2009; **50**: 59-68

74 **Carrión JA**, Navasa M, Bosch J, Bruguera M, Gilabert R, Forns X. Transient elastography for diagnosis of advanced fibrosis and portal hypertension in patients with hepatitis C recurrence after liver transplantation. *Liver Transpl* 2006; **12**: 1791-1798

75 **Vizzutti F**, Arena U, Romanelli RG, Rega L, Foschi M, Colagrande S, Petrarca A, Moscarella S, Belli G, Zignego AL, Marra F, Laffi G, Pinzani M. Liver stiffness measurement predicts severe portal hypertension in patients with HCV-related cirrhosis. *Hepatology* 2007; **45**: 1290-1297

76 **Bureau C**, Metivier S, Peron JM, Selves J, Robic MA, Gourraud PA, Rouquet O, Dupuis E, Alric L, Vinel JP. Transient elastography accurately predicts presence of significant portal hypertension in patients with chronic liver disease. *Aliment Pharmacol Ther* 2008; **27**: 1261-1268

77 **Robic MA**, Procopet B, Métivier S, Péron JM, Selves J, Vinel JP, Bureau C. Liver stiffness accurately predicts portal hypertension related complications in patients with chronic liver disease: a prospective study. *J Hepatol* 2011; **55**: 1017-1024

78 **Foucher J**, Chanteloup E, Vergniol J, Castéra L, Le Bail B, Adhoute X, Bertet J, Couzigou P, de Lédinghen V. Diagnosis of cirrhosis by transient elastography (FibroScan): a prospective study. *Gut* 2006; **55**: 403-408

79 **Masuzaki R**, Tateishi R, Yoshida H, Goto E, Sato T, Ohki T, Imamura J, Goto T, Kanai F, Kato N, Ikeda H, Shiina S, Kawabe T, Omata M. Prospective risk assessment for hepatocellular carcinoma development in patients with chronic hepatitis C by transient elastography. *Hepatology* 2009; **49**: 1954-1961

80 **Jacqueminet S**, Lebray P, Morra R, Munteanu M, Devers L, Messous D, Bernard M, Hartemann-Heurtier A, Imbert-Bismut F, Ratziu V, Grimaldi A, Poynard T. Screening for liver fibrosis by using a noninvasive biomarker in patients with diabetes. *Clin Gastroenterol Hepatol* 2008; **6**: 828-831

81 **Roulot D**, Buyck JF, Gambier N, Warzocha U, Czernichow S, Costes JL, Le Clesiau H, Beaugrand M. Liver stiffness measurement: an appropriate screening method to detect liver fibrosis in the general population (abstract). *J Hepatol* 2009; **50** (Suppl 1): S89

82 **Castéra L**, Foucher J, Bernard PH, Carvalho F, Allaix D, Merrouche W, Couzigou P, de Lédinghen V. Pitfalls of liver stiffness measurement: a 5-year prospective study of 13,369 examinations. *Hepatology* 2010; **51**: 828-835

83 **Foucher J**, Castéra L, Bernard PH, Adhoute X, Laharie D, Bertet J, Couzigou P, de Lédinghen V. Prevalence and factors associated with failure of liver stiffness measurement using FibroScan in a prospective study of 2114 examinations. *Eur J Gastroenterol Hepatol* 2006; **18**: 411-412

84 **de Lédinghen V**, Vergniol J, Foucher J, El-Hajbi F, Merrouche W, Rigalleau V. Feasibility of liver transient elastography with FibroScan using a new probe for obese patients. *Liver Int* 2010; **30**: 1043-1048

85 **Kemp W**, Graham M, Roberts S. Comparison of the M and XL probe in transient elastography using fibroscan. *J Gastroenterol Hepatol* 2010; **25**: A29

86 **Myers RP**, Pomier-Layrargues G, Kirsch R, Pollett A, Duarte-Rojo A, Wong D, Beaton M, Levstik M, Crotty P, Elkashab M. Feasibility and diagnostic performance of the FibroScan XL probe for liver stiffness measurement in overweight and obese patients. *Hepatology* 2012; **55**: 199-208

87 **Coco B**, Oliveri F, Maina AM, Ciccorossi P, Sacco R, Colombatto P, Bonino F, Brunetto MR. Transient elastography: a new surrogate marker of liver fibrosis influenced by major changes of transaminases. *J Viral Hepat* 2007; **14**: 360-369

88 **Arena U**, Vizzutti F, Corti G, Ambu S, Stasi C, Bresci S, Moscarella S, Boddi V, Petrarca A, Laffi G, Marra F, Pinzani M. Acute viral hepatitis increases liver stiffness values measured by transient elastography. *Hepatology* 2008; **47**: 380-384

89 **Millonig G**, Reimann FM, Friedrich S, Fonouni H, Mehrabi A, Büchler MW, Seitz HK, Mueller S. Extrahepatic cholestasis increases liver stiffness (FibroScan) irrespective of fibrosis. *Hepatology* 2008; **48**: 1718-1723

90 **Trifan A**, Sfarti C, Cojocariu C, Dimache M, Cretu M, Hutanasu C, Stanciu C. Increased liver stiffness in extrahepatic cholestasis caused by choledocholithiasis. *Hepat Mon* 2011; **11**: 372-375

91 **Millonig G**, Friedrich S, Adolf S, Fonouni H, Golriz M, Mehrabi A, Stiefel P, Pöschl G, Büchler MW, Seitz HK, Mueller S. Liver stiffness is directly influenced by central venous pressure. *J Hepatol* 2010; **52**: 206-210

92 **Grant A**, Neuberger J. Guidelines on the use of liver biopsy in clinical practice. *Gut* 1999; **45** Suppl 4: IV1-IV11

93 National Institutes of Health Consensus Development Conference Panel statement: management of hepatitis C. *Hepatology* 1997; **26**: 2S-10S

94 **Sherman M**, Shafran S, Burak K, Doucette K, Wong W, Girgrah N, Yoshida E, Renner E, Wong P, Deschênes M. Management of chronic hepatitis C: consensus guidelines. *Can J Gastroenterol* 2007; **21** Suppl C: 25C-34C

95 **Poordad F**, McCone J, Bacon BR, Bruno S, Manns MP, Sulkowski MS, Jacobson IM, Reddy KR, Goodman ZD, Boparai N, DiNubile MJ, Sniukiene V, Brass CA, Albrecht JK, Bronowicki JP. Boceprevir for untreated chronic HCV genotype 1 infection. *N Engl J Med* 2011; **364**: 1195-1206

96 **Jacobson IM**, McHutchison JG, Dusheiko G, Di Bisceglie AM, Reddy KR, Bzowej NH, Marcellin P, Muir AJ, Ferenci P, Flisiak R, George J, Rizzetto M, Shouval D, Sola R, Terg RA, Yoshida EM, Adda N, Bengtsson L, Sankoh AJ, Kieffer TL, George S, Kauffman RS, Zeuzem S. Telaprevir for previously untreated chronic hepatitis C virus infection. *N Engl J Med* 2011; **364**: 2405-2416

97 **European Association for the Study of the Liver**. EASL Clinical Practice Guidelines: management of hepatitis C virus infection. *J Hepatol* 2011; **55**: 245-264

98 **Ramachandran P**, Fraser A, Agarwal K, Austin A, Brown A, Foster GR, Fox R, Hayes PC, Leen C, Mills PR, Mutimer DJ, Ryder SD, Dillon JF. UK consensus guidelines for the use of the protease inhibitors boceprevir and telaprevir in genotype 1 chronic hepatitis C infected patients. *Aliment Pharmacol Ther* 2012; **35**: 647-662

99 **Ghany MG**, Nelson DR, Strader DB, Thomas DL, Seeff LB. An update on treatment of genotype 1 chronic hepatitis C virus infection: 2011 practice guideline by the American Association for the Study of Liver Diseases. *Hepatology* 2011; **54**: 1433-1444

100 **Bedossa P**, Carrat F. Liver biopsy: the best, not the gold standard. *J Hepatol* 2009; **50**: 1-3

101 **de Sant HA**. Non invasive methods for the evaluation of hepatic Fibrosis/cirrhosis: an update. Available from: URL: http: //www.has-sante.fr

102 **Castera L**, Denis J, Babany G, Roudot-Thoraval F. Evolving practices of non-invasive markers of liver fibrosis in patients with chronic hepatitis C in France: time for new guidelines? *J Hepatol* 2007; **46**: 528-529; author reply 529-530

S- Editor Lv S **L- Editor** Kerr C **E- Editor** Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i4.445

World J Gastroenterol 2013 January 28; 19(4): 445-456
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

TOPIC HIGHLIGHT

Anca Trifan, Professor, *Series Editor*

Update on adrenal insufficiency in patients with liver cirrhosis

Anca Trifan, Stefan Chiriac, Carol Stanciu

Anca Trifan, Stefan Chiriac, Carol Stanciu, "Gr. T. Popa" University of Medicine and Pharmacy, Institute of Gastroenterology and Hepatology, "St. Spiridon" University Hospital, 700111 Iaşi, Romania

Author contributions: Trifan A prepared the first draft, revised the article critically for important intellectual content, and performed the final edit of the paper; Chiriac S performed the literature research, wrote and edited the paper; Stanciu C contributed in the conception, design and drafting of this paper, revised and gave the final approval for the version to be published.

Correspondence to: Carol Stanciu, MD, PhD, FRCP, Professor, "Gr. T. Popa" University of Medicine and Pharmacy, Institute of Gastroenterology and Hepatology, "St. Spiridon" University Hospital, Independenței 1, 700111 Iași, Romania. stanciucarol@yahoo.com
Telephone: +40-72-2306020 Fax: +40-23-2264411
Received: November 5, 2012 Revised: December 3, 2012
Accepted: December 20, 2012
Published online: January 28, 2013

Abstract

Liver cirrhosis is a major cause of mortality worldwide, often with severe sepsis as the terminal event. Over the last two decades, several studies have reported that in septic patients the adrenal glands respond inappropriately to stimulation, and that the treatment with corticosteroids decreases mortality in such patients. Both cirrhosis and septic shock share many hemodynamic abnormalities such as hyperdynamic circulatory failure, decreased peripheral vascular resistance, increased cardiac output, hypo-responsiveness to vasopressors, increased levels of proinflammatory cytokines [interleukine(IL)-1, IL-6, tumor necrosis factor-alpha] and it has, consequently, been reported that adrenal insufficiency (AI) is common in critically ill cirrhotic patients. AI may also be present in patients with stable cirrhosis without sepsis and in those undergoing liver transplantation. The term hepato-adrenal syndrome defines AI in patients with advanced liver disease with sepsis and/or other complications, and it suggests that it could be a feature of liver disease *per se*, with a different pathogenesis from that of septic shock. Relative AI is the term given to inadequate cortisol response to stress. More recently, another term is used, namely "critical illness related corticosteroid insufficiency" to define "an inadequate cellular corticosteroid activity for the severity of the patient's illness". The mechanisms of AI in liver cirrhosis are not completely understood, although decreased levels of high-density lipoprotein cholesterol and high levels of proinflammatory cytokines and circulatory endotoxin have been suggested. The prevalence of AI in cirrhotic patients varies widely according to the stage of the liver disease (compensated or decompensated, with or without sepsis), the diagnostic criteria defining AI and the methodology used. The effects of corticosteroid therapy on cirrhotic patients with septic shock and AI are controversial. This review aims to summarize the existing published information regarding AI in patients with liver cirrhosis.

Key words: Liver cirrhosis; Adrenal insufficiency; Septic shock; Corticosteroid therapy

Trifan A, Chiriac S, Stanciu C. Update on adrenal insufficiency in patients with liver cirrhosis. *World J Gastroenterol* 2013; 19(4): 445-456 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i4/445.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i4.445

INTRODUCTION

Adrenocortical dysfunction in patients with liver cirrhosis has been described for over half a century[1], but was ignored until a decade ago when several studies reported that some septic patients had an inappropriately low response of adrenal glands to stimulation, and treatment with corticosteroids decreased mortality[2,3]. Relative adrenal insufficiency (RAI) is the term given to inadequate production of cortisol with respect to the severity of

illness[4,5]. More recently, another term, namely critical illness related corticosteroid insufficiency (CIRCI) defined as "inadequate cellular corticosteroid activity for the severity of the patient's illness"[6], has been used. Despite a large number of published studies during recent years, the concepts of RAI and CIRCI are still under debate.

Liver cirrhosis is a major cause of mortality worldwide[7], often with septic shock as the terminal event[8]. It is a well-established fact that cirrhotic patients have increased susceptibility to bacterial infections[9]. Both cirrhosis and septic shock share many hemodynamic abnormalities such as hyperdynamic circulatory failure, decreased peripheral vascular resistance, decreased mean arterial pressure, increased cardiac output, hyporesponsiveness to vasopressors, increased levels of proinflammatory cytokines [interleukine (IL)-1, IL-6, tumor necrosis factor-α (TNF-α)][5,10,11] and, consequently, several studies reported that adrenal insufficiency (AI) is common in critically ill cirrhotic patients[8,12-14]. Furthermore, AI may occur in compensated and decompensated cirrhosis without sepsis[14-20] or in early and late post-liver transplantation (LT)[12,21-23]. Nowadays, liver cirrhosis is considered to be among the major groups of high-risk diseases with a predisposition to AI[24]. The term hepatoadrenal syndrome is used to define AI in patients with advanced liver disease with sepsis and/or other complications[12,15], suggesting that adrenocortical insufficiency may be a feature of liver disease *per se*, with a different pathogenesis from that occurring in septic shock.

Mechanisms of AI in cirrhotic patients are not entirely known, but they may include impaired synthesis in total cholesterol, high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol, as well as increased levels of proinflammatory cytokines and circulating endotoxin (e.g., lipopolysaccharide)[25-27]. The effects of corticosteroid therapy on cirrhotic patients with septic shock and AI are controversial, some studies reporting favorable results[12-14,28], while a recent randomized control study[29] has shown no benefit.

This review aims to summarize the existing published data regarding all aspects of AI prevalence, diagnosis and treatment in patients with liver cirrhosis.

PHYSIOLOGY OF THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS: A SHORT REVIEW

Cortisol is the main glucocorticoid secreted by the adrenal cortex under the control of adrenocorticotropic hormone (ACTH) which is released from the pituitary gland. The stimulus for ACTH release is corticotropin-releasing hormone (CRH) secreted by the paraventricular nuclei of the hypothalamus. Among factors influencing cortisol synthesis and production (diurnal rhythm of ACTH secretion, negative feedback by cortisol), stress plays the most important role. During stress and severe illness, activation of the hypothalamic-pituitary-adrenal (HPA) axis by the action of cytokines and other factors results in increased secretion of CRH, which will stimulate the production of ACTH and, consequently, increased release of cortisol into the circulatory system[30]. Cortisol is an essential component of the global adaptation to stress, contributing to the maintenance of cellular and organ homeostasis. Adequate levels of cortisol are absolutely necessary to increase cardiac output and vascular tonus, and to decrease proinflammatory cytokines (IL-1, IL-6, TNF-α) released[31,32] in order to overcome critical illness.

Over 90% of circulating cortisol is bound to corticosteroid-binding-globulin (CBG) (also called transcortin) and albumin, with less than 10% in the free biologically active form[33]. CBG is the predominant binding site (85%), with albumin binding smaller amounts of circulating cortisol. During severe sepsis, CBG levels fall, determining a higher percentage of free cortisol[34]. Hypoalbuminemia, frequently present in cirrhotic patients, has also been suggested to increase the free cortisol fraction[35,36]. Approximately 80% of circulating cortisol is synthesized both at rest and during stress from plasma cholesterol (particularly in the form of HDL cholesterol) and this could be relevant in patients with liver cirrhosis where cholesterol is low and may limit the synthesis of cortisol[26]. In the liver, cortisol is converted to its inactive metabolite cortisone by the enzyme 11β - hidroxysteroid dehydrogenase. After diffusion across the cell membrane, cortisol binds to glucocorticoid receptor and translocates into the nucleus of the cell[37] where its effects are exerted (increased vascular tonus and cardiac output, protein catabolism, lipolysis, hyperglycemia, and decreased cytokine production)[38]. These effects of cortisol are beneficial in critical illness, and several studies have shown that corticosteroid therapy is beneficial in patients with severe sepsis or septic shock[12-14,39,40]. As adrenal glands do not store cortisol, this must urgently be synthesized from its precursor, cholesterol, under any conditions of stress. In cirrhotic patients there is a low substrate (HDL cholesterol) for the synthesis of cortisol, favoring AI in conditions of stress[26].

PATHOGENESIS

Mechanisms leading to AI in liver cirrhosis remain largely unknown, although some hypotheses such as endotoxemia, decreased levels of apolipoprotein A-1, HDL cholesterol and LDL cholesterol, increased levels of proinflammatory mediators, structural damage to the adrenal gland due to infarction or hemorrhage, bacterial translocation of enteric organisms, "exhaustion" of the adrenal cortex, and glucocorticoid resistance have been suggested[12,41-49]. Many (if not all) of these pathophysiologic mechanisms are also involved in the genesis of AI in critically ill patients with sepsis[50-56].

As we have mentioned, cholesterol is the main source of steroidogenic substrate in the adrenal gland[26,57]. Several studies reported a significant decrease in the level

of serum HDL in cirrhotic patients which was related to the severity of the disease[12,26,47]. Furthermore, increased levels of circulating endotoxin (lipopolysaccharide) and TNF-α inhibit cortisol synthesis, limiting the delivery of HDL cholesterol to the adrenal gland[58-60]. In addition to this, TNF-α, IL-1 and IL-6 decrease hepatocyte synthesis of apolipoprotein A-1[58], the major component of HDL cholesterol. The lack of substrate for steroidogenesis will eventually lead to the so-called "adrenal exhaustion syndrome"[42] which contributes to AI in cirrhotic patients.

Besides low levels of serum total cholesterol, HDL-cholesterol and LDL-cholesterol, other factors may play a definite role in the pathogenesis of AI in patients with liver cirrhosis. Thus, coagulopathy (frequent in liver cirrhosis) may cause adrenal hemorrhage and infarction leading to structural damage of the adrenal gland[5], resulting in AI. Systemic inflammation is common in cirrhotic patients[61]. Bacterial translocation of enteric organisms has been demonstrated in patients with advanced liver cirrhosis[41,62].

A high prevalence of AI reported in patients with stable cirrhosis[15-19,63], similar to that reported in cirrhosis complicated by sepsis/septic shock, suggests that AI may be a feature of liver disease *per se*, with a different pathogenesis from that occurring in septic shock. These findings are consistent with the observations of Marik *et al*[12] who put forward the term hepato-adrenal syndrome in order to define AI in patients with advanced liver disease.

DIAGNOSIS

Diagnosis of AI made on clinical grounds in critically ill cirrhotic patients is impossible because of the lack of typical addisonian features[5,13]. Hypotension refractory to vasopressors and fluid resuscitation is the most important clinical sign in such patients[52]. Therefore, the diagnosis of AI in patients with liver cirrhosis is based on the following laboratory tests.

Standard dose

Measurement of serum total cortisol, either at baseline or following stimulation by the standard dose-short synacthen test (SD-SST) or low dose-short synacthen test (LD-SST). Baseline serum total cortisol levels under 414 nmol/L[8,13,20,64-66], < 250 nmol/L[45] or < 138 nmol/L[67] have been used to define AI in different studies. The following thresholds were used to diagnose subnormal response to SD-SST or LD-SST: (1) a peak cortisol level (defined as the highest cortisol concentration after synacthen stimulation) < 690 nmol/L[16], < 552 nmol/L[12], < 500 nmol/L[14,15,18,45], < 442 nmol/L[17]; and (2) a delta cortisol (defined as the difference between peak and basal cortisol) less than 250 nmol/L[8,13,15-20,45,64-67].

As one can easily see, there are differences in the thresholds of serum total cortisol used to define AI in published studies, which may explain significant discrepancies in the prevalence of AI in cirrhotic patients. Moreover, the diagnosis of AI based on serum total cortisol in patients with cirrhosis may be inaccurate due to changes in serum concentrations of CBG and albumin (both synthesized in the liver) which are usually low[68-70]. It has been already shown that low levels of CBG and albumin lead to overestimation of the diagnosis of AI[45,67]. As we have mentioned before, over 90% of serum circulating cortisol is bound to CBG and albumin, with less than 10% in the free form. Standard laboratory assays of serum total cortisol measure the bound plus free fractions. This means that a decrease in the binding protein levels, as it often happens in cirrhosis, will reduce serum total cortisol, affecting the interpretation of SD-SST/LD-SST[35,44], and this may lead to the overestimation of AI in cirrhotic patients[45]. However, most of the studies evaluating adrenal function in critically ill patients with liver cirrhosis still rely on the measurement of serum total cortisol, both at baseline and after stimulation.

Serum free cortisol assays are considered the most reliable method to assess adrenal function in critically ill patients[71]. There are several methods used to measure serum free cortisol (gel filtration, ultrafiltration, equilibrium dialysis)[72], all of them expensive and inconvenient for routine clinical practice[73]. In patients with liver cirrhosis, the serum free cortisol level is not altered by a reduced concentration of CBG and albumin[74] and it therefore appears to be a more appropriate marker for assessing adrenal function in such patients[44,74]. Some studies reported significant differences in diagnosis of AI using serum total cortisol and free cortisol criteria in cirrhotic patients with septic shock[75] or in those with stable cirrhosis[15], while others found that assessing serum free cortisol had limited additive diagnostic value over serum total cortisol[76]. Serum free cortisol levels under 50 nmol/L at baseline or less than 86 nmol/L after synacthen stimulation are suggestive for the diagnosis of AI (in critically ill patients)[35], although the reference range for baseline values in healthy subjects varies from 8-25 nmol/L[71] to 12-70 nmol/L[44,77].

Due to the limitations of available assays to estimate serum free cortisol, surrogate markers may be used, such as Coolens equation "$U^2 \times K (1 + N) + U [1 + N + K (G - T)] - T = 0$", where T is total cortisol, G is CBG, U is unbound cortisol, K is the affinity of CBG for cortisol at 37 ℃ and N is the ratio of albumin-bound to unbound cortisol[68], free cortisol index (FCI) (serum total cortisol concentration divided by CBG level)[78], and salivary cortisol[71,79]. However, Coolens equation and FCI do not take into account the concentration of low serum albumin and CBG frequently present in cirrhotic patients and, therefore, both surrogates may not be suitable to estimate serum free cortisol in such patients[69-71]. By contrast, salivary cortisol, regardless of serum binding protein levels, correlates well with free cortisol levels[71,79]. Basal value of salivary cortisol < 1.8 ng/mL or a concentration after stimulation (SD-SST) < 12.7 ng/mL, an increment < 3 ng/mL[45] or a peak serum free cortisol < 33 nmol/L[15] are suggestive of AI. However, there are

significant variations in normal salivary cortisol values reported by different studies[74]. Other limits of salivary cortisol are represented by oral candidiasis, low salivary flow, and contaminated salivary samples from gingival bleeding, common in cirrhotic patients[44].

SD-SST

SD-SST measures total serum cortisol at baseline and 60 min after an intravenous injection of 250 μg of synthetic ACTH. Currently, there are two corticotropic analogues that can be used, namely tetracosactrin (synacthen, Novartis Pharma AG, Basel, Switzerland) and cosyntropin (Cortrosyn, Amphastar Pharmaceuticals, Rancho Cucamonga, CA, United States). Using a supraphysiological dose of 250 μg of corticotropin (which results in approximately 100 times higher than normal maximal stress ACTH levels)[17], SD-SST is not a "physiological test"[17,80]. In the context of critical illness, AI was defined by the International Task Force[6] as a delta cortisol of < 250 nmol/L (< 9 μg/dL) after SD-SST or a random serum total cortisol of < 276 nmol/L (< 10 μg/dL). There is no consensus on the diagnostic criteria of AI in cirrhotic patients, although a delta cortisol under 250 nmol/L has been used by most studies to define AI in such patients[81].

LD-SST

LD-SST uses 1 μg of synacthen given intravenously, and serum cortisol measured after 20 and 30 min (the latter time-point is mostly used). The normal response is a serum cortisol level > 500 nmol/L (> 18 μg/dL)[49]. In a meta-analysis[82] comprising the diagnostic value of SD-SST and LD-SST for diagnosing AI, LD-SST was found to be superior, contrary to another meta-analysis[83] which reported similar operative characteristics for both tests. LD-SST seems to be a more physiological and sensitive test than SD-SST for the diagnosis of AI, and appropriate for use in non-critically ill cirrhotic patients[49].

Insulin-induced hypoglycemia test

Insulin-induced hypoglycemia test (IIHT) has been considered to be the gold standard to evaluate the HPA axis. The test uses injection of 0.15 IU/kg regular insulin to achieve blood glucose less than 40 mg/dL or until symptoms of hypoglycemia develop. Blood samples are taken before and at 15, 30, 45, 60, 90 min post-stimulation. Peak cortisol cut points between 500 and 550 nmol/L (18-20 μg/dL) are used for the diagnosis of adrenal sufficiency. This test is contraindicated in patients with cardio- or cerebrovascular diseases and convulsive disorders. In addition, the IIHT is unpleasant for the patients and therefore it has been replaced by alternative tests (LS-SST, SD-SST) for evaluating the HPA axis[84].

Corticotrophin-releasing hormone test

Corticotrophin-releasing hormone test (CRHT) evaluates the entirety of the HPA axis. Blood samples for the measurement of ACTH and cortisol are taken at baseline and at 15, 30, 45 and 60 min after an intravenous injection of 1 μg/Kg of CRH. Although CRHT is free of serious side effects, it is both difficult and costly and therefore it has been used in few studies in liver disease.

To conclude, in the absence of a gold standard test, SD-SST remains the most used test to assess the adrenal function in critically ill cirrhotic patients, while LD-SST seems to be more appropriate in those with stable cirrhosis. At present, serum free cortisol and salivary cortisol are the most accurate methods for the diagnosis of AI in cirrhotic patients, but cannot be used in routine clinical practice. The use of salivary cortisol needs to be validated. As diagnosis of AI in cirrhotics is of major clinical importance, there is an urgent need for a consensus as to which is the most appropriate diagnostic test of AI in such category of patients.

PREVALENCE AND EXISTING EVIDENCE

Initial reports on AI in liver cirrhosis were followed by multiple studies (Tables 1 and 2) and, recently, by excellent systematic reviews[43,44,46,49,81]. There are significant discrepancies between studies on the prevalence of AI in patients with liver cirrhosis, mainly because of the different tests used for diagnosis of adrenal dysfunction and the criteria applied to define AI. Thus, the prevalence of AI varies between critically ill cirrhotic patients (10%-87%; Table 1), those with stable cirrhosis (7%-83%; Table 2), and patients with liver transplant (61%-92%; Table 1). Overall, several published studies have reported a high prevalence of AI both in critically and non-critically ill cirrhotic patients[17,29,63,64,69,85] as well as in those who had received liver transplant[12].

Critically ill patients with liver cirrhosis

Almost all studies that included critically ill patients with liver cirrhosis[8,13,20,29,64-66,74,85] used SD-SST for the diagnosis of AI and only two performed LD-SST[12,16]. With SD-SST, the reported prevalence of AI in critically ill cirrhotics varied between 10%[74] and 87%[85], while with LD-SST, the prevalence range was between 33%[12] and 60%[16].

Harry *et al*[14] reported a prevalence of AI (defined as peak cortisol levels less than 500 nmol/L) of 69% in critically ill cirrhotic patients requiring vasopressor support. In a prospective study including 25 cirrhotic patients with severe sepsis, Fernández *et al*[13] reported a very high incidence of AI (68%) using SD-SST and defining AI either as baseline serum total cortisol level less than 414 nmol/L or a delta cortisol lower than 250 nmol/L in those with a baseline concentration below 966 nmol/L. The AI prevalence rate was correlated with the severity of liver disease (76% Child-Pugh C *vs* 25% Child-Pugh B).

SD-SST was also used to evaluate adrenal function in a prospective study which included 101 critically ill patients with cirrhosis and severe sepsis[8]. Authors found that 51% of their patients met the criteria for AI (defined as baseline serum total cortisol values under 414 nmol/L

Table 1 Prevalence of adrenal insufficiency in critically ill patients with liver cirrhosis

Ref.	No. of patients (type of cirrhosis)	Diagnosis and definition of AI	Prevalence of AI
Harry *et al*[14]	20 (ALF/CLD)	SD-SST: Peak cortisol < 500 nmol/L[1]	69%
Marik *et al*[12]	340	LD-SST: Peak cortisol < 552 nmol/L or	72%
	(ALF: 24)	random cortisol level < 414 nmol/L in non-stressed patients or	33%
	(CLD: 146)	random cortisol level < 552 nmol/L in stressed patients	66%
	(recent LT: 119)		92%
	(remote LT: 51)		61%
Tsai *et al*[8]	101 (cirrhosis+ severe sepsis)	SD-SST: Baseline cortisol < 414 nmol/L or delta cortisol < 250 nmol/L if baseline cortisol between 414 and 938 nmol/L	51%
Fernandez *et al*[13]	25 (cirrhosis + septic shock)	SD-SST: Baseline cortisol < 414 nmol/L or delta cortisol < 250 nmol/L if baseline cortisol between 414 and 966 nmol/L	68%
Thierry *et al*[64]	14 (cirrhosis + septic shock)	SD-SST: Baseline cortisol < 414 nmol/L; delta cortisol < 250 nmol/L	77%
du Cheyron *et al*[65]	50 (critically ill cirrhosis)	SD-SST: Baseline cortisol < 414 nmol/L; delta cortisol < 250 nmol/L if baseline cortisol between 414 and 938 nmol/L	82%
Vasu *et al*[86]	24 (critically ill cirrhotics)	SD-SST: Definition of AI was not reported	62%
Arabi *et al*[29]	75 (cirrhosis + septic shock)	SD-SST: Delta cortisol < 250 nmol/L	76%
Mohamed *et al*[85]	15 (cirrhosis+septic shock)	SD-SST: Definition of AI was not reported	87%
Thevenot *et al*[74]	30 (cirrhosis + sepsis)	SD-SST: Peak serum total cortisol < 510 nmol/L	10%
Acevedo *et al*[89]	166 (decompensated cirrhosis)	SD-SST: Delta cortisol < 250 nmol/L	26%
Graupera *et al*[20]	37 (severe acute bleeding)	SD-SST: Baseline cortisol < 414 nmol/L and/or delta cortisol < 250 nmol/L	38%
Triantos *et al*[16]	20 (cirrhosis with variceal bleeding)	SD-SST: Baseline cortisol < 276 nmol/L or delta cortisol < 250 nmol/L	30%
		LD-SST: Peak serum cortisol < 690 nmol/L or a delta cortisol < 250 nmol/L	60%
El Damarawy *et al*[66]	45 (cirrhosis with septic shock or HRS, cirrhosis without septic shock or HRS)	SD-SST: Baseline cortisol < 414 nmol/L or delta cortisol < 250 nmol/L in patients with baseline cortisol < 966 nmol/L	73%

[1]To convert serum total cortisol concentrations from nanomoles per liter to micrograms per deciliter divide by 27.59[79]. ALF: Acute liver failure; CLD: Chronic liver disease; HRS: Hepatorenal syndrome; LT: Liver transplant; AI: Adrenal insufficiency; SD-SST: Standard dose short synacthen test; LD-SST: Low dose short synacthen test.

or delta cortisol lower than 250 nmol/L with a baseline value between 414 and 938 nmol/L) which was related to disease severity [Child-Pugh and model for end-stage liver disease (MELD) scores] and increased mortality. Recently, Arabi *et al*[29], using the same test (SD-SST) and definition for AI (delta cortisol < 250 nmol/L) in a similar group of critically ill patients (cirrhosis with septic shock) reported an even higher AI prevalence rate (76%).

The SD-SST test was also used in several other studies to assess adrenal function in critically ill cirrhotic patients[64-66,74,85,86].

Adrenal function has also been evaluated by SD-SST in cirrhotic patients with variceal bleeding[16,20]. Graupera *et al*[20] reported AI prevalence (defined as baseline serum cortisol < 414 nmol/L or delta cortisol < 250 nmol/L) in 38% of bleeding patients. AI was associated with increased risk of failure to control bleeding and lower survival rate at 6 wk. In a prospective observational study on 20 cirrhotic patients with variceal bleeding and 60 with stable cirrhosis, Triantos *et al*[16] reported an AI prevalence rate (defined as basal cortisol < 276 nmol/L or delta cortisol < 250 nmol/L following SD-SST) of 30% (similar to that in stable cirrhosis); with the use of LD-SST, AI prevalence (defined as a peak cortisol < 690 nmol/L or a delta cortisol < 250 nmol/L) was significantly higher in bleeders (60%) than in stable cirrhotics (48%).

LD-SST was also previously used by Marik *et al*[12] to evaluate adrenal function in 340 critically ill patients with liver disease (24 with fulminant hepatic failure, 146 critically ill cirrhotics, 51 with remote LT, and 119 having recently undergone LT). AI was defined as having a random cortisol level of < 552 nmol/L in highly stressed patients (hypotension, hepatic failure, respiratory failure) and a random cortisol level of < 414 nmol/L or a 30 min post LD-SST level of < 552 nmol/L in all other patients. Out of 340 patients studied, 245 (72%) met the criteria for AI (33% fulminant hepatic failure, 66% critically ill cirrhotics, 61% remote LT, 92% recent LT).

Non-critically ill cirrhotics

AI is also common in patients with stable liver cirrhosis (Table 2). However, as in critically ill cirrhotic patients, AI prevalence rate in those with stable liver cirrhosis varies significantly, depending on the diagnostic test used.

In a prospective study, Tan *et al*[15] evaluated adrenal function in 43 clinically stable cirrhotic patients. All patients underwent SD-SST, and AI was defined by delta cortisol < 250 nmol/L or a peak total cortisol < 500 nmol/L, or a peak serum free cortisol < 33 nmol/L. The prevalence of AI was 47% using delta cortisol < 250 nmol/L, 39% using peak total cortisol < 500 nmol/L, and 12% with serum free cortisol < 33 nmol/L. This study clearly shows that the reported prevalence of AI depends largely on the diagnostic test used and criteria for defining AI.

Galbois *et al*[45] have evaluated adrenal function in 88 patients hospitalized for complications of cirrhosis without bleeding and shock. Salivary and serum total cortisol were assessed 60 min before and after stimulation with SD-SST in all patients. Serum free cortisol was estimated

Table 2 Prevalence of adrenal insufficiency in patients with liver cirrhosis, not critically ill

Ref.	No. of patients (type of cirrhosis)	Diagnosis and definition of AI	Prevalence of AI
McDonald *et al*[69]	38 (stable cirrhosis)	IIHT: Reduction in maximal increments of plasma cortisol	64%
		SD-SST: Reduction in maximal increments of plasma cortisol	39%
Zietz *et al*[112]	52 (stable cirrhosis)	CRHT: Peak cortisol < 550 nmol/L or an increase < 250 nmol/L[1]	58%
		rise of plasma ACTH < twice the baseline	42%
Sigalas *et al*[87]	47 (stable cirrhosis)	SD-SST: Baseline cortisol < 250 nmol/L and delta cortisol < 250 nmol/L	36%
Alessandria *et al*[88]	25 (cirrhosis and ascites)	SD-SST: Delta cortisol < 250 nmol/L	36%
Jang *et al*[63]	18 (stable cirrhosis)	SD-SST: Baseline cortisol < 414 nmol/L delta cortisol < 250 nmol/L	83%
Acevedo *et al*[19]	198 (10 compensated and	SD-SST: Baseline cortisol < 414 nmol/L	64%
	188 decompensated cirrhosis)	delta cortisol < 250 nmol/L	27%
Galbois *et al*[45]	88 (stable cirrhosis)	SD-SST: (1) Serum total cortisol: Baseline cortisol < 250 nmol/L or peak cortisol < 500 nmol/L or delta cortisol < 250 nmol/L	33%
		(2) Salivary cortisol: Basal salivary cortisol < 1.8 ng/mL or post-stimulation values < 12.7 ng/mL or increase values < 3 ng/mL	9%
Tan *et al*[15]	43 (stable cirrhosis)	SD-SST: Peak total cortisol < 500 nmol/L;	39%
		delta cortisol < 250 nmol/L;	47%
		peak plasma free cortisol < 33 nmol/L;	12%
		any set of criteria	58%
Thevenot *et al*[67]	95 (stable cirrhosis)	LD-SST: Baseline cortisol < 138 nmol/L;	7%
		< 440 nmol/L after stimulation;	19%
		⩽ 500 nmol/L after stimulation;	27%
		delta cortisol < 250 nmol/L	49%
Fede *et al*[17]	101 (stable cirrhosis)	LD-SST: Peak serum cortisol < 500 nmol/L;	38%
		peak serum cortisol < 442 nmol/L;	29%
		delta cortisol < 250 nmol/L	60%
Triantos *et al*[16]	60 (stable cirrhosis)	SD-SST: Peak serum cortisol < 500 nmol/L	30%
		LD-SST: Peak serum cortisol < 500 nmol/L	48%
Mohamed *et al*[85]	15 (stable cirrhosis)	SD-SST: Definition of AI was not reported	53%
Risso *et al*[18]	85 (cirrhosis with ascites, without sepsis or shock)	SD-SST: Delta cortisol < 250 nmol/L and/or peak cortisol < 500 nmol/L	39%
Vincent *et al*[73]	26 (liver impairment)	SD-SST: Serum total cortisol < 550 nmol/L;	46%
		free cortisol index < 12	13%

[1]To convert serum total cortisol concentrations from nanomoles per liter to micrograms per deciliter divide by 27.59[79]. AI: Adrenal insufficiency; SD-SST: Standard dose short synacthen test; LD–SST: Low dose short synacthen test; CRHT: Corticotropin-releasing hormone test; IIHT: Insulin-induced hypoglycemia test; ACTH: Adrenocorticotropic hormone.

from serum total cortisol and CBG levels using Coolens' formula[68]. The following definitions of AI were used by the authors: (1) according to serum total cortisol assays: baseline < 250 nmol/L, or a peak total cortisol < 500 nmol/L, or delta cortisol < 250 nmol/L; (2) according to salivary cortisol assays: baseline < 1.8 ng/mL, or an increase < 3 ng/mL or a concentration < 12.7 ng/mL after stimulation. The results indicated a significant difference in AI prevalence depending on the test used: 33% when serum total cortisol was considered *vs* 9.1% using salivary cortisol.

Another study performed by Thevenot *et al*[74] has demonstrated that assessment of adrenal function with measurements of serum total cortisol overestimated AI prevalence in cirrhotic patients. In this study, baseline and post-synacthen serum total cortisol, serum free cortisol and salivary cortisol concentrations were measured in 125 cirrhotic patients (95 non-septic, 30 septic). AI was defined as serum total cortisol < 510.4 nmol/L after SD-SST. AI was found in nine patients (7.2%) (6 non-septic; 3 septic) and restricted to cirrhotics with Child-Pugh C. Serum total cortisol concentrations, CBG and albumin levels significantly decreased in non-septic patients as liver function deteriorated (from Child-Pugh A to C). Cirrhotic patients with or without AI had similar basal serum free cortisol and salivary cortisol levels. As the serum total cortisol level overestimated the prevalence of AI in cirrhotic patients, and serum free cortisol is not suitable for routine laboratory use, authors concluded that measurement of salivary cortisol is a useful approach in such patients. The same group of investigators[67] analyzed only the 95 hemodynamically stable cirrhotic patients from the previously mentioned study, who underwent a LD-SST. The serum total cortisol and serum free cortisol concentrations were measured 30 min before and after LD-SST. AI was defined as: (1) basal serum total cortisol < 138 nmol/L and < 440 nmol/L after stimulation; (2) serum total cortisol < 500 nmol/L after stimulation; and (3) cortisol increment < 250 nmol/L. AI prevalence rates varied significantly according to the threshold used: 7.4 % with basal serum total cortisol, 19% using serum cortisol < 440 nmol/L, 27.4 % with serum cortisol < 500 nmol/L, and 49.4% with delta cortisol. Serum free cortisol levels before and after LD-SST stimulation were higher in the more severe cirrhotic patients regardless of CBG and albumin concentrations, and directly associated with the risk of non-transplant-related mortality in hemodynamically stable patients with cirrhosis.

In opposition to the above mentioned studies, recently, in a prospective study, Molenaar *et al*[76], using SD-SST, assessed the value of free *vs* total cortisol levels while evaluating AI in 49 septic and 63 non-septic patients with treatment-insensitive hypotension and found that total cortisol correlated with free cortisol during critical illness. Moreover, in sepsis, hypoalbuminemia did not affect total and free cortisol, contrary to the findings of other published studies[45,67].

Others, using SD-SST or LD-SST to diagnose adrenal dysfunction in patients with stable liver cirrhosis reported high AI prevalence rates[16-19,63,69,73,85,87,88]. Fede *et al*[17] reported an AI prevalence of 38% in 101 patients with stable cirrhosis (absence of infections or hemodynamic instability). AI, defined as a peak serum total cortisol level < 500 nmol/L after LD-SST, was correlated with the severity of liver disease graded according to Child-Pugh or MELD scores.

Using SD-SST in 85 cirrhotics with ascites but without sepsis, Risso *et al*[18] reported AI (delta cortisol < 250 nmol/L and/or peak cortisol < 500 nmol/L) in 39% of patients.

Vincent *et al*[73] evaluated adrenal function by SD-SST in 26 patients with liver impairment. Authors defined AI as serum total cortisol < 550 nmol/L or FCI < 12. Three patients (13%) met both criteria, 12 patients (46%) had a serum total cortisol < 550 nmol/L but an FCI > 12. When serum total cortisol was used, 46% of patients had AI, while when using FCI only 13% fulfilled the criteria for AI. Authors suggested that FCI is better suited for the evaluation of AI in patients with liver impairment.

Acevedo *et al*[19], using SD-SST, evaluated the prevalence of AI in 198 patients with liver cirrhosis [10 with compensated, 188 with decompensated cirrhosis and complications (hepatic encephalopathy, spontaneous bacterial peritonitis, ascites, gastrointestinal bleeding, hepatorenal syndrome)]. AI defined as basal serum total cortisol < 414 nmol/L was found in 64% of patients, and only in 27% when delta cortisol < 250 nmol/L was used, with no differences between compensated and decompensated cirrhosis. The same group of researchers evaluated the prevalence and prognostic value of AI in 166 patients with advanced cirrhosis (no severe sepsis or septic shock)[89]. AI, defined as delta cortisol < 250 nmol/L after SD-SST, was found in 26% of patients. Those with AI had a higher degree of circulatory dysfunction, greater prevalence of systemic inflammatory response syndrome, increased probability to develop severe infections, and higher hospital mortality rates than patients without AI.

AI after LT

AI has been reported both early as well as late after LT[12,21-23,90].

With LD-SST, Marik *et al*[12] found that 92% of 119 patients undergoing recent LT and maintained on steroid-free immunosuppressive regimens had AI. The steroid-free immunosuppressive regimen may expose patients undergoing LT to an increased risk for AI, while the use of steroids intra and postoperatively in LT may reduce such a risk or mask an AI[46]. Furthermore, LD-SST is not recommended for the diagnosis of AI in high-stress conditions like LT[6] as it may lead to an overestimated AI prevalence in such patients.

Toniutto *et al*[21], using SD-SST, reported an AI prevalence rate of 26% in 87 patients having received LT for end-stage liver disease and maintained on prolonged immunosuppressive treatment.

Patel *et al*[90] reported significantly reduced requirements for fluid, vasopressors, invasive ventilation, and renal replacement therapy, and intensive care unit stay for patients undergoing LT who received 1000 mg methylprednisolone prior to the liver graft reperfusion.

TREATMENT

Cortisol has several beneficial effects such as an increase of the vascular tonus and cardiac output, enhancement of catecholamine responsiveness, inhibition of the production of nitric oxide, modulation of cytokine production in septic shock[32,91-97], but the effects of corticosteroid therapy in sepsis, severe sepsis and septic shock remain, however, controversial. Thus, a significant reduction in mortality rate with hydrocortisone therapy in patients with septic shock has been reported in several studies and meta-analyses[6,28,39,98-101], while others have shown no effect on the 28-d mortality rate[14,29,102]. Both doses and duration of corticosteroid therapy vary significantly in published studies[6,28,39,40,102,103]. Thus, some used a daily dose of hydrocortisone (or equivalent) of 200-300 mg ("low-dose", also called "physiologic-dose" or "stress-dose")[3,28,39,98,100-105] while others used a "supra-physiologic" dose (> 300 mg)[98,106-108].

None of the early studies using high doses of corticosteroids for short courses reported any benefit[98,106-108], while more recent studies using a "physiologic-dose" for longer durations have shown a significant reduction in vasopressor agents requirement and in intensive care unit length of stay, greater shock resolution, and decreased mortality[6,28,39,98,100,104,105,109-111]. A randomized, double-blind placebo controlled trial, CORTICUS (Corticosteroid Therapy of Septic Shock)[102] including 499 patients with septic shock randomized to hydrocortisone (50 mg intravenously every 6 h for 5 d, followed by 50 mg intravenously every 12 h for 3 d, and then by 50 mg daily for 3 d) or placebo, concluded that there was no benefit in terms of mortality, although steroid administration was associated with a greater shock reversal, but also with a higher incidence of episodes of new infections. On the other hand, Annane *et al*[28] in a randomized, double-blind controlled trial have found that the administration of hydrocortisone (50 mg intravenously every 6 h) and oral fludrocortisone (50 μg once daily) in patients with refractory septic shock and AI (delta cortisol < 250 nmol/L) resulted in a 30% decrease in 28-d mortality. It should be mentioned that consensus statements from an international task force[6] recommended corticosteroid therapy

Table 3 Published studies on corticosteroid therapy in patients with liver cirrhosis

Ref.	No. of patients (type of cirrhosis)	Study design	Steroid dose	Outcomes
Harry *et al*[14]	20 (ALF or ACLF)	Retrospective	Hydrocortisone 300 mg/d	Reduction in vasopressor doses, but higher incidence of infection and no survival benefit
Marik *et al*[12]	140 (ALF or CLD)	Not RCT	Hydrocortisone 300 mg/d	Reduction in the dose of norepinephrine at 24 h, and lower mortality rate increased survival
Fernandez *et al*[13]	17 (cirrhosis and septic shock)	Prospective but not RCT	Hydrocortisone 200 mg/d	Significant increase in shock resolution and high hospital survival rate
Arabi *et al*[29]	39 (cirrhosis and septic shock)	RCT	Hydrocortisone 200 mg/d	Reduction in vasopressor doses and higher rates of shock reversal, but no benefit in 28 d mortality, increase in gastrointestinal bleeding and shock relapse

ALF: Acute liver failure; ACLF: Acute-on-chronic liver failure; CLD: Chronic liver disease; RCT: Randomized controlled trial.

(intravenous hydrocortisone 200-300 mg/d in four divided doses for a week before tapering slowly) in patients with vasopressor-dependant septic shock.

Like in patients with severe sepsis/septic shock with other causes than liver cirrhosis, as mentioned above, the effects of steroid therapy in cirrhotic patients with AI remain controversial, some studies reporting beneficial results[12-14] while a recent randomized control study[29] has shown no benefit (Table 3).

Harry *et al*[14] evaluated the effects of stress doses of hydrocortisone in a retrospective comparative study including 40 patients. Twenty patients received hydrocortisone (300 mg/d) for 4-5 d. In patients with acute-on-chronic liver failure requiring norepinephrine support, the results showed a reduction in vasopressor doses, but no survival benefit; moreover, corticosteroid therapy was associated with a significant increase in infections.

Another study, carried out by Marik *et al*[12] evaluated the effect of 300 mg/d hydrocortisone given intravenously in vasopressor-dependant patients with acute or chronic liver disease. In patients with AI, treatment with hydrocortisone was associated with a significant reduction of the norepinephrine dosage at 24 h and with a lower mortality ($P = 0.02$), whereas in those patients without AI hydrocortisone did not affect the norepinephrine dose.

Fernández *et al*[13], in a prospective but non-randomized study have evaluated adrenal function by SD-SST and the effects of low-dose hydrocortisone in 25 patients with advanced cirrhosis and septic shock. Patients with AI received intravenous hydrocortisone (50 mg every 6 h) and results were compared with those obtained from a retrospective 50 cirrhotic patients with septic shock in whom adrenal function was not investigated and who did not receive corticosteroid therapy. Results showed that hydrocortisone therapy was associated with a significant increase in shock resolution and hospital survival rate. Authors suggested that all cirrhotic patients with AI should be treated with hydrocortisone.

Recently, Arabi *et al*[29] in a randomized controlled trial, have shown that low dose hydrocortisone therapy in cirrhotic patients with septic shock had a significant reduction in vasopressor doses and higher rates of shock reversal, but it did not reduce mortality and was associated with an increase in adverse effects (gastrointestinal bleeding) and shock relapse.

Based on the above mentioned studies, there are still several unsolved problems and questions awaiting answers. Thus, re-evaluation of both doses and duration of corticosteroid therapy is necessary. Obviously, further prospective randomized clinical studies are needed to assess the effect of corticosteroid therapy in critically ill cirrhotic patients with AI.

CONCLUSION

AI occurs frequently in patients with liver cirrhosis both during critical illness and in stable disease. Studies, however, do not agree on the prevalence of AI in cirrhotic patients, mostly because of the different criteria and the methodology used to define AI. Diagnosis of AI in patients with liver cirrhosis remains controversial (particularly in those critically ill) as all diagnostic tests proved their limitations. Pathogenesis of AI in liver cirrhosis is still unknown, although decreased levels of cholesterol (mainly HDL cholesterol) and increased levels of pro-inflammatory cytokines and circulating endotoxin have been put forward. Some data suggest that AI may be a feature of cirrhosis *per se*, with a pathogenesis subtly different from that occurring in septic shock from other causes. Yet, there is still controversy in what concerns treatment with corticosteroids, although some cirrhotic patients with vasopressor resistant shock may benefit. However, further prospective, randomized clinical trials are necessary to assess the effect of corticosteroid therapy in critically ill patients with cirrhosis.

REFERENCES

1 **Peterson RE**. Adrenocortical steroid metabolism and adrenal cortical function in liver disease. *J Clin Invest* 1960; **39**: 320-331 [PMID: 14432260 DOI: 10.1172/JCI104043]
2 **Annane D**, Sébille V, Troché G, Raphaël JC, Gajdos P, Bellissant E. A 3-level prognostic classification in septic shock based on cortisol levels and cortisol response to corticotropin. *JAMA* 2000; **283**: 1038-1045 [PMID: 10697064 DOI: 10.1001/jama.283.8.1038]
3 **Annane D**, Bellissant E, Bollaert PE, Briegel J, Keh D, Kupfer Y. Corticosteroids for severe sepsis and septic shock: a systematic review and meta-analysis. *BMJ* 2004; **329**: 480

[PMID: 15289273 DOI: 10.1136/bmj.38181.482222.55]
4 **Rothwell PM**, Udwadia ZF, Lawler PG. Cortisol response to corticotropin and survival in septic shock. *Lancet* 1991; **337**: 582-583 [PMID: 1671944 DOI: 10.1016/0140-6736(91)91641-7]
5 **Cooper MS**, Stewart PM. Corticosteroid insufficiency in acutely ill patients. *N Engl J Med* 2003; **348**: 727-734 [PMID: 12594318 DOI: 10.1056/NEJMra020529]
6 **Marik PE**, Pastores SM, Annane D, Meduri GU, Sprung CL, Arlt W, Keh D, Briegel J, Beishuizen A, Dimopoulou I, Tsagarakis S, Singer M, Chrousos GP, Zaloga G, Bokhari F, Vogeser M, American College of Critical Care Medicine. Recommendations for the diagnosis and management of corticosteroid insufficiency in critically ill adult patients: consensus statements from an international task force by the American College of Critical Care Medicine. *Crit Care Med* 2008; **36**: 1937-1949 [PMID: 18496365 DOI: 10.1097/CCM.0b013e31817603ba]
7 **Méndez-Sánchez N**, Villa AR, Zamora-Valdés D, Morales-Espinosa D, Uribe M. Worldwide mortality from cirrhosis. *Ann Hepatol* 2007; **6**: 194-195 [PMID: 17786150]
8 **Tsai MH**, Peng YS, Chen YC, Liu NJ, Ho YP, Fang JT, Lien JM, Yang C, Chen PC, Wu CS. Adrenal insufficiency in patients with cirrhosis, severe sepsis and septic shock. *Hepatology* 2006; **43**: 673-681 [PMID: 16557538 DOI: 10.1002/hep.21101]
9 **Gustot T**, Durand F, Lebrec D, Vincent JL, Moreau R. Severe sepsis in cirrhosis. *Hepatology* 2009; **50**: 2022-2033 [PMID: 19885876 DOI: 10.1002/hep.23264]
10 **Bouachour G**, Tirot P, Varache N, Gouello JP, Harry P, Alquier P. Hemodynamic changes in acute adrenal insufficiency. *Intensive Care Med* 1994; **20**: 138-141 [PMID: 8201094 DOI: 10.1007/BF01707669]
11 **Blendis L**, Wong F. The hyperdynamic circulation in cirrhosis: an overview. *Pharmacol Ther* 2001; **89**: 221-231 [PMID: 11516477 DOI: 10.1016/S0163-7258(01)00124-3]
12 **Marik PE**, Gayowski T, Starzl TE, Hepatic Cortisol Research and Adrenal Pathophysiology Study Group. The hepatoadrenal syndrome: a common yet unrecognized clinical condition. *Crit Care Med* 2005; **33**: 1254-1259 [PMID: 15942340 DOI: 10.1097/01.CCM.0000164541.12106.57]
13 **Fernández J**, Escorsell A, Zabalza M, Felipe V, Navasa M, Mas A, Lacy AM, Ginès P, Arroyo V. Adrenal insufficiency in patients with cirrhosis and septic shock: Effect of treatment with hydrocortisone on survival. *Hepatology* 2006; **44**: 1288-1295 [PMID: 17058239 DOI: 10.1002/hep.21352]
14 **Harry R**, Auzinger G, Wendon J. The effects of supraphysiological doses of corticosteroids in hypotensive liver failure. *Liver Int* 2003; **23**: 71-77 [PMID: 12654129 DOI: 10.1034/j.1600-0676.2003.00813.x]
15 **Tan T**, Chang L, Woodward A, McWhinney B, Galligan J, Macdonald GA, Cohen J, Venkatesh B. Characterising adrenal function using directly measured plasma free cortisol in stable severe liver disease. *J Hepatol* 2010; **53**: 841-848 [PMID: 20739086 DOI: 10.1016/j.jhep.2010.05.020]
16 **Triantos CK**, Marzigie M, Fede G, Michalaki M, Giannakopoulou D, Thomopoulos K, Garcovich M, Kalafateli M, Chronis A, Kyriazopoulou V, Jelastopoulou E, Nikolopoulou V, O'Beirne J, Burroughs AK. Critical illness-related corticosteroid insufficiency in patients with cirrhosis and variceal bleeding. *Clin Gastroenterol Hepatol* 2011; **9**: 595-601 [PMID: 21545846 DOI: 10.1016/j.cgh.2011.03.033]
17 **Fede G**, Spadaro L, Tomaselli T, Privitera G, Piro S, Rabuazzo AM, Sigalas A, Xirouchakis E, O'Beirne J, Garcovich M, Tsochatzis E, Purrello F, Burroughs AK. Assessment of adrenocortical reserve in stable patients with cirrhosis. *J Hepatol* 2011; **54**: 243-250 [PMID: 21056503 DOI: 10.1016/j.jhep.2010.06.034]
18 **Risso A,** Alessandria C, Elia C, Mezzabotta L, Andrealli A, Spandre M, Morgando A, Marzano A, Rizzetto M. Adrenal dysfunction in nonseptic cirrhotic patients with ascites: Impact on survival. *Dig Liv Dis* 2011; **43** Suppl 2: S74-75 [DOI: 10.1016/S1590-8658(11)60031-5]
19 **Acevedo J,** Fernandez J, Castro M, Roca D, Gines P, Arroyo V. Prognostic value of relative adrenal insufficiency in decompensated cirrhosis. *J Hepatol* 2010; **52** Suppl 1: S65 [DOI: 10.1016/S0168-8278(10)60148-1]
20 **Graupera I,** Hernandez-Gea V, Rodriguez J, Colomo A, Poca M, Llao J, Rigla M, Aracil C, Guarner C, Villanueva C. Incidence and prognostic significance of relative adrenal insufficiency in cirrhotic patients with severe variceal bleeding (abstract). The Liver Meeting® 2010 (AASLD). *Hepatology* 2010; **52** Suppl 1: 267A
21 **Toniutto P**, Fabris C, Fumolo E, Bitetto D, Fornasiere E, Falleti E, Rapetti R, Minisini R, Pirisi M. Prevalence and risk factors for delayed adrenal insufficiency after liver transplantation. *Liver Transpl* 2008; **14**: 1014-1019 [PMID: 18581463 DOI: 10.1002/lt.21465]
22 **Iwasaki T**, Tominaga M, Fukumoto T, Kusunoki N, Sugimoto T, Kido M, Ogata S, Takebe A, Tanaka M, Ku Y. Relative adrenal insufficiency manifested with multiple organ dysfunction in a liver transplant patient. *Liver Transpl* 2006; **12**: 1896-1899 [PMID: 17133575 DOI: 10.1002/lt.21006]
23 **Singh N**, Gayowski T, Marino IR, Schlichtig R. Acute adrenal insufficiency in critically ill liver transplant recipients. Implications for diagnosis. *Transplantation* 1995; **59**: 1744-1745 [PMID: 7604447 DOI: 10.1097/00007890-199506270-00020]
24 **Bornstein SR**. Predisposing factors for adrenal insufficiency. *N Engl J Med* 2009; **360**: 2328-2339 [PMID: 19474430 DOI: 10.1056/NEJMra0804635]
25 **Gaillard RC**, Turnill D, Sappino P, Muller AF. Tumor necrosis factor alpha inhibits the hormonal response of the pituitary gland to hypothalamic releasing factors. *Endocrinology* 1990; **127**: 101-106 [PMID: 2163305 DOI: 10.1210/endo-127-1-101]
26 **Cicognani C**, Malavolti M, Morselli-Labate AM, Zamboni L, Sama C, Barbara L. Serum lipid and lipoprotein patterns in patients with liver cirrhosis and chronic active hepatitis. *Arch Intern Med* 1997; **157**: 792-796 [PMID: 9125012 DOI: 10.1001/archinte.1997.00440280120012]
27 **Albillos A**, de la Hera A, González M, Moya JL, Calleja JL, Monserrat J, Ruiz-del-Arbol L, Alvarez-Mon M. Increased lipopolysaccharide binding protein in cirrhotic patients with marked immune and hemodynamic derangement. *Hepatology* 2003; **37**: 208-217 [PMID: 12500206 DOI: 10.1053/jhep.2003.50038]
28 **Annane D**, Sébille V, Charpentier C, Bollaert PE, François B, Korach JM, Capellier G, Cohen Y, Azoulay E, Troché G, Chaumet-Riffaud P, Bellissant E. Effect of treatment with low doses of hydrocortisone and fludrocortisone on mortality in patients with septic shock. *JAMA* 2002; **288**: 862-871 [PMID: 12186604 DOI: 10.1001/jama.288.7.862]
29 **Arabi YM**, Aljumah A, Dabbagh O, Tamim HM, Rishu AH, Al-Abdulkareem A, Knawy BA, Hajeer AH, Tamimi W, Cherfan A. Low-dose hydrocortisone in patients with cirrhosis and septic shock: a randomized controlled trial. *CMAJ* 2010; **182**: 1971-1977 [PMID: 21059778 DOI: 10.1503/cmaj.090707]
30 **Arlt W**, Stewart PM. Adrenal corticosteroid biosynthesis, metabolism, and action. *Endocrinol Metab Clin North Am* 2005; **34**: 293-313, viii [PMID: 15850843 DOI: 10.1016/j.ecl.2005.01.002]
31 **Snijdewint FG**, Kapsenberg ML, Wauben-Penris PJ, Bos JD. Corticosteroids class-dependently inhibit in vitro Th1- and Th2-type cytokine production. *Immunopharmacology* 1995; **29**: 93-101 [PMID: 7775161 DOI: 10.1016/0162-3109(94)00048-K]
32 **Yang S**, Zhang L. Glucocorticoids and vascular reactivity. *Curr Vasc Pharmacol* 2004; **2**: 1-12 [PMID: 15320828 DOI: 10.2174/1570161043476483]

33 **Stewart PM.** The adrenal cortex. In: Kronenberg HM, Melmed S, Polonsky KS, Larsen PR, editors. Williams textbook of endocrinology. 11th ed. Philadelphia: Saunders, 2008: 445-503

34 **Marik PE**. Mechanisms and clinical consequences of critical illness associated adrenal insufficiency. *Curr Opin Crit Care* 2007; **13**: 363-369 [PMID: 17599004 DOI: 10.1097/MCC.0b013e32818a6d74]

35 **Hamrahian AH**, Oseni TS, Arafah BM. Measurements of serum free cortisol in critically ill patients. *N Engl J Med* 2004; **350**: 1629-1638 [PMID: 15084695 DOI: 10.1056/NEJMoa020266]

36 **de Jong MF**, Beishuizen A, Spijkstra JJ, Groeneveld AB. Relative adrenal insufficiency as a predictor of disease severity, mortality, and beneficial effects of corticosteroid treatment in septic shock. *Crit Care Med* 2007; **35**: 1896-1903 [PMID: 17568326 DOI: 10.1097/01.CCM.0000275387.51629.ED]

37 **Duma D**, Jewell CM, Cidlowski JA. Multiple glucocorticoid receptor isoforms and mechanisms of post-translational modification. *J Steroid Biochem Mol Biol* 2006; **102**: 11-21 [PMID: 17070034 DOI: 10.1016/j.jsbmb.2006.09.009]

38 **Prigent H**, Maxime V, Annane D. Clinical review: corticotherapy in sepsis. *Crit Care* 2004; **8**: 122-129 [PMID: 15025773 DOI: 10.1186/cc2374]

39 **Briegel J**, Forst H, Haller M, Schelling G, Kilger E, Kuprat G, Hemmer B, Hummel T, Lenhart A, Heyduck M, Stoll C, Peter K. Stress doses of hydrocortisone reverse hyperdynamic septic shock: a prospective, randomized, double-blind, single-center study. *Crit Care Med* 1999; **27**: 723-732 [PMID: 10321661 DOI: 10.1097/00003246-199904000-00025]

40 **Oppert M**, Schindler R, Husung C, Offermann K, Gräf KJ, Boenisch O, Barckow D, Frei U, Eckardt KU. Low-dose hydrocortisone improves shock reversal and reduces cytokine levels in early hyperdynamic septic shock. *Crit Care Med* 2005; **33**: 2457-2464 [PMID: 16276166 DOI: 10.1097/01.CCM.0000186370.78639.23]

41 **Cirera I**, Bauer TM, Navasa M, Vila J, Grande L, Taurá P, Fuster J, García-Valdecasas JC, Lacy A, Suárez MJ, Rimola A, Rodés J. Bacterial translocation of enteric organisms in patients with cirrhosis. *J Hepatol* 2001; **34**: 32-37 [PMID: 11211904 DOI: 10.1016/S0168-8278(00)00013-1]

42 **Marik PE**. Adrenal-exhaustion syndrome in patients with liver disease. *Intensive Care Med* 2006; **32**: 275-280 [PMID: 16432671 DOI: 10.1007/s00134-005-0005-5]

43 **O'Beirne J**, Holmes M, Agarwal B, Bouloux P, Shaw S, Patch D, Burroughs A. Adrenal insufficiency in liver disease - what is the evidence? *J Hepatol* 2007; **47**: 418-423 [PMID: 17629587 DOI: 10.1016/j.jhep.2007.06.008]

44 **Thevenot T**, Borot S, Remy-Martin A, Sapin R, Penfornis A, Di Martino V, Monnet E. Assessing adrenal function in cirrhotic patients: is there a reliable test? *Gastroenterol Clin Biol* 2009; **33**: 584-588 [PMID: 19473798 DOI: 10.1016/j.gcb.2009.03.011]

45 **Galbois A**, Rudler M, Massard J, Fulla Y, Bennani A, Bonnefont-Rousselot D, Thibault V, Reignier S, Bourrier A, Poynard T, Thabut D. Assessment of adrenal function in cirrhotic patients: salivary cortisol should be preferred. *J Hepatol* 2010; **52**: 839-845 [PMID: 20385427 DOI: 10.1016/j.jhep.2010.01.026]

46 **O'Beirne J.** Adrenal function in liver disease. In: Gines P, Forns X, Abraldes JG, Fernndez J, Bataller R, Rodes J, Arroyo V, editors. Therapy in Liver Diseases. Barcelona: Elsevier, 2011: 217-225

47 **Etogo-Asse FE**, Vincent RP, Hughes SA, Auzinger G, Le Roux CW, Wendon J, Bernal W. High density lipoprotein in patients with liver failure; relation to sepsis, adrenal function and outcome of illness. *Liver Int* 2012; **32**: 128-136 [PMID: 22098564 DOI: 10.1111/j.1478-3231.2011.02657.x]

48 **Amarapurkar DN**. Adrenal function in cirrhosis: the pendulum swings. *J Gastroenterol Hepatol* 2012; **27**: 1543-1544 [PMID: 22994431 DOI: 10.1111/j.1440-1746.2012.07235.x]

49 **Fede G**, Spadaro L, Tomaselli T, Privitera G, Germani G, Tsochatzis E, Thomas M, Bouloux PM, Burroughs AK, Purrello F. Adrenocortical dysfunction in liver disease: a systematic review. *Hepatology* 2012; **55**: 1282-1291 [PMID: 22234976 DOI: 10.1002/hep.25573]

50 **Beishuizen A**, Thijs LG, Vermes I. Decreased levels of dehydroepiandrosterone sulphate in severe critical illness: a sign of exhausted adrenal reserve? *Crit Care* 2002; **6**: 434-438 [PMID: 12398784 DOI: 10.1186/cc1530]

51 **Marik PE**, Zaloga GP. Adrenal insufficiency in the critically ill: a new look at an old problem. *Chest* 2002; **122**: 1784-1796 [PMID: 12426284 DOI: 10.1378/chest.122.5.1784]

52 **Marik PE**, Zaloga GP. Adrenal insufficiency during septic shock. *Crit Care Med* 2003; **31**: 141-145 [PMID: 12545007 DOI: 10.1097/00003246-200301000-00022]

53 **van Leeuwen HJ**, Heezius EC, Dallinga GM, van Strijp JA, Verhoef J, van Kessel KP. Lipoprotein metabolism in patients with severe sepsis. *Crit Care Med* 2003; **31**: 1359-1366 [PMID: 12771603 DOI: 10.1097/01.CCM.0000059724.08290.51]

54 **van der Voort PH**, Gerritsen RT, Bakker AJ, Boerma EC, Kuiper MA, de Heide L. HDL-cholesterol level and cortisol response to synacthen in critically ill patients. *Intensive Care Med* 2003; **29**: 2199-2203 [PMID: 14566456 DOI: 10.1007/s00134-003-2021-7]

55 **Marik PE**. Adrenal insufficiency: the link between low apolipoprotein A-I levels and poor outcome in the critically ill? *Crit Care Med* 2004; **32**: 1977-1978; author reply 1977-1978 [PMID: 15343040 DOI: 10.1097/01.CCM.0000132895.89019.32]

56 **Chien JY**, Jerng JS, Yu CJ, Yang PC. Low serum level of high-density lipoprotein cholesterol is a poor prognostic factor for severe sepsis. *Crit Care Med* 2005; **33**: 1688-1693 [PMID: 16096442 DOI: 10.1097/01.CCM.0000171183.79525.6B]

57 **Yaguchi H**, Tsutsumi K, Shimono K, Omura M, Sasano H, Nishikawa T. Involvement of high density lipoprotein as substrate cholesterol for steroidogenesis by bovine adrenal fasciculo-reticularis cells. *Life Sci* 1998; **62**: 1387-1395 [PMID: 9585166 DOI: 10.1016/S0024-3205(98)00077-0]

58 **Ettinger WH**, Varma VK, Sorci-Thomas M, Parks JS, Sigmon RC, Smith TK, Verdery RB. Cytokines decrease apolipoprotein accumulation in medium from Hep G2 cells. *Arterioscler Thromb* 1994; **14**: 8-13 [PMID: 8274481 DOI: 10.1161/01.ATV.14.1.8]

59 **Baranova I**, Vishnyakova T, Bocharov A, Chen Z, Remaley AT, Stonik J, Eggerman TL, Patterson AP. Lipopolysaccharide down regulates both scavenger receptor B1 and ATP binding cassette transporter A1 in RAW cells. *Infect Immun* 2002; **70**: 2995-3003 [PMID: 12010990 DOI: 10.1128/IAI.70.6.2995-3003.2002]

60 **Vishnyakova TG**, Bocharov AV, Baranova IN, Chen Z, Remaley AT, Csako G, Eggerman TL, Patterson AP. Binding and internalization of lipopolysaccharide by Cla-1, a human orthologue of rodent scavenger receptor B1. *J Biol Chem* 2003; **278**: 22771-22780 [PMID: 12651854 DOI: 10.1074/jbc.M211032200]

61 **Cervoni JP**, Thévenot T, Weil D, Muel E, Barbot O, Sheppard F, Monnet E, Di Martino V. C-reactive protein predicts short-term mortality in patients with cirrhosis. *J Hepatol* 2012; **56**: 1299-1304 [PMID: 22314431 DOI: 10.1016/j.jhep.2011.12.030]

62 **Zapater P**, Francés R, González-Navajas JM, de la Hoz MA, Moreu R, Pascual S, Monfort D, Montoliu S, Vila C, Escudero A, Torras X, Cirera I, Llanos L, Guarner-Argente C, Palazón JM, Carnicer F, Bellot P, Guarner C, Planas R, Solá R, Serra MA, Muñoz C, Pérez-Mateo M, Such J. Serum and ascitic fluid bacterial DNA: a new independent prognostic factor in noninfected patients with cirrhosis. *Hepatology* 2008; **48**: 1924-1931 [PMID: 19003911 DOI: 10.1002/hep.22564]

63 **Jang JY,** Cho WY, Jeong SW, Kim SG, Cheon YK, Kim YS, Cho YD, Kim H, Lee JS, Shim CS, Kim BS. Relative adrenal insufficiency in patients with chronic liver disease. *Hepatology* 2008; **48:** 1088A

64 **Thierry S**, Giroux Leprieur E, Lecuyer L, Brocas E, Van de Louw A. Echocardiographic features, mortality, and adrenal function in patients with cirrhosis and septic shock. *Acta Anaesthesiol Scand* 2008; **52**: 45-51 [PMID: 17996000 DOI: 10.1111/j.1399-6576.2007.01491.x]

65 **du Cheyron D**, Bouchet B, Cauquelin B, Guillotin D, Ramakers M, Daubin C, Ballet JJ, Charbonneau P. Hyperreninemic hypoaldosteronism syndrome, plasma concentrations of interleukin-6 and outcome in critically ill patients with liver cirrhosis. *Intensive Care Med* 2008; **34**: 116-124 [PMID: 17906854 DOI: 10.1007/s00134-007-0864-z]

66 **El Damarawy M,** Hamed G, Heikal A, Darwish H,Badr M. Meld Score as a Predictor for Hepato Adrenal Syndrome. *J Am Sci* 2012; **8:** 208-211

67 **Thevenot T**, Dorin R, Monnet E, Qualls CR, Sapin R, Grandclement E, Borot S, Sheppard F, Weil D, Degand T, Di Martino V, Kazlauskaite R. High serum levels of free cortisol indicate severity of cirrhosis in hemodynamically stable patients. *J Gastroenterol Hepatol* 2012; **27**: 1596-1601 [PMID: 22647073 DOI: 10.1111/j.1440-1746.2012.07188.x]

68 **Coolens JL**, Van Baelen H, Heyns W. Clinical use of unbound plasma cortisol as calculated from total cortisol and corticosteroid-binding globulin. *J Steroid Biochem* 1987; **26**: 197-202 [PMID: 3560936 DOI: 10.1016/0022-4731(87)90071-9]

69 **McDonald JA**, Handelsman DJ, Dilworth P, Conway AJ, McCaughan GW. Hypothalamic-pituitary adrenal function in end-stage non-alcoholic liver disease. *J Gastroenterol Hepatol* 1993; **8**: 247-253 [PMID: 8390870 DOI: 10.1111/j.1440-1746.1993.tb01195.x]

70 **Wiest R**, Moleda L, Zietz B, Hellerbrand C, Schölmerich J, Straub R. Uncoupling of sympathetic nervous system and hypothalamic-pituitary-adrenal axis in cirrhosis. *J Gastroenterol Hepatol* 2008; **23**: 1901-1908 [PMID: 18554237 DOI: 10.1111/j.1440-1746.2008.05456.x]

71 **Arafah BM**. Hypothalamic pituitary adrenal function during critical illness: limitations of current assessment methods. *J Clin Endocrinol Metab* 2006; **91**: 3725-3745 [PMID: 16882746 DOI: 10.1210/jc.2006-0674]

72 **Vogeser M**, Groetzner J, Küpper C, Briegel J. Free serum cortisol during the postoperative acute phase response determined by equilibrium dialysis liquid chromatography-tandem mass spectrometry. *Clin Chem Lab Med* 2003; **41**: 146-151 [PMID: 12666999 DOI: 10.1515/CCLM.2003.024]

73 **Vincent RP,** Etogo-Asse FE, Dew T, Bernal W, Alaghband-Zadeh J, le Roux CW. Serum total cortisol and free cortisol index give different information regarding the hypothalamus-pituitary-adrenal axis reserve in patients with liver impairment. *Ann Clin Biochem* 2009; **46:** 505-507 [DOI: 10.1258/acb.2009.009030]

74 **Thevenot T**, Borot S, Remy-Martin A, Sapin R, Cervoni JP, Richou C, Vanlemmens C, Cleau D, Muel E, Minello A, Tirziu S, Penfornis A, Di Martino V, Monnet E. Assessment of adrenal function in cirrhotic patients using concentration of serum-free and salivary cortisol. *Liver Int* 2011; **31**: 425-433 [PMID: 21281437 DOI: 10.1111/j.1478-3231.2010.02431.x]

75 **Cohen J**, Smith ML, Deans RV, Pretorius CJ, Ungerer JP, Tan T, Jones M, Venkatesh B. Serial changes in plasma total cortisol, plasma free cortisol, and tissue cortisol activity in patients with septic shock: an observational study. *Shock* 2012; **37**: 28-33 [PMID: 21993448 DOI: 10.1097/SHK.0b013e318239b809]

76 **Molenaar N**, Johan Groeneveld AB, Dijstelbloem HM, de Jong MF, Girbes AR, Heijboer AC, Beishuizen A. Assessing adrenal insufficiency of corticosteroid secretion using free versus total cortisol levels in critical illness. *Intensive Care Med* 2011; **37**: 1986-1993 [PMID: 21850531 DOI: 10.1007/s00134-011-2342-x]

77 **Schlienger JL,** Sapin R, Gasser F, Briche-Prouveur S, Dreyfuss M. Intérêt cortisol libre plasmatique en pratique Clinique. *Sem Hop Paris* 1989; **65:** 2067-2070

78 **le Roux CW**, Chapman GA, Kong WM, Dhillo WS, Jones J, Alaghband-Zadeh J. Free cortisol index is better than serum total cortisol in determining hypothalamic-pituitary-adrenal status in patients undergoing surgery. *J Clin Endocrinol Metab* 2003; **88**: 2045-2048 [PMID: 12727952 DOI: 10.1210/jc.2002-021532]

79 **Arafah BM**, Nishiyama FJ, Tlaygeh H, Hejal R. Measurement of salivary cortisol concentration in the assessment of adrenal function in critically ill subjects: a surrogate marker of the circulating free cortisol. *J Clin Endocrinol Metab* 2007; **92**: 2965-2971 [PMID: 17535998 DOI: 10.1210/jc.2007-0181]

80 **Soni A**, Pepper GM, Wyrwinski PM, Ramirez NE, Simon R, Pina T, Gruenspan H, Vaca CE. Adrenal insufficiency occurring during septic shock: incidence, outcome, and relationship to peripheral cytokine levels. *Am J Med* 1995; **98**: 266-271 [PMID: 7872343 DOI: 10.1016/S0002-9343(99)80373-8]

81 **Fernández J,** Acevedo J. Adrenal Function in Chronic Liver Failure. In: Ginès P, Kamath PS, Arroyo V, editors. Chronic Liver Failure: Mechanisms and Management. New York: Humana Press Inc., 2011: 377-391

82 **Kazlauskaite R**, Evans AT, Villabona CV, Abdu TA, Ambrosi B, Atkinson AB, Choi CH, Clayton RN, Courtney CH, Gonc EN, Maghnie M, Rose SR, Soule SG, Tordjman K, Consortium for Evaluation of Corticotropin Test in Hypothalamic-Pituitary Adrenal Insufficiency. Corticotropin tests for hypothalamic-pituitary- adrenal insufficiency: a meta-analysis. *J Clin Endocrinol Metab* 2008; **93**: 4245-4253 [PMID: 18697868 DOI: 10.1210/jc.2008-0710]

83 **Dorin RI**, Qualls CR, Crapo LM. Diagnosis of adrenal insufficiency. *Ann Intern Med* 2003; **139**: 194-204 [PMID: 12899587]

84 **Agha A**, Tomlinson JW, Clark PM, Holder G, Stewart PM. The long-term predictive accuracy of the short synacthen (corticotropin) stimulation test for assessment of the hypothalamic-pituitary-adrenal axis. *J Clin Endocrinol Metab* 2006; **91**: 43-47 [PMID: 16249286 DOI: 10.1210/jc.2005-1131]

85 **Mohamed MB,** Hamed G Heikal A, Darwish H. Prevalence of adenocortical insufficiency in patients with liver cirrhosis, liver cirrhosis with septic shock and in patients with hepatorenal syndrome. *J Am Sci* 2011; **7:** 391-400

86 **Vasu TS,** Stewart J, Cavallazzi RS, Hirani A, Marik PE. Hepatoadrenal syndrome: prevalence and factors predicting adrenal insufficiency in critically ill patients with liver disease. *Am J Respir Crit Care Med* 2009; **179:** A1588

87 **Sigalas A,** Xirouchakis E, Manousou P, Corbani A, Calvaruso V, Patch D, Burroughs AK, O'Beirne J. Adrenal impairment is frequent finding in stable cirrhosis and is related to disease severity. *Hepatology* 2007; **46:** 573A

88 **Alessandria C,** Mezzabotta L, Carello M, Debernardi-Venon W, Martini S, Rizzetto M, Marzano A. Relative adrenal insufficiency in cirrhosis: relevance in patients with ascites and treatment with hydrocortisone in refractory ascites. *Digest Liver Dis* 2009; **41:** A13 [DOI: 10.1016/j.dld.2008.12.029]

89 **Acevedo J,** Fernández J, Castro M, Roca D, Ginès P, Arroyo V. Impact of relative adrenal insufficiency on circulatory function and mortality in advanced cirrhosis. *J Hepatol* 2011; **54** Suppl 1: S61 [DOI: 10.1016/S0168-8278(11)60141-4]

90 **Patel SBR,** Butt T, O'Beirne J, Mallett S. Steroid administration during liver transplantation reduces the need for physiological support post operatively - More evidence for relative adrenal insufficiency in liver failure. *Eur J Anaesthesiol* 2010; **27:** 176 [DOI: 10.1097/00003643-201006121-00563]

91 **Ullian ME**. The role of corticosteriods in the regulation of vascular tone. *Cardiovasc Res* 1999; **41**: 55-64 [PMID: 10325953 DOI: 10.1016/S0008-6363(98)00230-2]

92 **Beishuizen A**, Thijs LG. Relative adrenal failure in intensive care: an identifiable problem requiring treatment? *Best Pract*

Res Clin Endocrinol Metab 2001; **15**: 513-531 [PMID: 11800521 DOI: 10.1053/beem.2001.0167]
93 **Keh D**, Boehnke T, Weber-Cartens S, Schulz C, Ahlers O, Bercker S, Volk HD, Doecke WD, Falke KJ, Gerlach H. Immunologic and hemodynamic effects of "low-dose" hydrocortisone in septic shock: a double-blind, randomized, placebo-controlled, crossover study. *Am J Respir Crit Care Med* 2003; **167**: 512-520 [PMID: 12426230 DOI: 10.1164/rccm.200205-446OC]
94 **Radomski MW**, Palmer RM, Moncada S. Glucocorticoids inhibit the expression of an inducible, but not the constitutive, nitric oxide synthase in vascular endothelial cells. *Proc Natl Acad Sci USA* 1990; **87**: 10043-10047 [PMID: 1702214 DOI: 10.1073/pnas.87.24.10043]
95 **Annane D**, Bellissant E, Sebille V, Lesieur O, Mathieu B, Raphael JC, Gajdos P. Impaired pressor sensitivity to noradrenaline in septic shock patients with and without impaired adrenal function reserve. *Br J Clin Pharmacol* 1998; **46**: 589-597 [PMID: 9862249 DOI: 10.1046/j.1365-2125.1998.00833.x]
96 **Chrousos GP**. The hypothalamic-pituitary-adrenal axis and immune-mediated inflammation. *N Engl J Med* 1995; **332**: 1351-1362 [PMID: 7715646 DOI: 10.1056/NEJM199505183322008]
97 **Turnbull AV**, Rivier CL. Regulation of the hypothalamic-pituitary-adrenal axis by cytokines: actions and mechanisms of action. *Physiol Rev* 1999; **79**: 1-71 [PMID: 9922367]
98 **Minneci PC**, Deans KJ, Banks SM, Eichacker PQ, Natanson C. Meta-analysis: the effect of steroids on survival and shock during sepsis depends on the dose. *Ann Intern Med* 2004; **141**: 47-56 [PMID: 15238370]
99 **Bellissant E**, Annane D. Effect of hydrocortisone on phenylephrine-mean arterial pressure dose-response relationship in septic shock. *Clin Pharmacol Ther* 2000; **68**: 293-303 [PMID: 11014411 DOI: 10.1067/mcp.2000.109354]
100 **Bollaert PE**, Charpentier C, Levy B, Debouverie M, Audibert G, Larcan A. Reversal of late septic shock with supraphysiologic doses of hydrocortisone. *Crit Care Med* 1998; **26**: 645-650 [PMID: 9559600 DOI: 10.1097/00003246-199804000-00010]
101 **Meduri GU**, Marik PE, Chrousos GP, Pastores SM, Arlt W, Beishuizen A, Bokhari F, Zaloga G, Annane D. Steroid treatment in ARDS: a critical appraisal of the ARDS network trial and the recent literature. *Intensive Care Med* 2008; **34**: 61-69 [PMID: 18000649 DOI: 10.1007/s00134-007-0933-3]
102 **Sprung CL**, Annane D, Keh D, Moreno R, Singer M, Freivogel K, Weiss YG, Benbenishty J, Kalenka A, Forst H, Laterre PF, Reinhart K, Cuthbertson BH, Payen D, Briegel J, CORTICUS Study Groupf. Hydrocortisone therapy for patients with septic shock. *N Engl J Med* 2008; **358**: 111-124 [PMID: 18184957 DOI: 10.1056/NEJMoa071366]
103 **Marik PE**. Critical illness-related corticosteroid insufficiency. *Chest* 2009; **135**: 181-193 [PMID: 19136406 DOI: 10.1378/chest.08-1149]
104 **Confalonieri M**, Urbino R, Potena A, Piattella M, Parigi P, Puccio G, Della Porta R, Giorgio C, Blasi F, Umberger R, Meduri GU. Hydrocortisone infusion for severe community-acquired pneumonia: a preliminary randomized study. *Am J Respir Crit Care Med* 2005; **171**: 242-248 [PMID: 15557131 DOI: 10.1164/rccm.200406-808OC]
105 **Cicarelli DD**, Vieira JE, Benseñor FE. Early dexamethasone treatment for septic shock patients: a prospective randomized clinical trial. *Sao Paulo Med J* 2007; **125**: 237-241 [PMID: 17992396 DOI: 10.1590/S1516-31802007000400009]
106 **Cronin L**, Cook DJ, Carlet J, Heyland DK, King D, Lansang MA, Fisher CJ. Corticosteroid treatment for sepsis: a critical appraisal and meta-analysis of the literature. *Crit Care Med* 1995; **23**: 1430-1439 [PMID: 7634816 DOI: 10.1097/00003246-199508000-00019]
107 **The Veterans Administration Systemic Sepsis Cooperative Study Group.** Effect of high-dose glucocorticoid therapy on mortality in patients with clinical signs of systemic sepsis. *N Engl J Med* 1987; **317**: 659-665 [PMID: 2888017]
108 **Lefering R**, Neugebauer EA. Steroid controversy in sepsis and septic shock: a meta-analysis. *Crit Care Med* 1995; **23**: 1294-1303 [PMID: 7600840 DOI: 10.1097/00003246-199507000-00021]
109 **Schneider AJ**, Voerman HJ. Abrupt hemodynamic improvement in late septic shock with physiological doses of glucocorticoids. *Intensive Care Med* 1991; **17**: 436-437 [PMID: 1774404 DOI: 10.1007/BF01720688]
110 **Chawla K,** Kupfer Y, Goldman I. Hydrocorticsone reverses refractory septic shock. *Crit Care Med* 1999; **27** Suppl 1: A33 [DOI: 10.1097/00003246-199901001-00022]
111 **Yildiz O**, Doganay M, Aygen B, Güven M, Keleştimur F, Tutuû A. Physiological-dose steroid therapy in sepsis [ISRCTN36253388]. *Crit Care* 2002; **6**: 251-259 [PMID: 12133187]
112 **Zietz B**, Lock G, Plach B, Drobnik W, Grossmann J, Schölmerich J, Straub RH. Dysfunction of the hypothalamic-pituitary-glandular axes and relation to Child-Pugh classification in male patients with alcoholic and virus-related cirrhosis. *Eur J Gastroenterol Hepatol* 2003; **15**: 495-501 [PMID: 12702906 DOI: 10.1097/00042737-200305000-00007]

P- Reviewers LesmanaLA, Llompart-Pou J
S- Editor Song XX **L- Editor** A **E- Editor** Xiong L

Observational Study

Medicine®

OPEN

Efficacy and safety of paritaprevir/ritonavir, ombitasvir, and dasabuvir with ribavirin for the treatment of HCV genotype 1b compensated cirrhosis in patients aged 70 years or older

Anca Trifan, MD[a,b], Carol Stanciu, MD, FRCP[b,*], Liana Gheorghe, MD[c,d], Speranta Iacob, MD[c,d], Manuela Curescu, MD[e,f], Cristina Cijevschi Prelipcean, MD[a,b], Gabriela Stefanescu, MD[a,b], Irina Girleanu, MD[a,b], Stefan Chiriac[a], Catalina Mihai, MD[a,b], Ciprian Brisc, MD[g,h], Adrian Goldis, MD[i,j], Ioan Sporea, MD[i,j], Egidia Miftode, MD[k,l], Simona Bataga, MD[m,n], Ion Rogoveanu, MD[o,p], Carmen Preda, MD[c,d], Florin Alexandru Caruntu, MD[q,r], Ana-Maria Singeap, MD[a,b]

Abstract

Advanced age has been a major limitation of interferon-based treatment for chronic hepatitis C virus (HCV) infection because of its poor response and tolerability. Direct-acting antiviral (DAA) drug regimens are safe and highly effective, allowing administration of treatment also in elderly. This study aims to assess the efficacy and safety of paritaprevir/ritonavir, ombitasvir, and dasabuvir (PrOD) with ribavirin for the treatment of patients aged ≥70 years with HCV genotype 1b compensated cirrhosis.

A total of 1008 patients with HCV genotype 1b compensated cirrhosis were prospectively treated with PrOD + ribavirin for 12 weeks, between December 2015 and July 2016. Sustained virologic response 12 weeks after the end of treatment (SVR12), adverse effects (AEs), comorbidities, discontinuation, and death rates were recorded. Efficacy and safety of therapy were assessed in patients aged ≥70 years and compared with data from patients <70 years.

There were 117 patients aged ≥70 years, preponderantly females (58.9%), mean age 73.3 ± 2.8 years (range 70–82), and 37 (31.6%) were treatment-experienced. Comorbidities were reported in 60.6% of patients ≥70 years and in 39.8% of those <70 years ($P < .001$). SVR12 rates based on intention-to-treat and per-protocol analyses were 97.4% and 100%, respectively, in patients ≥70 years, compared to 97.8% and 99.6%, respectively, in patients <70 years (P = ns and P = ns). Severe AEs were reported in 4 (3.4%) patients ≥70 years, compared to 23 (2.6%) in those <70 years (P = ns). One death was recorded in a patient aged 79 years (0.9%) and 6 deaths (0.8%) in those <70 years (P = ns).

Treatment with PrOD + ribavirin in patients 70 years of age or older with HCV genotype 1b compensated cirrhosis proved as effective, safe, and well tolerated, as it did in younger patients.

Abbreviations: AE = adverse event, DAA = direct acting antiviral, EOT = end of treatment, HCV = hepatitis C virus, ITT = intention-to-treat, PP = per-protocol, PrOD = paritaprevir/ritonavir, ombitasvir and dasabuvir, SVR = sustain virologic response, SVR12 = sustained virologic response 12 weeks after the end of treatment.

Keywords: direct-acting antivirals, elderly patients, HCV infection, liver cirrhosis, paritaprevir/ritonavir ombitasvir and dasabuvir

Editor: Hirayuki Enomoto.

Authorship: AT, CS, LG, AMS, and CCP: conceptualized, designed, and drafted the study protocol; SI, GS, IG, SC, CM, and CP: collected, analyzed, and interpreted the data; MC, CB, AG, IS, EM, SB, IR, and FAC: enrolled and treated patients. All authors read and approved the final version of the manuscript.

The authors have no funding and conflicts of interest to disclose.

[a] "Grigore T. Popa" University of Medicine and Pharmacy Iasi, [b] Institute of Gastroenterology and Hepatology, Iasi, [c] Gastroenterology, "Carol Davila" University of Medicine and Pharmacy, [d] Gastroenterology and Hepatology Center, Fundeni Clinical Institute, Bucharest, [e] Infectious Diseases, "Victor Babes" University of Medicine and Pharmacy, [f] Department of Infectious Diseases, "Victor Babes" Hospital for Infectious and Lung Diseases, Timisoara, [g] Gastroenterology, Oradea University of Medicine and Pharmacy, [h] Department of Gastroenterology, County Clinical Hospital, Oradea, [i] Gastroenterology, "Victor Babes" University of Medicine and Pharmacy, [j] Department of Gastroenterology and Hepatology Timisoara, The County Hospital Timisoara, [k] Infectious Diseases, "Grigore T. Popa" University of Medicine and Pharmacy, [l] "Sf. Parascheva" Infectious Diseases Clinical Hospital, Iasi, [m] Gastroenterology, Targu Mures University of Medicine and Pharmacy, Targu Mures, [n] Department of Gastroenterology, Targu Mures Emergency County Hospital, [o] Internal Medicine, Craiova University of Medicine and Pharmacy, [p] Internal Medicine Department, Emergency Clinical County Hospital Craiova, [q] Infectious Diseases, "Carol Davila" University of Medicine and Pharmacy, [r] "Matei Bals" National Institute for Infectious Diseases, Bucharest, Romania.

[*] Correspondence: Carol Stanciu, Institute of Gastroenterology and Hepatology, Independentei Street, 700111 Iasi, Romania (e-mail: stanciucarol@yahoo.com).

Medicine (2017) 96:50(e9271)

Received: 22 March 2017 / Received in final form: 1 November 2017 / Accepted: 22 November 2017

http://dx.doi.org/10.1097/MD.0000000000009271

1. Introduction

Chronic hepatitis C virus (HCV) infection affects approximately 150 million people worldwide and is the leading cause of cirrhosis and hepatocellular carcinoma when left untreated.[1] Among the genotypes of HCV infection, genotype 1 is the most common, accounting for 60% to 70% of all infections, while subgenotype 1b is predominant in some parts of Europe.[2] It is well-known that in the era of interferon-based therapy, HCV genotype 1 infection was "difficult-to-treat," as these patients had sustain virologic response (SVR) rates of just 40%.[3]

The elderly population is most likely to be infected with HCV and has advanced liver disease as compared to the younger people.[4] Advanced age has been a major limitation of pegylated interferon and ribavirin therapy for chronic HCV infection because of its poor response and tolerability. Consequently, the great majority of elderly patients (if not all, in some countries), defined as those aged 65 years or older, were denied antiviral treatment solely on the basis of their advanced age.[5] In consequence, there is nowadays a large cohort of elderly patients with chronic HCV infection untreated (with interferon-based therapy) and in great need for a new treatment.

Interferon-free regimens are safe and highly effective, allowing treatment for elderly chronic HCV-infected patients without any age limit.[6–9] However, pivotal trials of all oral combinations with direct-acting antivirals (DAAs) included few elderly patients with compensated cirrhosis.[10–12] Twelve-week treatment of HCV genotype 1 compensated cirrhosis with paritaprevir/ritonavir, ombitasvir, and dasabuvir (PrOD) with ribavirin was approved in many countries, including Romania, based on the results of a phase III trial showing an SVR response 12 weeks after the end of therapy (sustained virologic response 12 weeks after the end of treatment [SVR12]) well above 90%.[13] More recently, the HCV regimen of 12-week PrOD without ribavirin reported 100% SVR12 in HCV genotype 1b-infected patients with compensated cirrhosis, meaning that ribavirin does not provide evidence of improving the effectiveness in such patients treated with PrOD.[14]

This study aims to assess the real-world efficacy and safety of PrOD with ribavirin for the treatment of HCV genotype 1b compensated cirrhosis in patients aged 70 years and older.

2. Methods

2.1. Patients

One thousand and eight patients with HCV genotype 1b compensated cirrhosis, treatment-experienced or naïve, were prospectively followed and treated with PrOD + ribavirin for 12 weeks across 10 academic centers of gastroenterology/infectious diseases from all over Romania, between December 1, 2015 and July 31, 2016. Eligible patients were enrolled and assessed following the criteria established by the Romanian National Health Insurance House: adults 18 years of age and above with HCV genotype 1, Child–Pugh class A compensated cirrhosis defined as F4 by Fibromax Biopredictive (Fibrotest score $\geq$0.75). Exclusion criteria were: decompensated liver cirrhosis, severe chronic kidney disease, documented malignant neoplastic disease, active alcohol consumption, and human immunodeficiency virus coinfection.

All eligible patients signed an informed consent and received treatment with PrOD + ribavirin according to the therapeutic protocol. The PrOD regimen contains paritaprevir 75 mg boosted with ritonavir 50 mg and ombitasvir 12.5 mg (Viekirax, AbbVie Deutschland Gmbh & Co Ludwigshafen, Germany) 2 tablets in a single daily dose, and dasabuvir (Exviera 250 mg AbbVie Deutschland GmbH & Co Ludwigshafen) twice-daily administration. The dose of ribavirin was 1000 mg/day in patients weighting $<$75 kg or 1200 mg/day in those weighting $>$75 kg.

This study was approved by National Ethics Committee, and written informed consent was obtained from each patient in accordance with the principles of the Declaration of Helsinki.

2.2. Methods

Blood and urine samples were taken for laboratory analyses at baseline, on weeks 4, 8, 12 (end of treatment [EOT]), 12 weeks after the treatment, and whenever it was necessary. Baseline clinical data referred to gender, age, treatment history, comorbidities, and concomitant medication. Laboratory data included HCV RNA level (at baseline, EOT, and SVR12), genotype and subgenotype, liver function tests (aspartate and alanine aminotransferases, bilirubin, alkaline phosphatase, gamaglutamyl transpeptidase, albumin, and international normalized ratio), serum creatinine and creatinine clearance, hemoglobin, platelet count, and alpha-fetoprotein. Child–Pugh and Model of End-Stage Liver Disease scores were calculated at baseline and 12 weeks after the end of therapy. Serum HCV RNA levels were measured with the COBAS TaqMan HCV Quantitative Test (Roche Molecular Systems, Inc. Branchburg, NJ) with a lower limit of quantification and detection of 15 IU/mL.

Efficacy of therapy was assessed by the percentage of patients achieving SVR12 (defined as HCV RNA below the limit of detection 12 weeks after the end of therapy) calculated based on intention-to-treat (ITT) and per-protocol (PP) analysis. ITT population was defined as all patients receiving at least 1 dose of medication while PP population included all patients who completed the 12 weeks of therapy. Safety and tolerability assessment included physical examinations, laboratory data analysis, and all adverse effects (AEs) recorded from the time of the 1st dose of treatment to the last one. Severe adverse events (SAEs), therapy discontinuation, and death rate were recorded.

2.3. Statistical analysis

Continuous variables with normal distribution were expressed as mean $\pm$ SD, while categorical variables were expressed as absolute values and percentages. The Chi-square test was used to compare categorical data. Quantitative variables with normal distribution were compared using the Student t test. For nonnormal data, we used nonparametric methods such as the Mann–Whitney U test, while the Kolmogorov–Smirnov test was used to check the normality of the data distributions. The efficacy analysis examined data concerning the total patient population by age at baseline ($\geq$70 or $<$70 years), whereas the safety analysis described the number and percent of patients with adverse effects or laboratory abnormalities. P value less than 0.05 was considered statistically significant. Statistical analysis was carried out using the SPSS 19.0 software (SPSS Inc., Chicago, IL).

3. Results

3.1. Baseline characteristics

Among the 1008 patients included in our analysis (51.7% females), mean age 59.2 $\pm$ 8.7 years (range 33–82), and 117 (11.6%) were aged $\geq$70 years. Most of the elderly patients were females (58.9%), mean age 73.3 $\pm$ 2.8 years (range 70–82), and

Table 1

Baseline demographics and laboratory characteristics in patients aged ≥70 and <70 years treated with paritaprevir/ritonavir, ombitasvir, and dasabuvir + ribavirin.

Characteristics	≥70 y (n = 117)	<70 y (n = 891)	P
Age, y, mean ± SD, range	73.3 ± 2.8 70–82	57.4 ± 7.5 33–69	**<.001**
Female, n, %	69 (58.9)	452 (50.7)	.093
Treatment experienced, n, %	37 (31.6)	503 (56.4)	**<.001**
Comorbidities, n, %	71 (60.6)	355 (39.8)	**<.001**
Cardiovascular	48 (41.0)	177 (19.9)	**<.001**
Diabetes mellitus	13 (11.1)	123 (13.8)	.42
Platelet count $\times 10^9$/L, mean ± SD	143.54 ± 6.1	142.63 ± 2.3	.94
Hemoglobin, g/dL, mean ± SD	13.62 ± 1.7	14.25 ± 1.6	**<.001**
Albumin, g/dL, mean ± SD	4.01 ± 0.4	4.02 ± 0.6	.97
eGFR, mL/min, mean ± SD	72.04 ± 23.3	101.7 ± 30.7	**<.001**
Total bilirubin, mg/dL, mean ± SD	1.04 ± 0.45	1.09 ± 0.5	.40
AST, UI/L, mean ± SD	101.25 ± 59.8	101.5 ± 86.5	.97
ALT, UI/L, mean ± SD	98.0 ± 56.0	100.7 ± 69.1	.50
INR, mean ± SD	1.19 ± 0.4	1.15 ± 0.26	.18
Child–Pugh score, n, %			
5	104 (88.8)	779 (87.4)	.65
6	13 (11.2)	112 (12.6)	
MELD score, mean ± SD	8.01 ± 1.2	7.95 ± 1.6	.87

ALT = alanine aminotransferase, AST = aspartate aminotransferase, eGFR = glomerular filtration rate, INR = international normalized ratio, MELD = Model of End-Stage Liver Disease, SD = standard deviation.

37 of them (31.6%) were treatment-experienced. Comorbidities were reported in 60.6% of patients aged ≥70 years compared to 39.8% of those below 70 years ($P < 0.001$). The most frequently met comorbidity in the patients ≥70 years was cardiovascular disease (hypertension, ischemic heart disease, and atrial fibrillation) (Table 1). At baseline, a significant number of patients aged ≥70 years had reduced estimated glomerular filtration rate and hemoglobin level than those <70 years (Table 1). Improvement in the laboratory results was noted at the EOT, while aspartate aminotransferase and alanine aminotransferase values in both age groups were normalized in most of the patients at 4 weeks of therapy. There were no differences in Child–Pugh and Model of End-Stage Liver Disease scores between patients ≥70 and those <70 years of age.

3.2. Efficacy

SVR12 rates based on ITT analysis were 97.4% in patients ≥70 years, compared to 97.8% in those <70 years of age ($P = .82$), while SVR12 rates based on PP were 100% in the older group compared to 99.6% in the younger group ($P = .61$), as shown in Table 2. The SVR12 in treatment-naïve patients was 97.5% (78/80) for those ≥70 years of age and 98.2% (381/388) for those <70 years, while for treatment-experienced patients the SVR12 was 97.0% (36/37) for those ≥70 years and 99.4% for those <70 years of age, the differences not being statistically significant.

3.3. Safety

A total of 37.6% of patients aged ≥70 years and 34.6% of those <70 years of age ($P = .51$) reported at least 1 AE considered by their physicians as treatment-related (Table 2). The great majority of AEs were mild and manageable, none leading to treatment discontinuation. The most frequent reported AEs in both age groups were: asthenia, pruritus, insomnia, and headache

Table 2

Efficacy and safety of paritaprevir/ritonavir, ombitasvir, and dasabuvir + ribavirin treatment by age.

Characteristics	≥70 y (n = 117)	<70 y (n = 891)	P
Efficacy			
ITT SVR12, n, %	114 (97.4)	872 (97.8)	0.82
PP SVR12, n, %	114 (100)	872 (99.6)	0.61
Safety			
Any AE, n, %	44 (37.6)	308 (34.6)	0.51
Common AEs	13 (11.1)	92 (10.3)	0.79
Asthenia	5 (4.3)	67 (7.5)	0.20
Pruritus	4 (3.4)	33 (3.7)	0.87
Insomnia	3 (2.6)	31 (3.5)	0.60
Headache	3 (2.6)	26 (2.9)	0.82
SAEs, n, %	4 (3.4)	23 (2.6)	0.54
Decompensation of liver cirrhosis	1 (0.9)	14 (1.5)	0.74
Variceal bleeding	0	5 (0.5)	
Ascites	1 (0.9)	3 (0.3)	0.48
Hepatic encephalopathy	0	4 (0.4)	
Isolated grade 4 increase of direct bilirubin	0	2 (0.2)	
Cardiovascular			
Heart failure	1 (0.9)	1 (0.1)	**0.03**
Stroke	1 (0.9)	1 (0.1)	**0.03**
Malignant arrhythmia	0	1 (0.1)	
Acute pancreatitis	0	1 (0.1)	
Sepsis	0	1 (0.1)	
Severe depression	0	2 (0.2)	
Nonvariceal upper digestive bleeding	0	2 (0.2)	
Acute kidney failure	1 (0.9)	0	
Treatment discontinuation, n, %	3 (2.6)	15 (1.7)	0.36
Death	1 (0.9)	6 (0.8)	0.88
Decompensation of liver cirrhosis			
Ascitis	1 (0.9)	0	
Hepatic encephalopathy	0	3 (0.3)	
Isolated grade 4 increase of direct bilirubin	0	2 (0.2)	
Cardiovascular			
Heart failure	0	1 (0.1)	
Stroke	1 (0.1)	1 (0.1)	**0.03**
Severe depression	0	2 (0.2)	
Death, n, %	1 (0.9)	6 (0.8)	0.88
Liver disease related death			
Variceal bleeding	0	2 (0.2)	
Severe liver decompensation	0	2 (0.2)	
Nonliver disease related death			
Heart failure	1 (0.9)	0	
Sepsis	0	1 (0.1)	
Malignant arrhythmia	0	1 (0.1)	
Ribavirin treatment			
Dose reduction	31 (26.5)	162 (18.2)	0.14
Discontinuation	11 (9.4)	51 (5.7)	0.13

AE = adverse effects, ITT = intention-to-treat, PP = per-protocol, SAE = severe adverse effects, SVR = sustained virologic response.

(Table 2). Severe AEs were reported in 4 patients (3.4%) aged ≥70 years (1 decompensation of liver cirrhosis, 1 heart failure, 1 stroke, and 1 acute kidney failure), compared to 23 patients (2.6%) in the group <70 years of age ($P = .54$) (14 decompensation of liver cirrhosis: 5 variceal bleeding, 3 ascites, 4 hepatic encephalopathy, 2 isolated grade 4 increase of direct bilirubin).

One death occurred (0.9%) in a patient aged 79 years (heart failure, not related in any way to PrOD/RBV therapy), and 6 deaths were reported (0.7%) in those under 70 years (2 variceal bleeding, 2 severe liver decompensation, 1 sepsis, and 1 malignant arrhythmia) ($P = .88$) (Table 2). In the elderly group,

of the 4 patients with SAEs, 3 discontinued therapy (1 death, 1 liver decompensation, and 1 stroke), and the 1 with acute kidney failure continued therapy after withdrawal of ribavirin. In the younger group, among the 23 patients with SAEs, 15 of them discontinued therapy (6 deaths, 3 hepatic encephalopathy, 2 isolated grade 4 increase of direct bilirubin, 2 severe depression, 1 stroke, and 1 heart failure). Modification of the ribavirin dose (due to anemia and/or increased bilirubin levels) was required in 31 (23.1%) of the patients aged ≥70 years and in 162 (18.2%) of those <70 years ($P = .14$).

4. Discussion

The elderly patients with chronic HCV infection, defined in most studies as those aged 65 years or older, were usually denied previous pegylated interferon and ribavirin therapy because of severe adverse effects and poor response.[5,15] Therefore, there is a large cohort in real clinical practice setting of untreated elderly patients with chronic HCV infection and with advanced liver disease. This cohort is in great need for a treatment due to the progressive nature of their disease. Fortunately, interferon-free HCV therapy with DAAs is highly effective and safe, allowing treatment for elderly patients in whom several studies reported similar SVR rates as those obtained in younger patients.[6–9]

Controlled clinical trials with PrOD + ribavirin in patients with chronic HCV genotype 1 infection have reported SVR12 rates ranging from 91.8% to 98.3% in cirrhotic and noncirrhotic patients,[10,11,13,16] while with PrOD without ribavirin in HCV genotype 1b noncirrhotic patients SVR12 rates varied from 96.7% to 99.5%.[11,13,16] Poordad et al[13] in a phase 3 clinical trial of patients with HCV genotype 1 compensated cirrhosis (Child–Pugh class A) treated with PrOD + ribavirin for 12 weeks reported SVR12 rates of 91.8% (98.5% in HCV genotype 1b patients). Based on the results of this study, PrOD + ribavirin for 12 weeks regimen has been recommended for patients with HCV genotype 1 compensated cirrhosis.[17,18] More recently, Feld et al[14] have demonstrated that PrOD regimen without ribavirin for 12 weeks was highly effective (100% SVR 12) and well tolerated in HCV genotype 1b patients with compensated cirrhosis, and now this regimen is recommended by both American Association for the Study of Liver Diseases/Infectious Diseases Society of America and European Association for the Study of the Liver guidelines.[19,20] Eliminating ribavirin from this regimen without reducing efficacy will certainly improve the safety profile.

In our real-world cohort of HCV genotype 1b patients aged 70 years or older with compensated cirrhosis treated with PrOD + ribavirin for 12 weeks, the SVR12 rates based on ITT or PP analyses were 97.4% and 100%, respectively, compared to 97.8% and 99.6%, respectively, in cirrhotic patients aged <70 years, the differences being statistically nonsignificant. Of the patients aged ≥70 years, 37.6% reported at least 1 AE considered as treatment-related, a proportion slightly higher but with no statistical significance compared with patients under 70 years of age (34.6%). Most AEs were mild and none was leading to treatment discontinuation. Also, the percentage of SAEs was not significantly higher in patients aged ≥70 years when compared to those less than 70 years of age (3.4% vs 2.6%; $P = .54$). This safety profile is even better than one might expect, considering that all subjects included in the study were older patients with cirrhosis; the safety profile in our study was undoubtedly better than in other studies.[9,21] Such high SVR 12 rates and good safety profiles obtained in our study may be partially explained by the requirements imposed by our national regulations according to which treatment was conducted only in tertiary centers and under the close monitoring of experienced gastroenterologists and infectious diseases specialists.

Our study was carried out in a real-life setting on a homogeneous elderly population (≥70 years of age) with HCV-genotype 1b compensated cirrhosis only, which is what makes it uniquely interesting among many others of its kind. There are but few published studies regarding efficacy of PrOD ± ribavirin in patients with HCV genotype 1 compensated cirrhosis in real life setting.[9,21–23] Thus, Chamorro-de-Vega et al[21] from Spain evaluated in a prospective study the effectiveness and safety in real clinical practice of PrOD ± ribavirin for 12 weeks in patients with chronic HCV genotype 1 (82% genotype 1b) infection and reported a SVR12 rate of 93.8% in cirrhotic patients and 100% in noncirrhotic patients, while AEs occurred in 91.7% of patients (in mild forms, mostly), although none led to premature discontinuation. Of note, patients' average age was 60 years. The study of Walker et al[22] assessed real-world effectiveness of 2 therapeutic regimens (PrOD and sofosbuvir/ledipasvir) in patients with HCV genotype 1 infection and reported similar high SVR12 rates in both regimens, consistent with results from registrations trials; however, for PrOD regimens with 100% SVR12 rates, the sample size was very low (n = 15) and included only 1 cirrhotic patient and, therefore, no direct comparison with our study is possible. Another published study assessing real-world effectiveness and safety of PrOD ± ribavirin comes from Poland and reported an SVR12 rate of 98.3% in patients with liver cirrhosis, and a higher rate of AEs (72% of cases) than in our study.[9] From Asia (Hong Kong), Chan et al[23] in a retrospective, real-life study including 41 patients with chronic HCV genotype 1 infection (85% had genotype 1b and 61% had compensated liver cirrhosis), PrOD + ribavirin regimen for 12 weeks achieved 95% SVR12 rate, results comparable to the pivotal studies from the West. Similar results have been reported by other studies which included elderly patients treated with PrOD ± ribavirin or other DAAs regimens.[6,7,24–32] Recently, Conti et al,[7] evaluated the efficacy and safety of some DAA regimens in elderly patients, defined as those over 65 years of age with HCV-related advanced fibrosis/cirrhosis, in a real-life clinical setting, and reported that all DAAs regimens used (including PrOD ± ribavirin) were effective and safe in elderly patients with genotype 1b cirrhosis, with SVR12 of 95%. Ioannou et al[32] also reported high SVR rates in the Veteran Affair National Health System patients with HCV genotype 1 and cirrhosis, either treatment-naïve or experienced, treated with PrOD and ribavirin, similar to that obtained under sofosbuvir-based regimens. Saab et al[6] evaluated four open-label phase 3 clinical trials and reported SVR12 of 94% in patients >65 years with HCV genotype 1 cirrhosis who had received ledipasvir/sofosbuvir for 12 weeks, and this regimen proved safe and tolerable for elderly patients.

To our knowledge, our study represents the largest one yet published on PrOD + ribavirin efficacy and safety in patients aged ≥70 years with HCV-genotype 1b compensated cirrhosis in a real-life setting. This study has some strengths such as being prospective, multicentered and including a large number of homogeneous patients ≥70 years of age with HCV genotype 1b compensated cirrhosis only, treated with PrOD + ribavirin. However, our study has also some limitations, the most important one being the absence of assessment concerning long-term impact of SVR12 on the progression of liver disease in elderly patients.

In conclusion, our results demonstrate that a 12-week regimen of PrOD + ribavirin is highly effective, safe, and well-tolerated treatment for patients aged 70 years or older with HCV-genotype 1b compensated cirrhosis, adding new evidence that advanced age should not be a barrier anymore in treating this growing subgroup of HCV patients.

References

[1] World Health Organization. Hepatitis C. WHO fact sheet 164. [Online, 28 December 2016]. Available at: http://www.who.int/mediacentre/factsheets/fs164/en. Accessed January 10, 2017.

[2] Messina JP, Humphreys I, Flaxman A, et al. Global distribution and prevalence of hepatitis C virus genotypes. Hepatology 2015;61:77–87.

[3] McHutchison JG, Lawitz EJ, Shiffman ML, et al. Peginterferon alfa-2b or alfa-2a with ribavirin for treatment of hepatitis C infection. N Engl J Med 2009;361:580–93.

[4] Thabut D, Le Calvez S, Thibault V, et al. Hepatitis C in 6,865 patients 65 yr or older: a severe and neglected curable disease? Am J Gastroenterol 2006;101:1260–7.

[5] Gramenzi A, Conti F, Felline F, et al. Hepatitis C Virus-related chronic liver disease in elderly patients: an Italian cross-sectional study. J Viral Hepat 2009;17:360–6.

[6] Saab S, Park SH, Mizokami M, et al. Safety and efficacy of ledipasvir/sofosbuvir for the treatment of genotype 1 hepatitis C in subjects aged 65 years or older. Hepatology 2016;63:1112–9.

[7] Conti F, Brillanti S, Buonfiglioli F, et al. Safety and efficacy of direct acting antivirals for the treatment of chronic hepatitis C in a real-world population aged 65 years and older. J Viral Hepat 2017;24:454–63.

[8] Flamm SL, Gane E, DuFour JF, et al. Safety of ABT-450/r/ombitasvir + dasabuvir with or without ribavirin in HCV genotype 1-infected patients ≥65 years of age: results from phase 2 and 3 trials. Hepatology 2014;60 (Suppl 4):1157A.

[9] Flisiak R, Janczewska E, Wawrzynowicz-Syczewska M, et al. Real-world effectiveness and safety of ombitasvir/paritaprevir/ritonavir ± dasabuvir ± ribavirin in hepatitis C: AMBER study. Aliment Pharmacol Ther 2016;44:946–56.

[10] Ferenci P, Bernstein D, Lalezari J, et al. ABT-450/r-ombitasvir and dasabuvir with or without ribavirin for HCV. N Engl J Med 2014;370:1983–92.

[11] Feld JJ, Kowdley KV, Coakley E, et al. Treatment of HCV with ABT-450/r-ombitasvir and dasabuvir with ribavirin. N Engl J Med 2014;370:1594–603.

[12] Afdhal N, Zeuzem S, Kwo P, et al. Ledipasvir and sofosbuvir for untreated HCV genotype 1 infection. N Engl J Med 2014;370:1889–98.

[13] Poordad F, Hezode C, Trinh R, et al. ABT-450/r-ombitasvir and dasabuvir with ribavirin for hepatitis C with cirrhosis. N Engl J Med 2014;370:1973–82.

[14] Feld JJ, Moreno C, Trinh R, et al. Sustained virologic response of 100% in HCV genotype 1b patients with cirrhosis receiving ombitasvir/paritaprevir/r and dasabuvir for 12 weeks. J Hepatol 2016;64:301–7.

[15] Roeder C, Jordan S, Schulze Zur Wiesch J. Age-related differences in response to peginterferon alfa-2a/ribavirin in patients with chronic hepatitis C infection. World J Gastroenterol 2014;20:10984–93.

[16] Zeuzem S, Jacobson IM, Baykal T, et al. Retreatment of HCV with ABT-450/r-ombitasvir and dasabuvir with ribavirin. N Engl J Med 2014;370:1604–14.

[17] AASLD/IDSA HCV Guidance PanelHepatitis C guidance: AASLD-IDSA recommendations for testing, managing, and treating adults infected with hepatitis C virus. Hepatology 2015;62:932–54.

[18] European Association for the Study of the LiverEASL recommendations on treatment of hepatitis C 2015. J Hepatol 2015;63:199–236.

[19] AASLD/IDSA HCV Guidance Panel. Recommendations for Testing, Managing, and Treating Hepatitis C. [Online, 28 December 2016] Available at http://hcvguidelines.org/sites/default/files/HCV-Guidance_October_2016_a.pdf. Accessed January 2, 2017.

[20] European Association for the Study of the LiverEASL Recommendations on Treatment of Hepatitis C 2016. J Hepatol 2016;66:153–94.

[21] Chamorro-de-Vega E, Gimenez-Manzorro A, Rodriguez-Gonzalez CG, et al. Effectiveness and safety of ombitasvir-paritaprevir/ritonavir and dasabuvir with or without ribavirin for HCV genotype 1 infection for 12 weeks under routine clinical practice. Ann Pharmacother 2016;50:901–8.

[22] Walker DR, Pedrosa MC, Manthena SR, et al. Early view of the effectiveness of new direct-acting antiviral (DAA) regimens in patients with hepatitis C virus (HCV). Adv Ther 2015;32:1117–27.

[23] Chan HL, Tsang OT, Hui YT, et al. Real-life efficacy and safety of paritaprevir/ritonavir, ombitasvir and dasabuvir in chronic hepatitis C patients in Hong Kong. Gastroenterol Hepatol 2017;32:1230–3.

[24] Andreone P, Colombo MG, Enejosa JV, et al. ABT-450, ritonavir, ombitasvir, and dasabuvir achieves 97% and 100% sustained virologic response with or without ribavirin in treatment-experienced patients with HCV genotype 1b infection. Gastroenterology 2014;147:359.e1–65.e1.

[25] Lawitz E, Makara M, Akarca US, et al. Efficacy and safety of ombitasvir, paritaprevir, and ritonavir in an open-label study of patients with genotype 1b chronic hepatitis C virus infection with and without cirrhosis. Gastroenterology 2015;149:971–80.

[26] Fox DS, McGinnis JJ, Tonnu-Mihara I, McCombs JS. Comparative treatment effectiveness of direct acting antiviral regimens for hepatitis C: data from the veterans administration. J Gastroenterol Hepatol 2017;32:1136–42.

[27] Backus LI, Belperio PS, Shahoumian TA, et al. Real-world effectiveness of ledipasvir/sofosbuvir in 4,365 treatment-naive, genotype 1 hepatitis C-infected patients. Hepatology 2016;64:405–14.

[28] Sulkowski MS, Vargas HE, Di Bisceglie AM, et al. Effectiveness of simeprevir plus sofosbuvir, with or without ribavirin, in real-world patients with HCV genotype 1 infection. Gastroenterology 2016;150:419–29.

[29] Calleja JL, Rincon D, Ruiz-Antoran B, et al. Effectiveness and safety of ombitasvir, paritaprevir, ritonavir and dasabuvir patients with genotype 1 chronic hepatitis C virus infection: results from the Spanish Real World Cohort. J Hepatol 2016;64(Suppl2):S218–9.

[30] Hinrichsen H, Wedemeyer H, Christensen S, et al. Real-world safety and effectiveness of ombitasvir/paritaprevir/R with dasabuvir and/or ribavirin in the German Hepatitis C Registry. J Hepatol 2016;64(Suppl 2):S159.

[31] Lubel JS, Pianko S, Thompson A, et al. Real world evaluation of viekira pak (ritonavir boosted paritaprevir, ombitasvir and dasabuvir +/− ribavirin) in HCV genotype 1 targeting advanced liver disease (The Revital Study). J Hepatol 2016;64(Suppl 2):S780.

[32] Ioannou GN, Beste LA, Chang MF, et al. Effectiveness of sofosbuvir, ledipasvir/sofosbuvir, or paritaprevir/ritonavir/ombitasvir and dasabuvir regimens for treatment of patients with hepatitis C in the Veterans Affairs National Health Care System. Gastroenterology 2016;151:457–71.

Research Article

EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY

Relative performances of FibroTest, Fibroscan, and biopsy for the assessment of the stage of liver fibrosis in patients with chronic hepatitis C: A step toward the truth in the absence of a gold standard

Thierry Poynard[1,*], Victor de Ledinghen[2], Jean Pierre Zarski[3], Carol Stanciu[4], Mona Munteanu[5], Julien Vergniol[2], Julie France[6], Anca Trifan[4], Gilles Le Naour[1], Jean Christophe Vaillant[1], Vlad Ratziu[1], Frederic Charlotte[2], The Fibrosis-TAGS group [†]

[1]*APHP UPMC Liver Center, France;* [2]*Service d'Hépato-Gastroentérologie, CHU de Bordeaux, France;* [3]*Clinique Universitaire d'Hépato-Gastroentérologie, Grenoble, France;* [4]*Gastroenterology and Hepatology Institute, Iasi, Romania;* [5]*Biopredictive, Paris, Franc e;* [6]*Centre d'Investigation Clinique INSERM CIC003, CHU Grenoble, France*

Background & Aims: Liver fibrosis stage is traditionally assessed with biopsy, an imperfect gold standard. Two widely used techniques, FibroTest®, and liver stiffness measurement (LSM) using Fibroscan® have been validated using biopsy, and therefore the true performances of these estimates are still unknown in the absence of a perfect reference.

The aim was to assess the relative accuracy of FibroTest, LSM, and biopsy using methods without gold standard in patients with chronic hepatitis C (CHC) and controls.

Methods: A total of 1289 patients with CHC and 604 healthy volunteers, with assessment of fibrosis stage by the three techniques, and alanine aminotransferase (ALT) taken as a control test, were analyzed by latent class method with random effects. In the volunteers, the false positive risk of biopsy was obtained from a large surgical sample of four normal livers.

Results: The latent class model with random effects permitted to conciliate the observed data and estimates of test performances. For advanced fibrosis, the specificity/sensitivity was for FibroTest 0.93/0.70, LSM 0.96/0.45, ALT 0.79/0.78 and biopsy 0.67/0.63, and for cirrhosis FibroTest 0.87/0.41, LSM 0.93/0.39, ALT 0.78/0.08 and biopsy 0.95/0.51. The analysis of the discordances between pairs suggested that the variability of the model was mainly related to the discordances between biopsy and LSM (residuals >10; p <0.0001).

Conclusions: A method without the use of a gold standard confirmed the accuracy of FibroTest and Fibroscan for the diagnosis of advanced fibrosis and cirrhosis in patients with chronic hepatitis C. The variability of the model was mostly due to the discordances between Fibroscan and biopsy.

Keywords: Liver biopsy; FibroTest; FibroSure; FibroScan; Transaminases; Latent class; Accuracy; Methods without gold standard; Hepatitis C; Non-invasive biomarker.

Received 14 April 2011; received in revised form 30 July 2011; accepted 9 August 2011; available online 1 September 2011

* Corresponding author. Address: 47-83 Boulevard de l'Hôpital, 75651 Paris Cedex 13, France. Tel.: +33 1 42 16 10 22.

E-mail address: tpoynard@teaser.fr (T. Poynard).

† List of members of the Fibrosis-TAGS group (Truth in the Absence of a Gold Standard) is available in Supplementary data.

Introduction

New tests generally are evaluated in comparison with a reference test, often termed a "gold standard", whose sensitivity and specificity are both assumed to be 100%. If the reference test is not perfect, classical estimates of accuracy (sensitivity, specificity and AUROC) of the new diagnostic test are false [1].

One example of major debate surrounds the efforts to find the best means of evaluating and managing the increasing numbers of patients with chronic liver disease [2,3]. Liver biopsy, due to its risks and limitations, is no longer considered mandatory as the first-line indicator of liver injury, and several markers have been developed as non-invasive alternatives [2,3]. Among patients with chronic viral hepatitis, the assessment of liver fibrosis by two validated non-invasive techniques, biomarkers [FibroTest® (FT)] Biopredictive Paris, France [4] and liver stiffness measurements (LSM) by Fibroscan® Echosens, Paris, France [5], is now widely done in countries where these techniques are available and approved [6].

The true liver disease status, the "true gold standard", is the histological analysis of large surgical biopsies [7]. Therefore, the definitive diagnosis is impossible to obtain in routine practice, and liver biopsy, an "imperfect gold standard", is used as a standard against which new tests are evaluated.

In this situation with several tests and no perfect gold standard, latent class analysis has been recommended to better estimate the rate of false positives and false negatives [1], and we previously performed a pilot study using this methodology [8].

The aim was then to apply this methodology to estimate the relative accuracy of FT, LSM and biopsy for the diagnosis of fibrosis in the absence of a gold standard in a large group of patients,

Research Article

with CHC, independent of our institution, and in healthy volunteers. The reference was the model which fitted the best the observed distribution of the estimates of fibrosis.

Materials and methods

Patients

The final database included 1893 subjects retrospectively extracted from four prospective cohorts (Fig. 1): three populations of patients with CHC (n = 1289 out of 2675), and one population of apparently healthy volunteers (Healthy cohort, n = 604 out of 766). HCV patients belonged to one tertiary center in Bordeaux, France (Bordeaux cohort, n = 768) [9], one multicenter French study (Fibrostar cohort, n = 378) [10] and one multicenter Romanian study (Romanian cohort, n = 143) [11].

The inclusion criteria were retrospectively determined: patients had to have chronic hepatitis C, be PCR positive, and have the results of liver biopsy, FibroTest, LSM and alanine aminotransferase [ALT] interpretable according to the usual recommendations and precaution of use [4,9]. In all these cohorts, each of the four tests was performed without knowledge of the three others.

Controls

This group was analyzed in order to define the specificity of each test, as the probability of true advanced fibrosis was very low. Among a prospective cohort of healthy volunteers, a group of 604 subjects without any risk of liver disease was retrospectively selected [12]. The inclusion criteria were: no liver disease history, no or low alcohol consumption ($\leqslant$10 g/day for females, $\leqslant$20 g/day for males), HBsAg negative, HCV antibodies negative, and FibroTest and LSM results interpretable.

As it was not possible to perform liver biopsy in these healthy volunteers, we used large surgical biopsies obtained from four subjects without liver disease. From the digitized image of the whole section, 626 virtual biopsy specimens of 20 mm length were produced [13] (Supplementary Table 1).

FibroTest and ALT

FibroTest was performed according to published recommendations [4]. The following usual recommended cut-offs were used to estimate the presumed fibrosis stages: 0.48, and 0.74 for the F2 and F4 staging, respectively. ALT was used as a control liver test as a nonspecific biomarker of liver injury. As there is no consensual definition for the upper limit of normal for ALT, the following simple cut-offs were predetermined: 50 IU/L and 100 IU/L for F2 stage and F4 stage METAVIR, respectively.

Liver stiffness measurements

Patients were studied using transient elastography. The LSM results are expressed in kilopascals (kPa). For LSM reliability, the recommended criteria were a success rate greater than 60%, at least 10 valid LSM and interquartile range/median LSM <30% [9]. The following usual recommended cut-offs were used to estimate the presumed fibrosis stages: 8.8, and 14.5 kPa for the F2, and F4 staging, respectively [9,14,15].

Biopsy among patients with chronic hepatitis C

Staging and grading were performed blinded to the non-invasive methods. In the three groups, liver biopsies were performed with a 1.6 mm needle (Hepafix, Brown, Melsungen, Germany), and were formalin-fixed and paraffin embedded. Sections (4 mm) were stained with hematoxylin-eosin-saffron and picrosirius red. The liver fibrosis stage was evaluated according to the METAVIR scoring system [16] by one senior pathologist in the Bordeaux cohort and in the Romanian study, by two senior liver pathologists in Fibrostar. In Fibrostar, slides were simultaneously reviewed to reach a consensus in case of disagreement; to be eligible for scoring, biopsies less than 20 mm had to measure at least 15 mm and/or contain at least 11 portal tracts, except for cirrhosis. The reliability of biopsy was decided by each pathologist in the Romanian study and Bordeaux cohorts.

Design and modeling

Concept

The first concept was to estimate the performances of four estimates (tests) of liver fibrosis using methods without a gold standard.

The second concept was to use a control population without any risk of chronic liver disease, therefore with a very low risk of advanced fibrosis. This concept will permit to assess the performance of the fibrosis tests in screening strategies. As a biopsy cannot be directly performed in a large group of non-selected healthy volunteers, the distribution of subjects according to the results of a virtual biopsy (fibrosis present or absent) was calculated using the prevalence of fibrosis observed using large surgical biopsies from normal livers. For each eight possible combinations of FibroTest, LSM and ALT results (fibrosis present or absent), the number of virtual biopsy results (fibrosis present or absent) was calculated by multiplying the number of subjects in each eight possible combinations by the

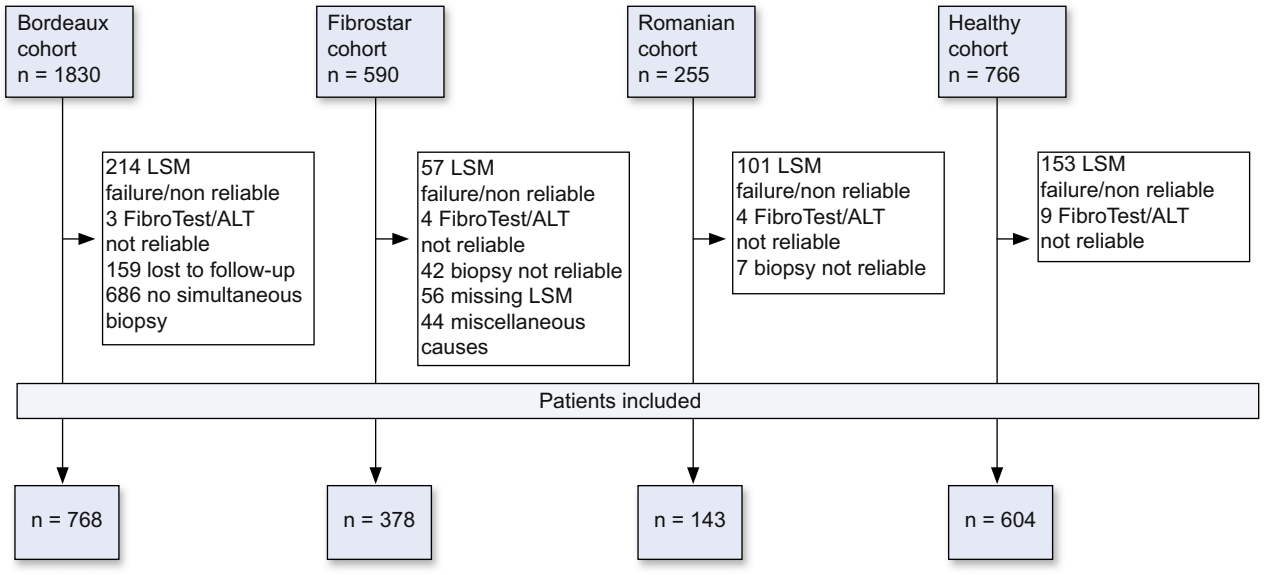


Fig. 1. Cohort and number of patients included and excluded.

mean prevalence of fibrosis observed using large surgical biopsies from normal livers. This method has permitted to generate the 16 distributions of subjects according to the four test results (Supplementary Table 3).

Basic model

Four different tests (FibroTest, LSM, ALT, biopsy) were applied in all patients, with each test producing a dichotomous test result (e.g. the test was either positive or negative). None of these tests was error-free. For a single test, the probability of obtaining a positive test result could be written as the sum of finding a positive test in a patient who has fibrosis and a positive test result in a patient without fibrosis. These probabilities can be written as a function of the following unknown measures: prevalence, sensitivity and specificity of the test. Therefore, nine parameters were unknown in this study: one prevalence parameter and the sensitivity and specificity for each of the four tests.

With four different dichotomous tests, there were 16 possible combinations. By using the probabilities for a positive or negative test result, the likelihood of observing each pattern of test results could be calculated. We observed the number of subjects for each of the 16 patterns of test results. Standard maximum likelihood methods could be used to obtain a (unique) solution [1,17,18].

Latent class analysis

Latent class uses the standard maximum likelihood method to combine the test results from each patient for constructing a reference standard [1,17–19]. This method acknowledges that there is no gold standard and that the available tests are all related to the unknown true status: fibrosis present or absent. These unobservable outcomes are named latent classes.

The fact that a two-class model might not fit the data is either seen as an artifact of the measurement instrument or as a result of within-class heterogeneity. To allow for local dependencies and within-class heterogeneity, we used a LCM model with a random-factor, the LCM-R model [1,17–19]. The LCM-R model incorporates random effects and thus relaxes the conditional independence assumption (see Supplementary statistical method details).

The specific assumptions for random effects were the following: the dependency between tests for FibroTest and LSM which were initially validated by biopsy; the intra-class heterogeneities for biopsy due to inter-observer variability and sampling error; for LSM, the inter-observer variability and the impact of inflammation and steatosis.

In LCM-R, it is assumed that the outcome of a diagnostic test is governed by two mechanisms or factors: the disease status of the subject, and the individual biological process or the diagnostic test technological characteristics.

Sources of fit impairment

We assessed which test dependency or heterogeneity significantly impaired the fit of the standard LCM without random effects by using bivariate residuals of the baseline latent class analysis. The pair of tests was excluded step by step up until a model fitting the observed results was obtained. The fit was reached when the likelihood-ratio goodness-of-fit value [likelihood squared (L^2)] L^2 significance was >0.05 [1,17–19].

Standard performance analysis using biopsy as a gold-standard

The standard performances of FibroTest, LSM and ALT were assessed using the fibrosis stage obtained by liver biopsy, the classical gold standard, expressed using the METAVIR scoring system. The thresholds for test positivity were the usual ones. The standard area under the Receiver Operating Characteristics Curves (AUROC) was estimated by the empirical (non-parametric) method, and compared using the paired method of Zhou *et al.* [20].

Sensitivity analyses

To assess possible variability due to the sampling population, we performed successive LCM-R models (excluding each populations): excluding false positives from each test, one without any false positive, one with lower cutoff for cirrhosis 10.1% of the area of fibrosis, and two with lower LSM cut-offs: 7.1 for advanced fibrosis and 12.5 kPa for cirrhosis. We performed also a meta-analysis using random effect model of weighted AUROCs (Obuchowski measure) to identify significant heterogeneity between the different populations of patients [21].

Statistical analysis and software

We used NCSS software (Kaysville, Utah, USA) [22] for standard statistics and LatentGold-4.5 software (Statistical Innovation, Belmont, MA, USA) for estimating the model parameters [19]. We used the following criteria to identify a good model: the *p*-value of the likelihood squared (L^2) had to be greater than 0.05, and the Bayesian information criterion (BIC), defined as $L^2 - \log(N) \times Df$ (degrees of freedom of the data), had to be the smallest among all competing models. Standard error of L^2 was calculated used bootstrap method [19].

This study was conducted according to the principles expressed in the declaration of Helsinki. Signed informed consent was obtained for all controls and for patients for whom tests were not routinely performed according to the standard of care.

Results

Failure and non-reliable results were observed in 15.3% (525/3441) of LSM and in 0.6% for FibroTest (20/3441).

Subjects included

The characteristics of included patients are described in Table 1. Healthy controls were more often female and older than HCV patients. Patients of the Romanian population were more often female, and had less cirrhosis at biopsy. The median length of biopsy was 17 mm in the Bordeaux group, 25 mm in the French multicenter group and 20 in the Romanian multicenter group.

Standard assessment of biomarker performance using biopsy as the reference (imperfect gold-standard)

Performances of FibroTest, LSM and ALT using the standard AUROCs (95% CI), observed among patients with biopsy, were similar to those of the extensive literature [8,10]; for the diagnosis of advanced fibrosis: 0.75 (95% CI 0.72–0.77), 0.76 (0.73–0.79) and 0.62 (0.59–0.65), and for cirrhosis 0.85 (0.82–0.88), 0.90 (0.87–0.92) and 0.61 (0.57–0.66) respectively. As expected, performances of ALT were significantly lower than those of FibroTest and LSM (p <0.0001).

Assessment of the specificity of liver biopsy using large surgical biopsies

The distribution of the area of fibrosis estimated by virtual biopsies of different lengths is shown in Fig. 2 and Supplementary Table 1. Cases with areas of fibrosis above 5.3% were considered to be false positives of biopsy for the diagnosis of advanced fibrosis, and those above 16.5% as false positives for the diagnosis of cirrhosis. The specificity of a 20 mm length biopsy for the diagnosis of advanced fibrosis was 83.71% (Supplementary Table 2).

Assessment of test performances in the absence of a gold standard

The distribution of the subjects according to the 16 possible combinations of the four test results are shown in Supplementary Table 3 for presuming advanced fibrosis, and in Supplementary Table 4 for cirrhosis. Perfect concordance between the tests for the diagnosis of advanced fibrosis was observed in 1059 (55.4%) subjects (728 all negatives and 321 all positives) and for the diagnosis of cirrhosis in 1340 (70.8%) (1292 all negatives and 48 all positives). Details of the assessment in healthy volunteers are given in Supplementary data 2 for the diagnosis of advanced fibrosis.

Models using LCM-R were interpretable as they fit (Table 2) the observed distribution of test results. For advanced fibrosis, the ranking for the specificities was LSM (0.96), FibroTest (0.93)

Research Article

Table 1. Characteristics of the 1893 included subjects.

Characteristics	HCV patients' group				Healthy volunteers
	Bordeaux	Multicenter France	Multicenter Romania	All patients	
	n = 768	n = 378	n = 143	n = 1289	n = 604
Age, yr[1]	48 (47-49)	50 (49-51)	49 (48-52)	49 (48-50)	58 (56-59)
Male, (%)	441 (57%)	239 (63%)	48 (34%)	728 (56%)	209 (44%)
Biopsy stage[2]	2 (2-2)	1 (1-2)	2 (2-2)	2 (2-2)	0 (0-0)
Presumed fibrosis	523 (68%)	176 (47%)	89 (62%)	788 (61%)	16%[3]
Presumed cirrhosis	136 (18%)	57 (15%)	6 (4%)	199 (15%)	3%[3]
FibroTest	0.47 (0.43-0.50)	0.58 (0.53-0.64)	0.48 (0.40-0.53)	0.50 (0.48-0.53)	0.16 (0.15-0.16)
Presumed fibrosis	370 (48%)	229 (61%)	69 (48%)	668 (52%)	19 (3%)
Presumed cirrhosis	171 (22%)	123 (33%)	18 (13%)	312 (24%)	2 (0.3%)
LSM, kPa (8.8/14.5)	7.0 (6.8-7.3)	7.0 (6.7-7.7)	7.7 (7.2-8.8)	7.1 (6.9-7.4)	5.4 (3.6-6.7)
Presumed fibrosis	251 (33%)	126 (33%)	58 (41%)	435 (34%)	19 (3%)
Presumed cirrhosis	124 (16%)	54 (14%)	28 (20%)	206 (16%)	2 (0.3%)
LSM, kPa (7.1/12.5)					
Presumed fibrosis	368 (48%)	185 (49%)	84 (59%)	637 (49%)	38 (6%)
Presumed cirrhosis	151 (20%)	70 (19%)	36 (25%)	257 (20%)	5 (1%)
ALT, IU/L	65 (61-68)	72 (65-78)	93 (83-105)	69 (66-74)	22 (21-23)
Presumed fibrosis	491 (64%)	271 (72%)	117 (82%)	879 (68%)	23 (4%)
Presumed cirrhosis	207 (27%)	105 (28%)	64 (48%)	376 (29%)	4 (0.7%)

[1]Median (95% confidence interval).
[2]METAVIR scoring system.
[3]False positive of a 20 mm length biopsy as assessed using large surgical specimens.

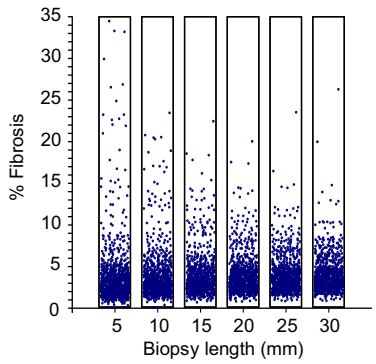


Fig. 2. Area of fibrosis estimated by biopsy according to its length (mm) in subjects scoring METAVIR F0 (no fibrosis) on the large surgical section. Cases with area of fibrosis above 5.3% were considered false positives of biopsy for the diagnosis of advanced fibrosis and those above 16.5% were false positives for the diagnosis of cirrhosis.

and biopsy (0.67); the ranking for the sensitivities was FibroTest (0.70), biopsy (0.63) and LSM (0.45). For cirrhosis, the ranking for the specificities was biopsy (0.95), LSM (0.93), and FibroTest (0.87); all sensitivities were low with the following ranking: biopsy (0.51), FibroTest (0.41), and LSM (0.39).

Compared to their performances assessed by biopsy, the performances of FibroTest and assessed by LCM-R were all increased for the diagnosis of advanced fibrosis and decreased for the diagnosis of cirrhosis. The performances of LSM were lower using LCM-R except for an increase in the specificity for advanced fibrosis (Table 3).

Models using LCM without random effects did not fit the observed distribution, suggesting a random effect due to dependency between tests (as expected due to previous validation of FibroTest and LSM by biopsy) and intra-class heterogeneity such as inter-observers variability for biopsy and LSM (Supplementary Table 5).

Assessment of significant sources of impairment in modeling

Biopsy-LSM and biopsy-ALT were identified as the two main sources of impairment in LCM models both for advanced fibrosis and cirrhosis. Bivariate residuals of LSM-ALT and biopsy-FibroTest were lower but also significantly impaired the model fit for advanced fibrosis (Table 4).

Sensitivity analyses

The population that impaired the goodness of fit the most was the healthy population results, since when excluded, the baseline BIC decreased from 34.4 to −17.7 for advanced fibrosis and from 21.6 to 9.6 for cirrhosis (Supplementary Table 6). The exclusion of healthy volunteers strongly modified the estimates, reducing specificities both for advanced fibrosis and cirrhosis and increasing sensitivities for advanced fibrosis (Supplementary Table 7). None of the other LCM-R analyses showed a major decrease of the fit assessed by BIC value (Supplementary Table 6). Results were not different when the diagnosis of cirrhosis used >10.1% area of fibrosis in healthy volunteers (Supplementary Table 8). When lower cut-offs (7.1 *vs.* 8.8 kPa) were used for LSM, this induced an expected dramatic increase in the sensitivity of LSM for advanced fibrosis from 0.45 to 0.88 but a decrease of specificity from 0.96 to 0.83 (Supplementary Table 9).

Table 2. Best latent class model with random effect of fibrosis estimate performances.

Best model for advanced fibrosis (n = 1893)		
L-Squared (standard error calculated using bootstrap)	3.2 (0.02)	
Goodness of fit likelihood ratio test statistics: *p* value[1]	0.20	
Bayesian information criterion	-11.9	
Performance of test	**Specificity[2]**	**Sensitivity[2]**
FibroTest	0.93	0.70
LSM	0.96	0.45
ALT	0.79	0.78
Biopsy	0.67	0.63
Best model for cirrhosis (n = 1893)		
L-Squared (standard error calculated using bootstrap)	0.61 (0.01)	
Goodness of fit likelihood ratio test statistics: *p* value[1]	0.74	
Bayesian information criterion	-14.5	
Performance of test	**Specificity[2]**	**Sensitivity[2]**
FibroTest	0.87	0.41
LSM	0.93	0.39
ALT	0.78	0.08
Biopsy	0.95	0.51

[1]Model fit when $p > 0.05$.
[2]No confidence interval for the LCM-derived sensitivity and specificity estimates because these estimates are calculated from combinations of conditional probabilities, which have individual maximum-likelihood estimated standard errors.

Table 3. Sensitivity and specificity of fibrosis biomarkers according to the choice of the reference: biopsy (an imperfect gold standard) or a model without gold standard (latent class model with random effect [LCM-R] as reference) in 1893 subjects.

Estimate[1]	Advanced fibrosis				Cirrhosis			
Reference	Biopsy		Latent class		Biopsy		Latent Class	
	Sp	Se	Sp	Se	Sp	Se	Sp	Se
FibroTest	0.85	0.66	0.93	0.70	0.89	0.68	0.87	0.41
LSM	0.93	0.48	0.96	0.45	0.95	0.65	0.93	0.39
ALT	0.70	0.73	0.79	0.78	0.83	0.42	0.78	0.08
Biopsy	1.00[2]	1.00[2]	0.67	0.63	1.00[2]	1.00[2]	0.95	0.51

The standard test cut-offs used for the diagnosis of advanced fibrosis and cirrhosis were 0.48 and 0.74 for FibroTest, 8.8 and 14.5 kPa for stiffness, 50 IU/L and 100 IU/L for ALT, and for biopsy in LCM-R model F2 stage and F4 stage METAVIR for real biopsy, and 5.3% and 16.5% area of fibrosis for virtual biopsies in healthy volunteers respectively.
[1]Standard errors or 95% confidence interval are not given as for the LCM-derived sensitivity and specificity estimates, because they are calculated from combinations of conditional probabilities.
[2]In this model, biopsy is considered as the reference ("gold standard") with 100% accuracy.

The meta-analysis using random effect model of weighted AUROCs showed no significant heterogeneity between the different populations of patients (Supplementary Table 10) contrarily to nonweighted AUROCs (Supplementary Table 11). The details of the 95% confidence intervals of standard sensitivity and specificities (using biopsy as reference) are given in Supplementary Table 12.

Discussion

This study is the first using appropriate methods for better reconciliation of the estimates of sensitivity and specificity of noninvasive fibrosis biomarkers, as well as those of biopsy, the former gold standard, which cannot be 100% accurate [23]. The main result is that a model without using reference is compatible with the distribution of biomarkers and biopsy results.

The high specificity (>0.85) of FibroTest and LSM was confirmed for the diagnosis of both advanced fibrosis and cirrhosis. As already observed in standard analysis and in a preliminary latent class study [8], the results confirmed that the sensitivity of FibroTest (0.70) was higher than that of LSM (0.48) for the diagnosis of advanced fibrosis. The performance for the diagnosis of cirrhosis was similar between FibroTest and LSM.

One original result of the present study is the relative lower level of biopsy performance, in comparison with FibroTest and LSM when evaluated similarly for the diagnosis of advanced fibrosis. For cirrhosis, biopsy had the best performance with the highest specificity, and the highest sensitivity but far from perfection, with 49% of presumed false negativity rate, as FibroTest and LSM.

Strengths of the study

Population included

The first strength was the wide spectrum of liver injury, from healthy volunteers to cirrhotic patients, with two multicenter studies in two different countries.

The second strength was the inclusion of a large healthy population with biomarkers, together with the presumed results of biopsies generated from normal livers. The inclusion of a healthy population in the model changed it very significantly. One major

Research Article

Table 4. Direct effects of pairs of variables that impaired the fit of the baseline latent class model. Effects are estimated by bivariate residuals of the baseline latent class analysis, without random effects. The effect of the most significant pair was excluded to achieve non-significance.

	Bivariate residuals			Model improvement after excluding residuals		
	FibroTest	LSM	ALT	Pair excluded (Cumulative)	Fit (L^2) (Cumulative)	Significance after pair exclusion[2]
Advanced fibrosis				None	79.7[1]	<0.0001
LSM	0.44			Biopsy-LSM	38.3	<0.0001
ALT	2.9	0.14		Biopsy-ALT	30.1	<0.0001
Biopsy	0.11	11.6	0.47	LSM-ALT	13.8	0.003
				Biopsy-FibroTest	0.32	0.85
Cirrhosis				None	66.0[1]	<0.0001
LSM	0.24			Biopsy-LSM	27.7	<0.0001
ALT	3.52	0.29		Biopsy-ALT	9.96	0.04
Biopsy	0.95	10.8	10.7			

[1]Baseline fit.
[2]Model fit when $p > 0.05$.

weakness of previous overviews of LSM performance was the absence of conciliation between the LSM accuracy estimated in patients [5] with the positive rate observed in healthy populations [12,15]. The 95th percentiles of LSM in a healthy non-obese population without metabolic syndrome, 7.8 kPa for females and 8.0 kPa for males, observed by Roulot *et al.* [15], were in accordance with the 3% positive rate of LSM (above 8.8 kPa) observed in our healthy volunteers (Table 1) and with the 4% of false positive for advanced fibrosis estimated by our LCM-R model (Table 2 and Supplementary Table 5). LSM should not be used at the 8.8 kPa cutoff for screening purposes, as the specificity was 96% but only applicable in 45% of patients.

Use of latent class with random effects

The third strength was the use of a latent class paradigm with random effect which introduces a random variability factor in the model. FibroTest and LSM were initially validated using biopsy, and therefore it was rational to use a method which takes into account this non-independence between tests.

All tests can then be compared without the systematic bias of the absence of error for biopsy. FibroTest performances were similar to that of a 20 mm biopsy for the diagnosis of advanced fibrosis.

As expected, performances of ALT were lower than those of FibroTest for the diagnosis of both advanced fibrosis and cirrhosis. The main interest of ALT used as a negative control test was to better understand the possible sources of variability among LSM and biopsy.

Sources of major variability among tests

The fourth strength was the identification of the major sources of test dependency and heterogeneity. Indeed, LCM failed to obtain a model that fits with the observed distribution, without including a "random factor" that is unknown sources of discordances not related to the diagnostic performance of tests (Table 3). As FibroTest and LSM were validated using biopsy, the first rational variability factor was this initial "dependency".

The variability was mainly related to the biopsy-LSM residual. It was rational to observe the greater variability for the biopsy-LSM pair, as these indicators have both significant intra- and inter-observer variability [7,8,9,15,24,25] in comparison with the smaller analytical variability of FibroTest [26]. Furthermore the biopsy-LSM pair variability is impacted by the (fibrosis stages) spectrum effect to a greater degree than the biopsy-FibroTest pair. LSM has no diagnostic value for the initial fibrosis stages (METAVIR F0 and F1), a limited accuracy between stages F1 *vs.* F2, and a higher accuracy between F2, F3 and F4. Contrary to LSM, FibroTest has a consistent accuracy between adjacent stages [3,4,8,10].

The biopsy-ALT pair was the second source of residuals for the diagnosis of advanced fibrosis, without obvious bias as the pathologists were not aware of the ALT value. However, a bias related to an overestimation of liver fibrosis stage cannot be ruled out during biopsy readings, when biopsies showed higher activity grades.

The LSM-ALT pair was the third most important residual with a documented rationale, as necrosis and inflammation increased LSM independent of fibrosis stage [8,27,28].

The various sensitivity analyses (LSM cut-offs, area of fibrosis cut-offs, population, false positive rate in healthy volunteers) did not induced any absence of fit (Supplementary Tables 6 and 8). In the LCM-R model, despite no change in the fit, there was indeed a "cutoff effect" of LSM on FibroTest performances but limited to the sensitivity for cirrhosis, which was lower to the impact observed on biopsy (Supplementary Table 10).

Limitations of the study

Biopsy estimates in healthy volunteers

The results of biopsy in volunteers were directly estimated in only 4 subjects with large, normal liver biopsies, the specificity being assessed using 626 generated virtual biopsies. This method is imperfect. However, the observed false-positive rates were compatible with other assessments using virtual biopsies, or surgical samples [7]. The distribution of area of fibrosis was similar to that of Bedossa *et al.* (Supplementary Table 1) [7]. Furthermore the change for another more sensitive cutoff for cirrhosis (Supplementary Tables 8 and 9) and the exclusion of all false-positive cases of biopsies (Supplementary Tables 6 and 7) did not impair the model. The model was constructed with a median of biopsy around 20 mm and if the length had been around 40 mm the expected performance of biopsy would have been better but less realistic [25].

Other test performances

The present study compared the accuracy of tests, which is considered only one part of the performance. The failure rates and

reliability were not assessed as well as the other features that could be provided by each test. For liver biopsy, pathologists recommend lengths of at least 20–25 mm [7], which could correspond to a reliability rate of 50% according to the length distribution in large cohorts [25]. For LSM using Fibroscan, the failure rate is 3.8% and the reliability rate 15.8% [9]. For FibroTest, the failure rate is 0% and reliability rate is 98% [28].

Biopsy has an obvious advantage by providing activity grade, steatosis grade and features of other liver diseases. FibroTest assessment includes ActiTest, validated for activity grade diagnosis [29]; SteatoTest, which assesses steatosis grade, can also be associated with FibroTest but has been less validated [30,31].

Variability factors not analyzed

We did not directly analyze the impact of factors from individual data, such as histological steatosis and activity, metabolic factors, age, gender, ethnicity or operator effects that could be related to diagnostic performance [9], and the pathologist variability [24]. As for LSM, the inter-observer variability is a pragmatic weakness of biopsy in comparison with serum biomarkers.

How can the comparisons between liver fibrosis indicators be improved?

First, clear guidelines must be provided defining the reliability criteria of each indicator. For FibroTest, pre-analytical and analytical recommendations must be applied [28]. Other studies have previously demonstrated for LSM that few changes in the precautions of use had a direct impact on its reliability rate or on its risk of false-positives or negatives [8,9]. Publications not applying the precautions of use concerning IQR/LSM and success rate made hazardous conclusions, such as the suggestion that five valid shots could be sufficient for cirrhosis diagnosis [14]. For liver biopsy, it would be wise to consider the results of specimens shorter than 20 mm reliable only after checking the concordance with the reliable results of a validated biomarker.

Second, the intra-indicator variability should be reduced. For FibroTest, the improvement of analytical calibration should reduce the inter-laboratory variability [4,26]. For LSM [8,9] and biopsy [24], the major concern is the operator variability, even if the results are reliable. New methodology such as the concordance rate between LSM and FibroTest can identify observers with too high variability [8]. This method could also be applied to pathologists.

Third, these results must be confirmed by independent groups. However, in the present study all the included cohorts of patients were independent of the FibroTest inventor.

Conclusions

In a model without gold-standard, the high specificity (>0.85) of FibroTest and LSM was confirmed for the diagnosis of both advanced fibrosis and cirrhosis. However, from the analysis of the tests that impaired the fit of the model, more studies should be performed to identify the causes of the high discordances rates between biopsy and LSM, including their intra- and inter-observers' variability.

If the accuracy paradigm cannot convince the users in this field, it is possible to replace it by a new one: the concept of the validation of medical tests [1]. The present results were consistent with the recent prognostic validation of fibrosis biomarkers. In patients with chronic hepatitis C [32,33] as well as in patients with chronic hepatitis B [34] and alcoholic liver disease [35], the prognostic value of FibroTest was at least similar to that of biopsy.

The present results confirm that balanced discussions are needed when discordances are observed between estimates of fibrosis. Biopsy, even of 20 mm, is no more the reference. This model confirms the first guidelines and reimbursement by French health authorities recommending either FibroTest or LSM as first line fibrosis estimates in adult patients with uncomplicated chronic hepatitis C [36]. Finally to move forward such models without gold standard should permit also to better estimate the forthcoming new test performances.

Authors' involvement

T.P. study concept and design, analysis and interpretation of data, drafting; statistical analysis; study supervision. V.dL., J.P.Z., C.S., J.V., J.F., A.T., G.L.N., J.C.V., V.R., F.C.: acquisition of data; V.R., M.M.: critical revision of the manuscript.

Conflict of interest

TP is the inventor of FibroTest and has a capital interest in Biopredictive the company marketing the test. The patents belong to Assistance Publique Hôpitaux de Paris, a public organization. M.M. is a full time employee of Biopredictive and participated in the critical analysis of the manuscript. Biopredictive has no role in the study design, in the collection, analysis, and interpretation of data.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhep.2011.08.007.

References

[1] Rutjes AW, Reitsma JB, Coomarasamy A, Khan KS, Bossuyt PM. Evaluation of diagnostic tests when there is no gold standard. A review of methods. Health Technol Assess 2007;11:1–51.
[2] Castera L, Pinzani M. Non-invasive assessment of liver fibrosis: are we ready? Lancet 2010;375:1419–1420.
[3] Poynard T. First-line assessment of patients with chronic liver disease with non-invasive techniques and without recourse to liver biopsy. J Hepatol 2011;54:586–587.
[4] Halfon P, Munteanu M, Poynard T. FibroTest–ActiTest as a non-invasive marker of liver fibrosis. Gastroenterol Clin Biol 2008;32:22–38.
[5] Friedrich-Rust M, Ong MF, Martens S, Sarrazin C, Bojunga J, Zeuzem S, et al. Performance of transient elastography for the staging of liver fibrosis: a meta-analysis. Gastroenterology 2008;134:960–974.
[6] Castera L, Denis J, Babany G, Roudot-Thoraval F. Evolving practices of non-invasive markers of liver fibrosis in patients with chronic hepatitis C in France: time for new guidelines? J Hepatol 2007;46:528–529.
[7] Bedossa P, Dargère D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. Hepatology 2003;38:1449–1457.
[8] Poynard T, Ingiliz P, Elkrief L, Munteanu M, Lebray P, Morra R, et al. Concordance in a world without a gold standard: a new non-invasive methodology for improving accuracy of fibrosis markers. PLoS One 2008;3:e3857.
[9] Castéra L, Foucher J, Bernard PH, Carvalho F, Allaix D, Merrouche W, et al. Pitfalls of liver stiffness measurement: a 5-year prospective study of 13,369 examinations. Hepatology 2010;51:828–835.
[10] Zarski, Sturm N, Guechot J, Paris A, Zafrani ES, Asselah T, et al. Comparison of 10 blood tests and transient elastography for liver fibrosis in chronic hepatitis C: the ANRS HCEP-23 study. J Hepatol 2012;56:55–62.

Research Article

[11] Sporea I, Sirli R, Deleanu A, Tudora A, Popescu A, Curescu M, et al. Liver stiffness measurements in patients with HBV vs. HCV chronic hepatitis: a comparative study. World J Gastroenterol 2010;16:4832–4837.

[12] Poynard T, Lebray P, Ingiliz P, Varaut A, Varsat B, Ngo Y, et al. Prevalence of liver fibrosis and risk factors in a general population using non-invasive biomarkers (FibroTest). BMC Gastroenterol 2010;10:40.

[13] Poynard T, Lenaour G, Charlotte F, Munteanu M, Vaillant JC, Ngo Y, et al. Comparison of Fibrotest–Fibrosure and liver stiffness measurement, using fibrosis area and large surgical samples. Hepatology 2010;52:282A, [abstract].

[14] Kettaneh A, Marcellin P, Douvin C, Poupon R, Ziol M, Beaugrand M, et al. Features associated with success rate and performance of FibroScan measurements for the diagnosis of cirrhosis in HCV patients: a prospective study of 935 patients. J Hepatol 2007;46:628–634.

[15] Roulot D, Czernichow S, Le Clésiau H, Costes JL, Vergnaud AC, Beaugrand M, et al. Liver stiffness values in apparently healthy subjects: influence of gender and metabolic syndrome. J Hepatol 2008;48:606–613.

[16] Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. Hepatology 1996;24:289–293.

[17] Qu Y, Tan M, Kutner MH. Random effects models in latent class analysis for evaluating accuracy of diagnostic tests. Biometrics 1996;52:797–810.

[18] Hadgu A, Qu Y. A biomedical application of latent class models with random effects. Appl Stat 1998;47:603–616.

[19] Vermunt JK, Magidson J. Technical guide to latent gold 4.5. Belmont, Massachusetts, USA: Statistical Innovations Inc.; 2007.

[20] Zhou X, Obuchowski N, McClish D. Statistical methods in diagnostic medicine. John Wiley and Sons; 2002.

[21] Lambert J, Halfon P, Penaranda G, Bedossa P, Cacoub P, Carrat F. How to measure the diagnostic accuracy of noninvasive liver fibrosis indices: the area under the ROC curve revisited. Clin Chem 2008;54:1372–1378.

[22] Hintze JL. NCSS 2007 user guide. Number cruncher statistical systems software. Kaysville, Utah: NCSS; 2007.

[23] Mehta SH, Lau B, Afdhal NH, Thomas DL. Exceeding the limits of liver histology markers. J Hepatol 2009;50:36–41.

[24] Rousselet MC, Michalak S, Dupré F, Croué A, Bedossa P, Saint-André JP, et al. Hepatitis network 49. Sources of variability in histological scoring of chronic viral hepatitis. Hepatology 2005;41:257–264.

[25] Poynard T, Halfon P, Castera L, Charlotte F, Bail BL, Munteanu M, et al. Variability of the area under the receiver operating characteristic curves in the diagnostic evaluation of liver fibrosis markers: impact of biopsy length and fragmentation. Aliment Pharmacol Ther 2007;25:733–739.

[26] Poynard T, Munteanu M, Deckmyn O, Ngo Y, Drane F, Messous D, et al. Applicability and precautions of use of liver injury biomarker FibroTest. A reappraisal at 7 years of age. BMC Gastroenterol 2011;11:39.

[27] Coco B, Oliveri F, Maina AM, Ciccorossi P, Sacco R, Colombatto P, et al. Transient elastography: a new surrogate marker of liver fibrosis influenced by major changes of transaminases. J Viral Hepat 2007;14:360–369.

[28] Poynard T, Ngo Y, Munteanu M, Thabut D, Massard J, Moussalli J, et al. Biomarkers of liver injury for hepatitis clinical trials: a meta-analysis of longitudinal studies. Antivir Ther 2010;15:617–631.

[29] Poynard T, Munteanu M, Ngo Y, Castera L, Halfon P, Ratziu V, et al. ActiTest accuracy for the assessment of histological activity grades in patients with chronic hepatitis C, an overview using Obuchowski measure. Gastroenterol Clin Biol 2010;34:388–396.

[30] Poynard T, Ratziu V, Naveau S, Thabut D, Charlotte F, Messous D, et al. The diagnostic value of biomarkers (SteatoTest) for the prediction of liver steatosis. Comp Hepatol 2005;4:10.

[31] Poynard T, Munteanu M, Colombo M, Bruix J, Schiff E, Terg R, et al. FibroTest is an independent predictor of virologic response in chronic hepatitis C patients retreated with pegylated interferon alfa-2b and ribavirin in the EPIC(3) program. J Hepatol 2011;54:227–235.

[32] Ngo Y, Munteanu M, Messous D, Charlotte F, Imbert-Bismut F, Thabut D, et al. A prospective analysis of the prognostic value of biomarkers (FibroTest) in patients with chronic hepatitis C. Clin Chem 2006;52:1887–1896.

[33] Vergniol J, Foucher J, Terrebonne E, Bernard PH, Le Bail B, Merrouche W, et al. Non-invasive tests for fibrosis and liver stiffness predict 5-year outcomes of patients with chronic hepatitis C. Gastroenterology 2011;40:1970–1979.

[34] Ngo Y, Benhamou Y, Thibault V, Ingiliz P, Munteanu M, Lebray P, et al. An accurate definition of the status of inactive hepatitis B virus carrier by a combination of biomarkers (FibroTest–ActiTest) and viral load. PLoS One 2008;2:e2573.

[35] Naveau S, Gaudé G, Asnacios A, Agostini H, Abella A, Barri-Ova N, et al. Diagnostic and prognostic values of noninvasive biomarkers of fibrosis in patients with alcoholic liver disease. Hepatology 2009;49:97–105.

[36] La Haute Autorité de Santé (HAS) in France – The HAS recommendations for the management of the chronic hepatitis C using non-invasive biomarkers. Available from: <http://www.has-sante.fr/portail/display.jsp?id=c_476486> [accessed August 2007].